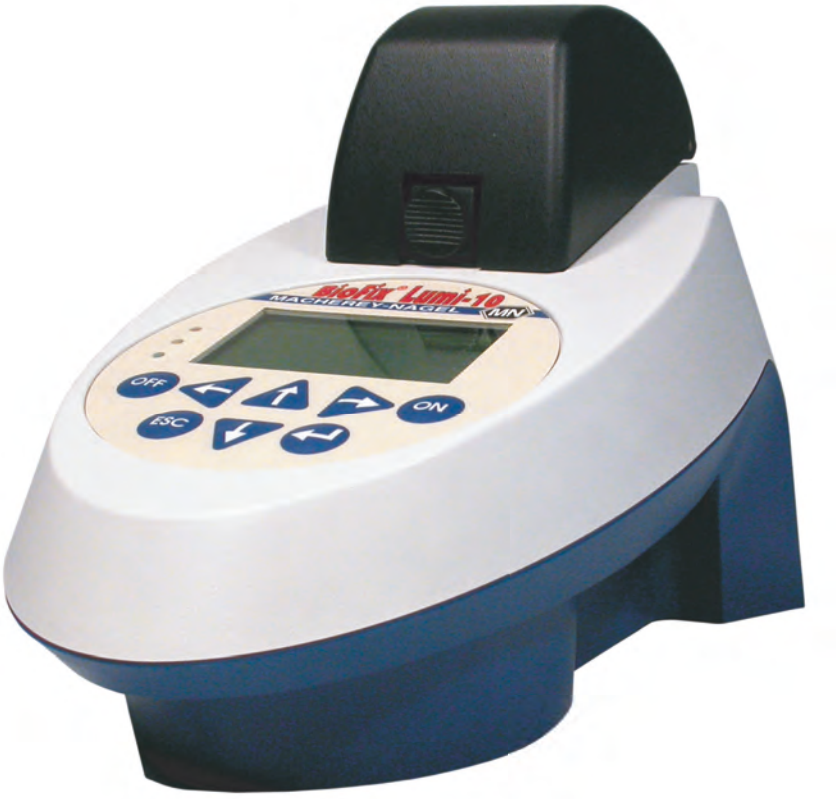
Water Analysis

MACHEREY-NAGEL

BioFix® Lumi-10



Manual

CE

The CE labelling refers to the fact that this instrument conforms with the the EMC 89/336/EEC directive, DIN VDE 0700.1 (EN 60 335-1).

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Content

Safety notices

Use in accordance with the regulations

The small **BioFix®** ***Lumi-10*** luminometer is used to measure bioluminescent or chemical luminescent reactions. In this process all the reagents that lead to a relatively constant light emission can be used. The ranges of application include, for instance, environmental analysis, ecotoxicological examinations, clinical diagnosis, hygiene monitoring as well as molecular biological/biochemical research.

For your safety

The **BioFix®** ***Lumi-10*** luminometer is a state of the art instrument and corresponds to the safety regulations.

The manufacturer has done everything that it can to ensure its safe operation. The user must ensure that the instruments are set and installed in such a way that their safe usage is not impaired.

The instruments are work's checked and were delivered in a safe operational state.

The operating manual at hand contains information and warnings that must be followed by the user in order to enable the secure operation of the instruments.

The following safety instructions must be observed at all times both prior to the commissioning and also during the operation of the instrument:

- The instrument may only be put into operation by authorised persons and only operated by personnel who have been trained to use it. All the users who work with the instrument must read these operating instructions first.
- Only the maintenance and service work described in this manual may be carried out by the user. Only use the stated parts in this process.
- Ensure that the mains power supply that has been delivered is appropriate for your mains voltage.
- Service work may only be carried out by the authorised service technicians of the **MACHEREY-NAGEL** company.
- Always keep the cuvette shaft clean!
- Avoid electrostatic charging!

MACHEREY-NAGEL does not give any warrantees, not even for losses *vis-à-vis* third parties that are caused by the improper handling of the instrument.

The commissioning steps, recommendations and maintenance measures, which are recommended by the manufacturer, must be implemented by the manufacturer in order to ensure the user's safety and the functioning of the instruments. All the servicing and maintenance tasks which go beyond those described in the operator's manual may only be carried out by authorised technicians.

1. Introduction

1.1 Description of the BioFix® *Lumi-10 luminometer*

The **BioFix®** ***Lumi-10*** luminometer is a small portable luminometer for the measurement of biological and chemical luminescence reactions with relatively constant light emission. As a mobile test system the **BioFix®** ***Lumi-10*** luminometer is appropriate for a wide range of applications due to its highly sensitive detector (Ultra Fast Single Photon Counter):

- Environmental analysis/ecological toxicology: acute and chronic luminous bacteria tests, mutagenicity and genotoxicity tests
- Hygiene monitoring: ATP and biological mass analyses
- Molecular biological and biochemical diagnostics: Reporter gene assays, NADP(H)-measurements, DNA probe assays, luminescence immunoassays

The option of net-free operation via the battery integrated within the instrument additionally makes it possible to also insert the luminometer for on-site measurements above all in the area of mobile environmental analysis. A fully charged battery facilitates net-free non-stop operation of 6 to 8 hours. The measured data are automatically stored and can, if necessary, be transferred for further processing and documentation to a standard computer. The maximum storage capacity amounts to 2000 readings. In addition the results can be immediately classified by means of user-defined boundary values. **BioFix®** ***Lumi-10*** should be operated at ambient temperatures of +15 °C to +30 °C.

Warning signs signify to the user that the ambient temperatures are too low or too high, instrument and operating errors, lighting intensities that are too high or too low as well as the extent to which the battery is loaded.

BioFix® ***Lumi-10*** provides all the advantages of a modern and contemporary as well as user-friendly luminometer:

- Can be operated from the mains or using batteries
- High resolution graphic display
- Menu mode optionally in German or English
- Serial 9 PIN RS232 interface for selective data transfer to a standard PC
- Test result memory for up to 2000 test results
- Stopwatch integrated within the apparatus with variable settings
- Selective data administration (calling/deleting) by means of the location identification digit, sample number, date, time parameters
- Password entry option
- 6 programming spaces that can be individually set for user-specific measuring programmes
- Classification of test results by means of previously defined boundary values
- Variable measurement times in order to obtain optimum results even in the event of weak luminosity

1.2 Test protocols and ranges of application

With the aid of the **BioFix® *Lumi-10*** both toxicity and mutagenicity tests can be carried out using luminous bacteria and also a series of molecular biological and biochemical investigations (e.g. ATP measurements, Reporter gene assays, DNA probe assays).

During the **toxicity measurement** with the aid of luminous bacteria the light produced by the *Vibrio fischeri* luminous bacteria will be measured following the reaction of the sample and compared with the light intensity of a control preparation. The results are stated as the % inhibition or % stimulation of the light intensity compared with the unhindered control. The extent of the percentage deviation of the light intensity in the sample compared to the control demonstrates a disruption of the metabolism of the *Vibrio fischeri* luminous bacteria and is deemed as being a measure of the relative toxicity of the sample.

The **BioFix® *Lumi-10*** luminometer provides the option of carrying out the luminous bacteria tests in two different ways. With the aid of the **<BioTox-S>** test protocol only the final light intensity of the test preparations following the expiry of the incubation time is used for an estimate of the toxicity. This method is suitable for rapid screening measurements if a rough estimate of the degree of toxicity of the samples is sufficient.
Alternatively the **<BioTox-B>** test protocol provides the option of also analysing the initial light intensity of the test preparations in addition to the final light intensity prior to the addition of the luminous bacteria. We precisely recommend this if more precise measuring results are desired.

The **<RLU>** test protocol serves to implement the luminescence test from the hygiene monitoring sector (**ATP and Biological mass analyses**) and molecular biological/ biochemical diagnostics (**Reporter gene assays**, **DNA probe assays**, **luminescence immunoassays** etc.). Also in the case of these methods light is generated – in a different manner depending on the test procedure – that is measured with the aid of the luminometer. The results are stated in these cases as "relative light units" (RLU).

In addition the **<RLU>** test protocol provides the special option of carrying out a **Mutatox® genotoxicity test** that is based on a dark mutant of the *Vibrio fischeri* luminous bacteria (M 169 "Light$^-$-mutant" strain). Genotoxicological terms or mutagenic effective agents cause a reversion of the "Light$^-$-mutant" to the luminous variant of the luminous bacteria ("Light$^+$") and thus re-establishes the bioluminescence. The light intensity in the sample by comparison with a non-luminous control is deemed as being the measure of the genotoxicity/mutagenicity or for the potential of sample to change the genotype.

The software of the **BioFix® *Lumi-10*** that is integrated within the instrument facilitates the simple, user-friendly implementation of the aforementioned applications. The software option provides the option of storing the user-specific measuring programmes that can be individually set on 6 programming spaces! In addition the test results obtained following a measurement can be automatically classified by means boundary values, which can also be individually defined. The measuring convenience is further enhanced by means of a stop watch that can be variably set, which is integrated in the instrument. An idle tone that rings out following the expiry of the incubation time provides a signal for the implementation of the measurements.

2. Instrument structure

During the development of the **BioFix®** ***Lumi-10*** the focus was upon functionality, quality and reliability.
The housing is splashwater resistant in accordance with IP54 and can be employed in a temperature range of up to + 30 °C.

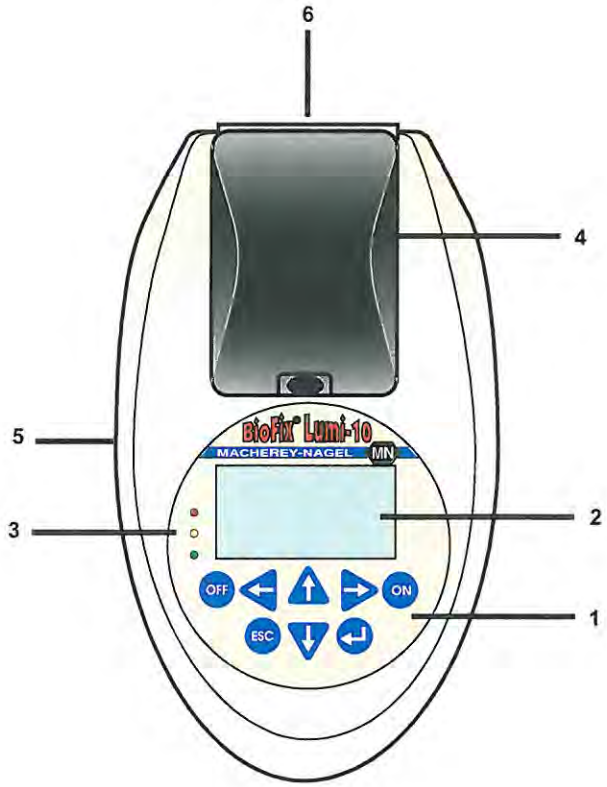


Diagram 1: **BioFix®** ***Lumi-10*** luminometer (supervision).

1 Keypad

8-part standard keyboard covered with foil for all commands to do with the entering of parameters, test implementation and data administration.

2 Graphic display

Backlit graphic display with a resolution of 128 x 64 pixels. The back lighting of the display is automatically switched to the economy mode if the keyboard is not operated for 10 seconds. This is automatically switched on by pressing the **[ON]** key. The contrast and brightness can be set by means of the software (see chapter 5.2.6).

3 Warning and signal lights

One green, yellow and red light respectively for the classification of the measurement results depending on the set limit and threshold values (see chapter 5.2.2). The red light additionally serves as a warning signal in the event of ambient temperatures that are too high or too low, instrument or operating errors, lighting intensities that are too high or too low.

4 Measurement unit (cuvette shaft and photomultiplier)

The light-proof lockable measurement unit consists of a black sealing cap, the cuvette shaft and a photomultiplier as a detector.

Opening of the measurement unit: The sealing cap can be opened after pressing the black pushbutton.

Closing of the measurement unit: Press the sealing cap down until it audibly clicks into place. Only then is the cuvette shaft locked in a light-proof manner and the measurement start is released.

Cuvette formats of 47 x 12 mm to 75 x 15 mm can be used. All the cuvette formats can be optimally fitted in by using the adapter that came with the delivery.

In the case of the photomultiplier it is an single photon counter (Ultra Fast Single Photon Counter) that works in the spectral wave range of 380 to 630 nm. The linear range covers 6 decades. In the **<RLU>** test protocol the counts measured are directly displayed as RLU.

5 Battery compartment

The battery compartment is located on the bottom side of the instrument and is sealed with a cover plate, that is attached with a screw. Observe the right polarity when loading the battery compartment!

Remove the cuvettes from the cuvette shaft before the instrument is turned! Risk of damage due to liquid seeping out!

6 Mains supply and RS232 interface

Both connections are located on the rear of the instrument and can be protected by means of a sliding plate:

Serial RS232 interface: Is used for data transmission to a standard computer. You need a standard null modem cable to do this.

Mains supply: Socket to connect the mains power supply that came with the delivery.

3. Keypad

The keypad consists of six pushbuttons covered with foil for the operation of the software and two pushbuttons covered with foil for to switch the luminometer on or off.

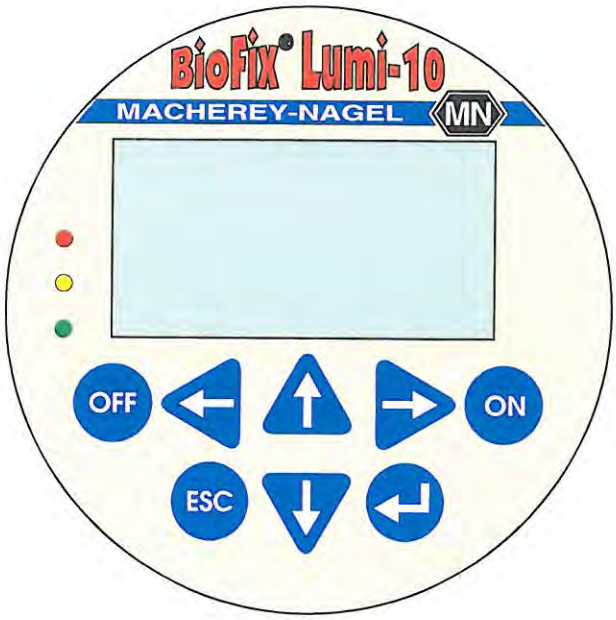


Diagram 2: Keypad of the **BioFix® *Lumi-10*** luminometer.

Switching on the instrument and (if the instrument is switched on) the display illumination.

Switching off the instrument.

Enter key to activate a selected option, confirmation of an entry, confirmation of a selection.

Escape key to return to the next highest hierarchy level or to cancel an entry without assuming the change.

Selection of a line, scrolling in a selection list, changing a numeric value or text.

Moving the cursor, yes/no selection.

4. Commissioning and main menu

Unpacking

Unpack the measuring instruments and the mains supply circuit and check that both parts are intact. Immediately inform **MACHEREY-NAGEL** in Dueren, Germany in the event of any damage.

Power supply

BioFix® *Lumi-10* works with an input voltage of 6 V. The instrument can be operated by means of a mains supply circuit with the mains or free of the mains by means of a battery.

If the instrument is connected to the mains then the rechargeable batteries will be recharged. If the **BioFix® *Lumi-10*** is switched on with the **[ON]** key then it works with mains current (with a simultaneous charging function). The integrated real time clock will be operated by means of a lithium cell when the instrument is switched off (serviceable life at least 5 years, typically 10 years).

IMPORTANT:
- **The charging of the rechargeable batters is only possible via the mains supply circuit when the instrument is switched on !**
- **Do not charge the rechargeable batteries using an external battery charger!**

Rechargeable battery type	NiCd baby cells
Serviceable life without an electric mains connection	6 hours
Charging time when operated at the same time	4 – 5 hours

The capacity of the rechargeable battery declines in the event of frequent discharging and charing and as they get older. The rechargeable batteries should be discharged at regular intervals and recharged again in order to counteract this.

The **<empty battery>** warning notice appears 15 minutes before the rechargeable batteries are exhausted!
The instrument is then switched off when the batteries are completely exhausted.

Connecting the instrument and switching it on

1. Push the protective plate on the rear side of the instrument downwards in order to uncover the electric mains socket.
2. Insert the mains supply circuit in a mains socket and the pin plug in the socket on the rear side of the instrument. In this way the rechargeable batteries are recharged in the interior of the instrument.
3. Switch on the instrument by pressing the **[ON]** key.
4. After being switched on the instrument description appears for a few seconds with the software version number and following that the main menu (see diagram 3).

Switch the instrument on in the mains-free rechargeable battery operation:

*If the instrument is not connected to the mains then it will also be switched on by pressing the **[ON]** key.*

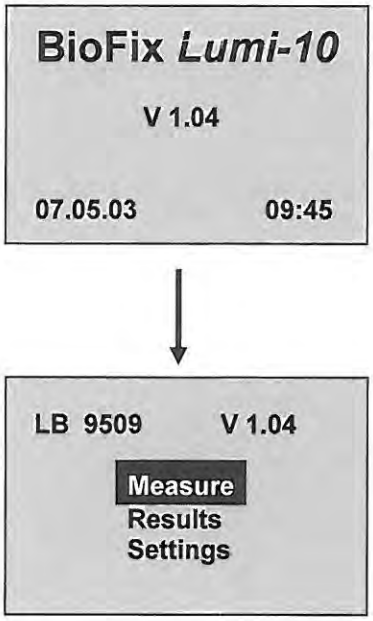


Diagram 3: Main menu after the instrument has been switched on.

5. Software and menu mode

The user software is designed with a menu type design and is operated by means of six function keys. The software, the instrument and test parameters as well as a maximum of 2000 measurement values are stored in the non-volatile memory. The integral values of a measurement are comprised of 5 ms intervals.

5.1 Design and operation

The user is directly in the main menu after switching on the instrument. There the users has a choice between the **<Measure>**, **<Results>** and **<Settings>** options:

<Measure> This option is called up if measurements should be carried out.

<Results> The data administration is accessed via this option (call up or delete measurement results, data transfer to the computer).

<Settings> The submenu for the setting of instrument and test parameters is accessed by calling up this option.

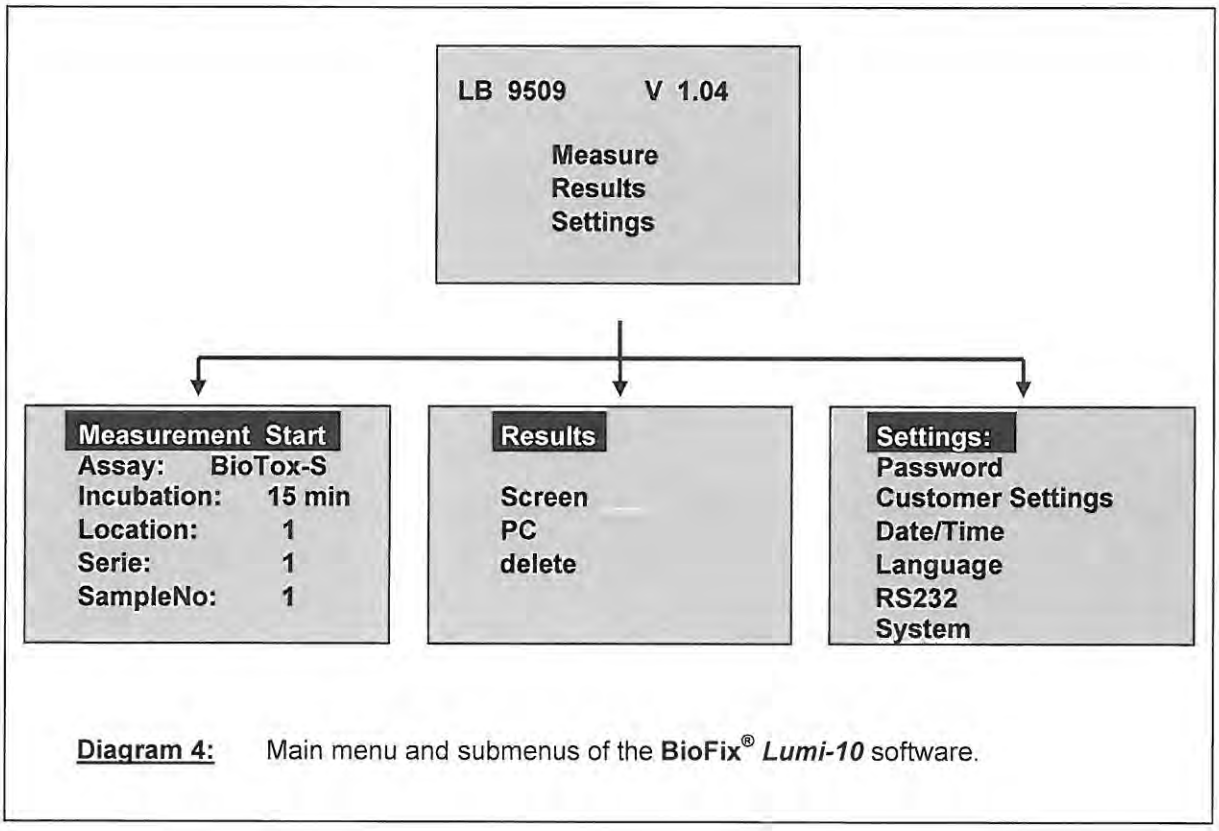


Diagram 4: Main menu and submenus of the **BioFix®** ***Lumi-10*** software.

Operation

The instrument is operated with 8 keys. This display is carried out on a high resolution graphic display.
After switching on the instrument the display automatically converts to the display mode. Here it is possible to move the cursor bars from one line to the next with the arrow keys [↑] / [↓]. In this process either the entire line is highlighted or a field. One can distinguish between the following field types for the processing:

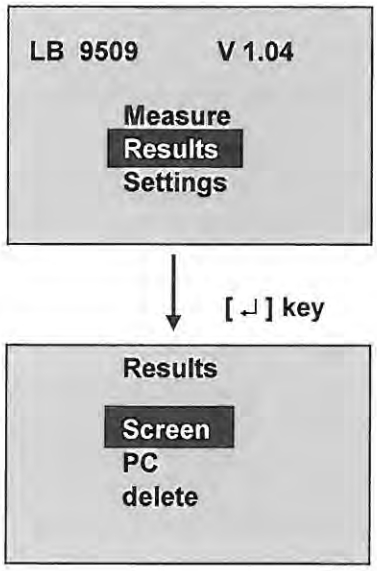
- **Entry fields** for the entry of numerical values or texts
- **Selection fields** for the selection of texts
- **Optional fields** for the selection of an option or a menu

Diagram 5: Field types in the **<Measurement Start>** mode.

Optional fields

You can recognise a highlighted menu or a highlighted option by the fact that the cursor bar extends across the whole line. If a menu or an option is highlighted with the cursor bare and the [↵] key is pressed then the selected menu is activated. The programme jumps to the selected menu or activates the option.

Example 1: Move from the main menu to the **<Results>** submenu with its sub-options.

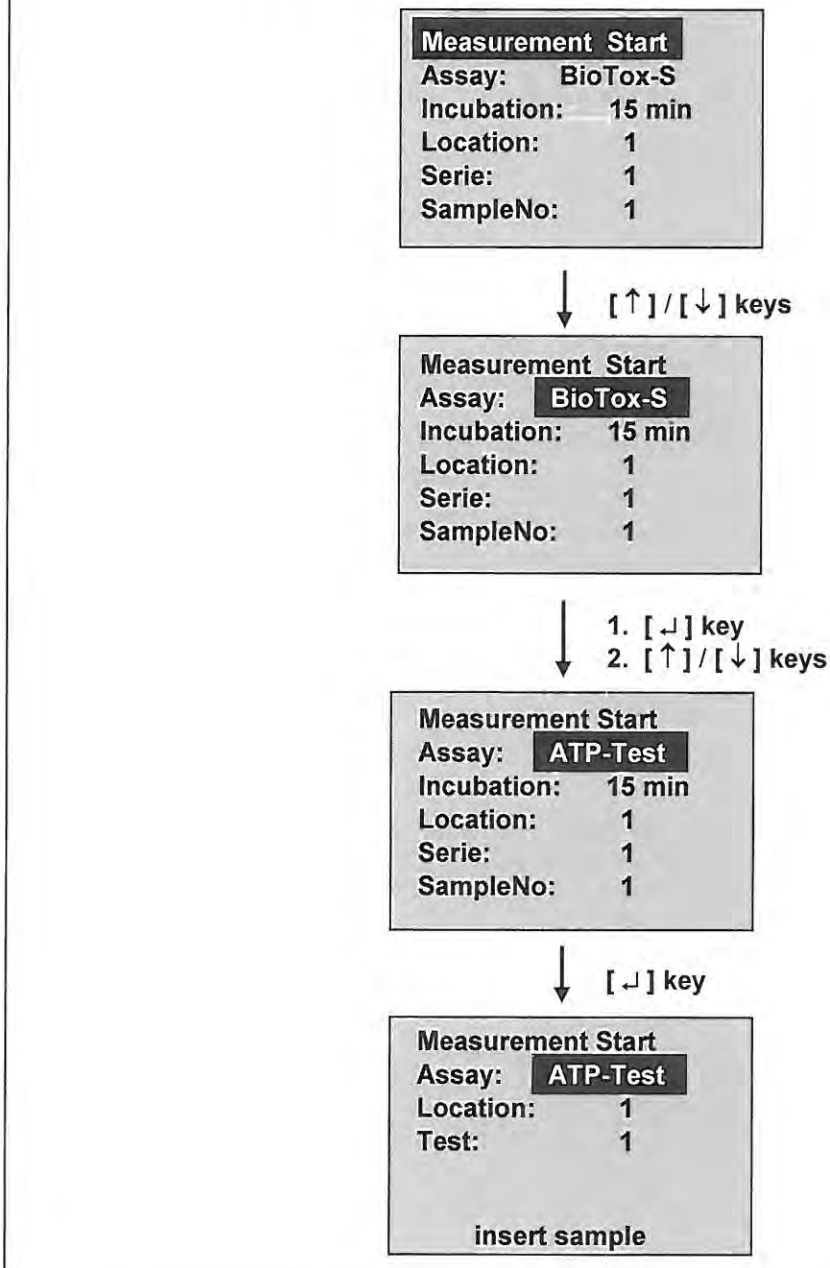


Selection fields

If you highlight a selection with the cursor bar and press the [↵] key then you activate the selection mode so that you can browse in the selection list with the arrow keys [↑] / [↓]. The selection mode can be recognised by the flashing cursor.

The selection is confirmed by pressing the [↵] key once more. The programme returns to the display mode.

Example 2: In the **<Measurement Start>** menu switch from Assay **<BioTox-S>** to Assay **<ATP-Test>**.



Entry fields

If you highlight an entry field with the cursor bar (number entry or text) and press the **[↵]** key then the entry mode will be activated so that the displayed value or text can adjusted with the aid of the 4 arrow keys. The entry mode can also be recognised by means of the flashing cursor.

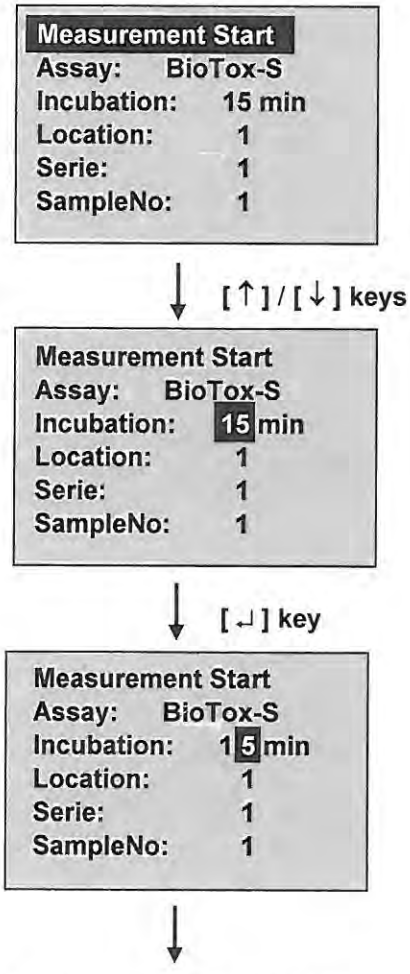
The flashing cursor highlights the point that can be adjusted. The cursor is move to the next position by means of the **[←] / [→]** keys.

The numeric value of the highlighted position counts up from 0 to 9 or down from 9 to 0 by means of the **[↑] / [↓]** keys.

The letter of the highlighted position counts up or down with the **[↑] / [↓]** keys.

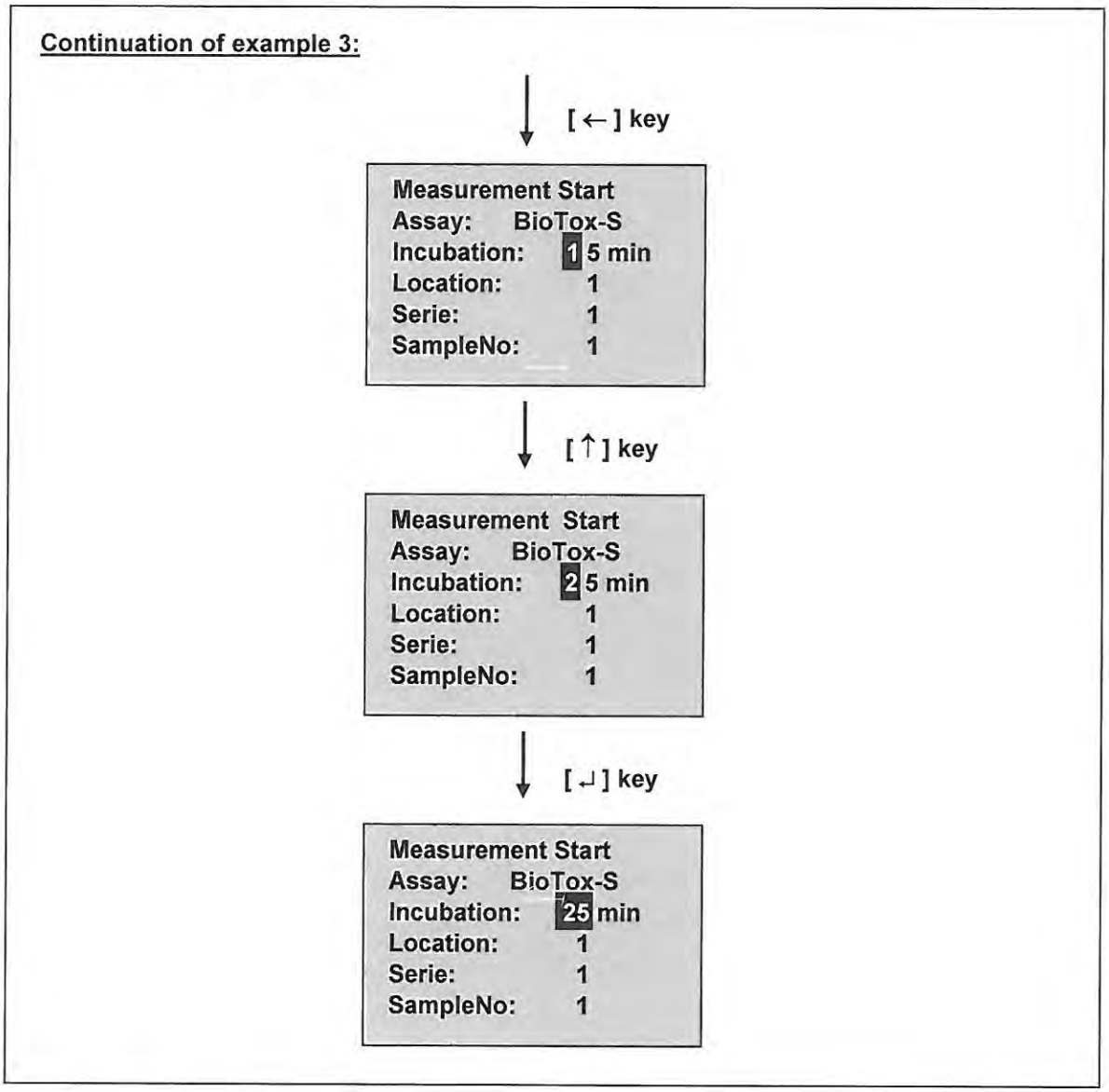
The entry is confirmed by pressing the **[↵]** key once more and you exit the entry mode.

Example 3: Adjustment of the incubation time from 15 minutes to 25 minutes in the **<Measurement Start>** menu in the case of Assay **<BioTox-S>**.



Continuation see next page

Continuation of example 3:



5.2 Setting of instrument and test parameters (<Settings> menu)

The user-specific instrument and test parameters are defined in the **<Settings>** submenu.

The **<Settings>** submenu is called up when the **<Settings>** option in the main menu is initially called up with the aid of the **[↑] / [↓]** cursor keys and following this is confirmed with the **[↵]** key (see diagram 6). The selection of instrument and test parameters that can be set appears in the display.

The following parameters can be individually selected and set in accordance with the user's specific requirements:

- **<Password>**
- **<Customer Settings>** (Test protocols and measurement parameters)
- **<Date/Time>**
- **<Language>**
- **<RS232>** (Interface configuration for data transfer to the PC)
- **<System>**

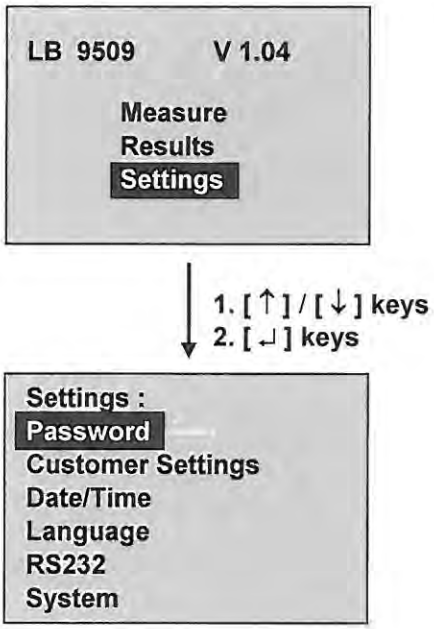


Diagram 6: Calling up of the **<Settings>** submenu.

5.2.1 <Password> option

If the alteration of the parameter settings and the deletion of the measurement results should be only be permitted for persons who are entitled to access the instrument, then a password (max. 4 figures) can be defined with the aid of this option. Upon delivery the password of the **BioFix® *Lumi-10*** luminometer is **<0000>**. If this presetting is retained then every password query can be skipped by pressing the **[↵]** key.

Change the password (see diagram 7):

- Select the **<Password>** option in the **<Settings>** submenu and confirm with the **[↵]** key.
- To begin with enter the old password in order to obtain entitlement to make an alteration.
- The cursor jumps to the **<New Password>** line following the confirmation of the old password with the **[↵]** key. The entry mode is automatically activated.
- Enter the new password (max. 4 figures) with the aid of the **[↑] / [↓] / [←] / [→]** cursor keys and confirm with the **[↵]** key.
- The new password is now assumed as the access entitlement and the program skips to the **<Settings>** submenu.

The aforementioned sequenced can be aborted at any time with the **[ESC]** key. In this case the old password is retained and the programme skips to the **<Settings>** submenu.

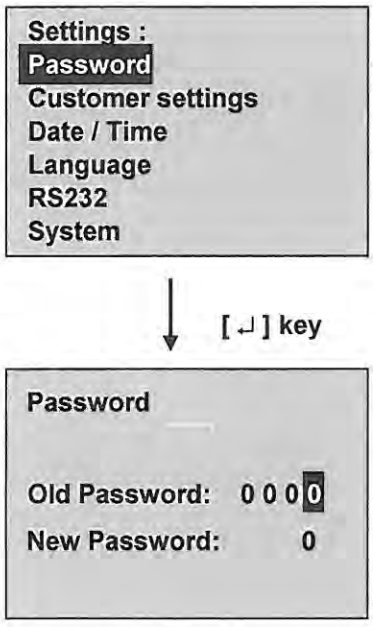


Diagram 7: Activation of the **<Password>** option.

5.2.2 <Customer Settings> option

The protocol type and measurement parameters are set and defined in the **<Customer Settings>** submenu. A maximum of 6 different measurement protocols can be issued with their own name and stored with the protocol types and measurement parameters that were set for them. Call up the desired measurement protocol prior to the implementation of a measurement in the **<Measurement Start>** submenu under the **<Assay>** option.

A measurement protocol contains all the parameters that define a measurement. You can distinguish between the following procedures in the case of the **protocol types** that are available:

- **<BioTox-S>**: Implementation of luminous bacteria toxicity tests only with the analysis of the final light intensity of the test preparations.
- **<BioTox-B>**: Implementation of luminous bacteria toxicity tests with analysis of initial and final light intensity.
- **<RLU>**: Evaluation of luminescence tests (e.g. ATP tests, Mutatox® gene toxicity tests, Reporter gene assays) with respect to the relative light unit (RLU) measurable variable.

Calling up the stored measurement protocols (see diagram 8)**:**

- ☛ Highlight the **<Customer Settings>** option in the **<Settings>** submenu and confirm with the **[↵]** key.
- ☛ Enter the current password and confirm with the **[↵]** key. (If the password **<0000>** is retained then it suffices if you immediately press the **[↵]** key.)
- ☛ Now the **<Customer Settings>** appears in which the store measurement protocols are listed.
- ☛ Upon delivery of the **BioFix® *Lumi-10*** luminometer the 6 programming spaces are preset as follows:

  Program space 1: **<BioTox-B>** measurement protocol for the implementation of luminous bacteria tests with the **<BioTox-B>** protocol type of the same name and a set incubation time of 15 minutes.

  Program space 2: **<BioTox-S>** measurement protocol for the implementation of luminous bacteria tests with the **<BioTox-S>** protocol type of the same name and a set incubation time of 15 min.

  Program space 3: **<ATP-Test>** measurement protocol with the **<RLU>** protocol type for the implementation of ATP/biomass analyses.

  Program spaces 4 - 6: Program spaces for the storage of additional, user defined measurement protocols.

In the case of the measurement protocols on the programming space 1 to 3 they are pre-settings upon the delivery of the BioFix® Lumi-10 luminometer. It is, of course, possible for the users to also reset and define these programming spaces in accordance with their own requirements and also to save them under other names.

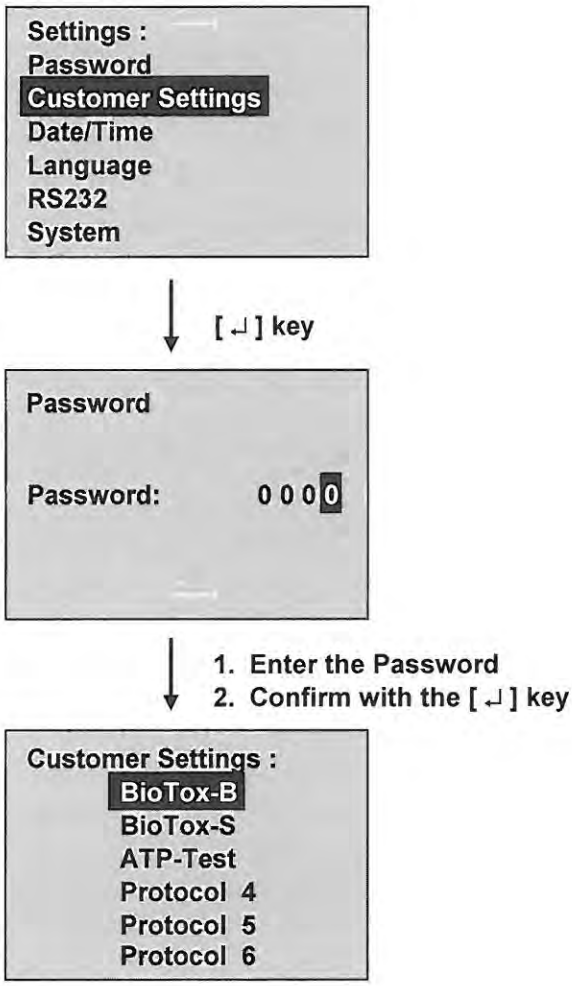


Diagram 8: Calling up the stored measurement protocols. The presetting of the instrument upon delivery is represented.

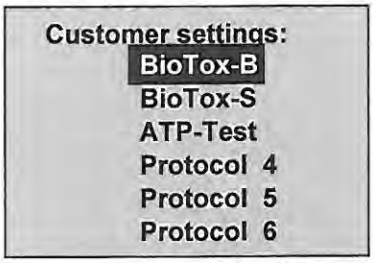
5.2.2.1 <BioTox-S> protocol type

The **<BioTox-S>** protocol type serves as a rapid test (rapid = german: schnell) for the implementation of luminous bacteria toxicity tests where only the final light intensity of the test preparations should be measured. This protocol type is advisable for rapid screening measurements if a rough estimate of the degree of toxicity of the samples is sufficient.

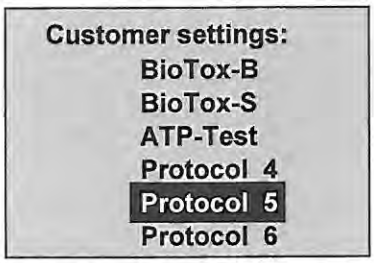
The results are stated as the % inhibition or % stimulation of the light intensity in the sample preparations compared to the light intensity in an unhindered control.

Setting of the measurement parameters in the <BioTox-S> protocol type:

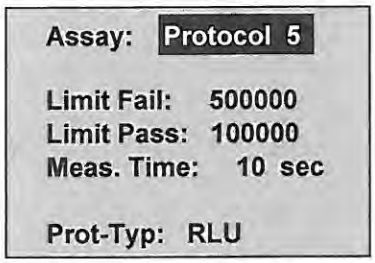
- Call up the **<Customer settings>** submenu with the list of the stored measurement protocols.



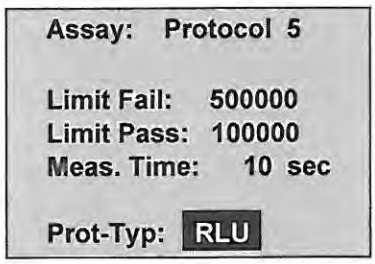
- Select any programming space and confirm with with the [↵] key.



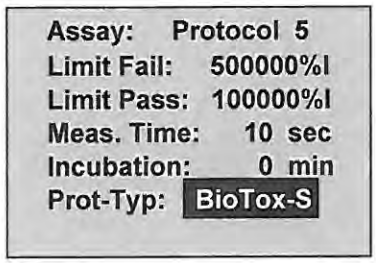
- Now the submenu for the setting of the measurement parameters appears.



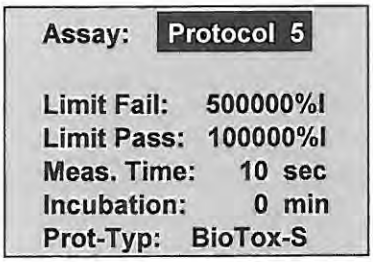
- To begin with selection the **<Prot-Typ>** option with the aid of the cursor keys [↑] / [↓] and activate the selection mode with the [↵] key.



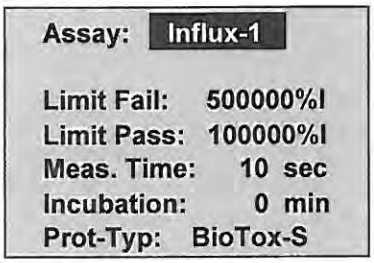
- Select the **<BioTox-S>** option with the [↑] / [↓] keys and confirm with the [↵] key.



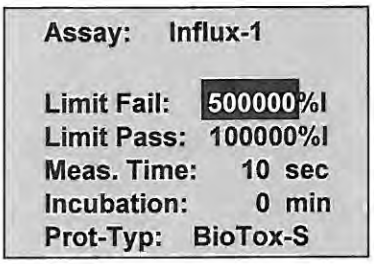
- Now move the cursor bar back to the **<Assay>** option with the aid of the **[↑]** cursor key and activate the selection mode with the **[↵]** key.



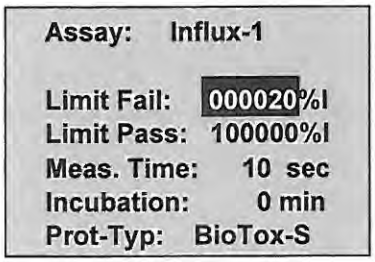
- In the selection mode any **name for the measurement protocol** can now be entered in the selection mode (max. figures) with the **[↑] / [↓] / [←] / [→]** keys, e. g. "Influx-1". Confirm the name that has been entered by pressing the **[↵]** key.



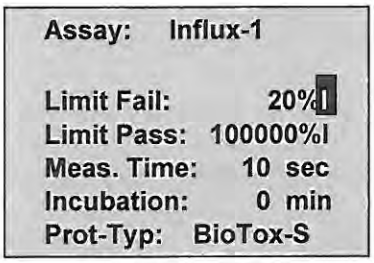
- Now move the cursor to the option **<Limit Fail>** with the aid of the **[↓]** cursor to the option and activate the selection mode with the **[↵]** key.



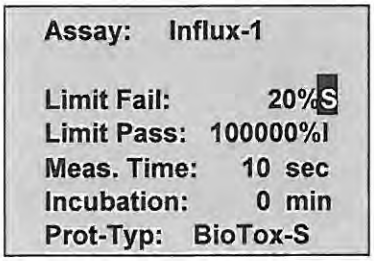
- Enter an upper **limit fail** with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "000020". Confirm the limit fail entered by pressing the **[↵]** key.



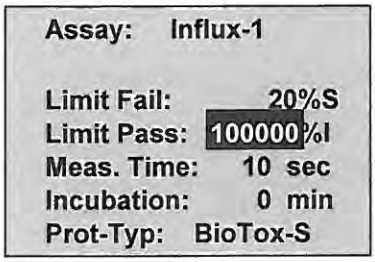
- Move to the **< S / I >** option with the aid of the **< ↓ >** cursor keys and activate the selection mode with the **[↵]** key.



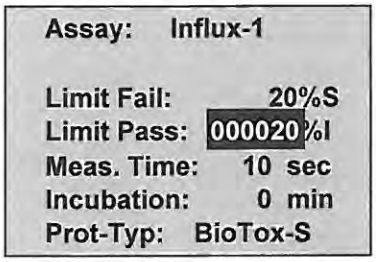
- You can now stipulate in the selection mode with the **[↑] / [↓]** cursor keys whether the limit fail should refer to the % inhibition (**< I >**) or % stimulation (**< S >**)I, e.g. "S". Confirm the entry by pressing the **[↵]** key.



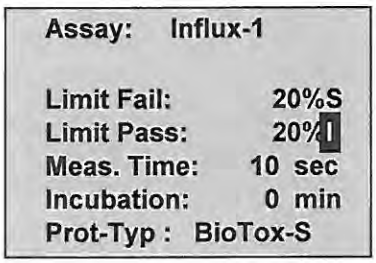
- Now move the cursor to the option **<Limit Pass>** with the aid of the **[↓]** key and activate the selection mode with the **[↵]** key.



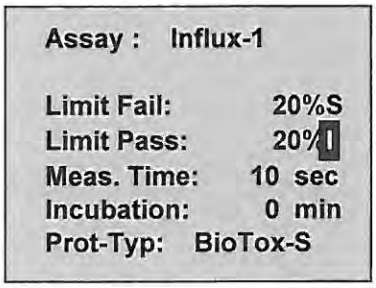
- In the selection mode enter a lower **limit pass** e.g. "000020" with the **[↑] / [↓] / [←] / [→]** keys.
  Confirm the limit pass entered by pressing the **[↵]** key.



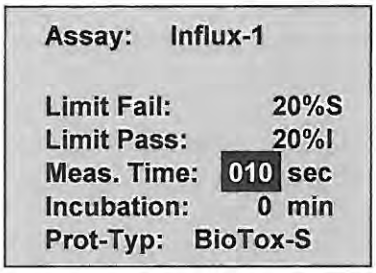
- Now move the cursor to the **< S / I >** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



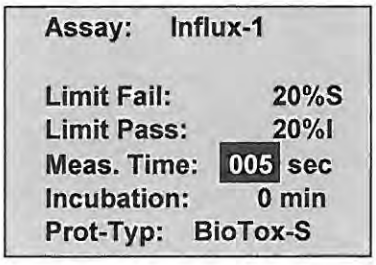
- You can now stipulate in the selection mode whether the limit pass refers to the % inhibition (**< I >**) or % stimulation (**< S >**) measured variable, e.g. "I" with the **[↑] / [↓]** keys.
  Confirm entry by pressing the **[↵]** key.



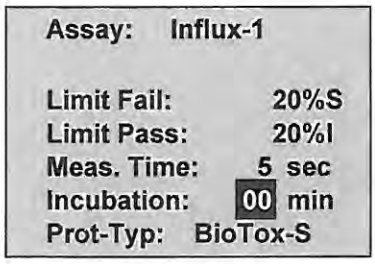
- Now move the cursor to the **<Meas. Time>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



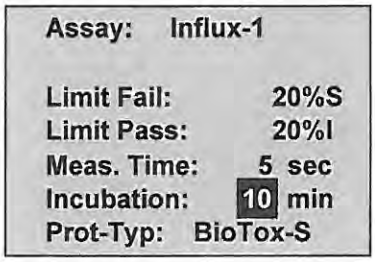
- Enter any **measuring time** between 0 and 999 sec with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "005".
  Confirm the measuring time entered by pressing the **[↵]** key.



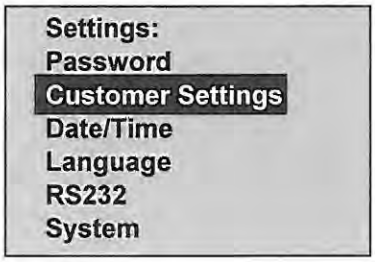
- Now move the cursor to the **<Incubation>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



- Select any **incubation time** between 0 and 39 min with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "10".
  Confirm the incubation time entered by pressing the the **[↵]** key.



- If all the parameters have now been set press the **[ESC]** key and you return to the **<Settings>** submenu.



Setting of <Limit Fail> and <Limit Pass> :

The entry of defined limit values in the **<BioTox-S>** serves to classify and evaluate the measurement results displayed. Individual measuring ranges can be defined by the entry of 2 limit values on the basis of which the samples can be classified as unsafe or safe in terms of their toxicity against luminous bacteria. It is possible by means of the variable entry of limit values depending on the sampling location to take account of changing basic levels of toxicity or to compensate for them during the results classification.

In the **<BioTox-S>** protocol type the measurement results are evaluated as follows:

Measurement result	Evaluation	Signal light
Measurement value between limit fail and limit pass	O.K.	Green
Measurement outside of the limit fail and limit pass boundaries	Warning	Red

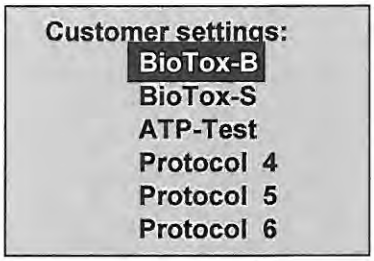
The yellow signal light is only active when using the **<RLU>** protocol type.

5.2.2.2 <BioTox-B> protocol type

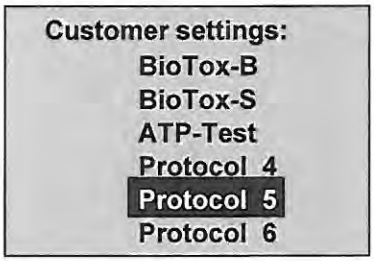
The **<BioTox-B>** protocol type serves as the Basic test for the implementation of luminous bacteria toxicity tests for which a higher level of precision of the measurement results is desired. For this reason in the case of this protocol type the initial light intensity of the test preparations is also measured as well as the final light intensity.
As with the **<BioTox-S>** protocol type the results are stated as % inhibition or % stimulation of the light intensity in the sample preparations compared to the light intensity in an unhindered control.

Setting of the measurement parameters in the <BioTox-B> protocol type:

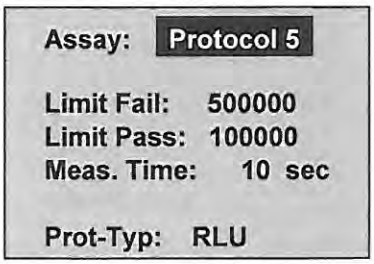
- Call up the **<Customer Settings>** submenu with the list of the stored measurement protocols.



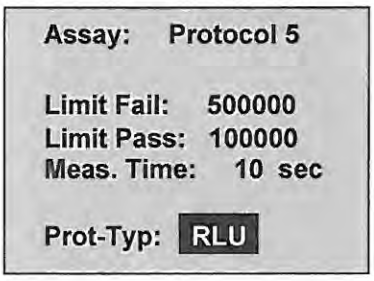
- Select any program space and confirm with the the [↵] key.



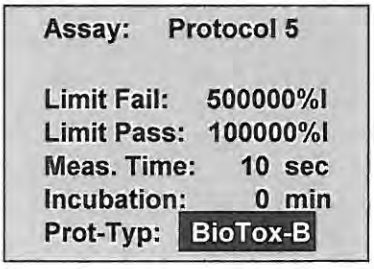
- Now the submenu for the setting of the measurement parameters appears.



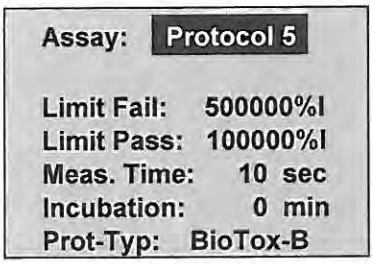
- To begin with select the **<Prot-Typ>** option with aid of the [↑] / [↓] cursor keys and activate the selection mode with the [↵].



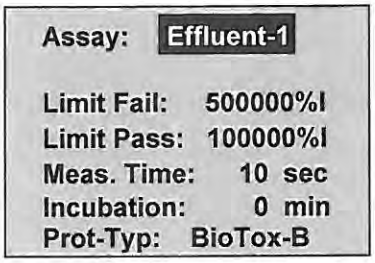
- In the selection mode select the **<BioTox-B>** option with the [↑] / [↓] keys and confirm it with the [↵] key.



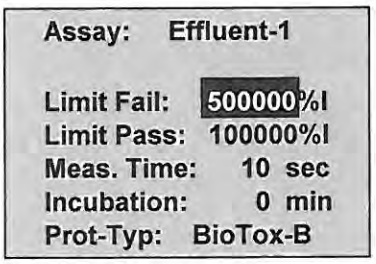
- Now move the cursor bar back to the **<Assay>** option with the aid of the **[↑]** cursor key and activate the selection mode with the **[↵]** key.



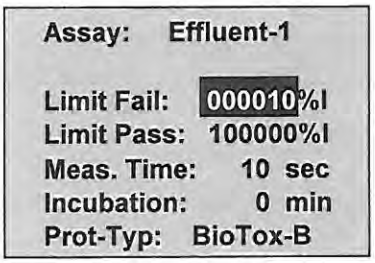
- Now any **name for the measurement protocol** can be entered in the selection mode (max. 10 figures), e. g. "Effluent-1" with the **[↑] / [↓] / [←] / [→]** keys. Confirm the name entered by pressing the **[↵]** key.



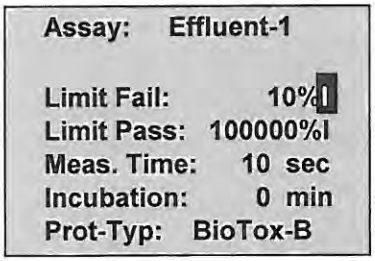
- Now move the cursor to the option **<Limit Fail>** with the aid of the **[↓]** key and activate the selection mode with the **[↵]** key.



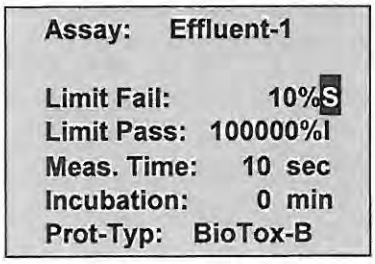
- Enter an upper **limit fail** with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e. g. "000010". Confirm the limit fail entered by pressing the **[↵]** key.



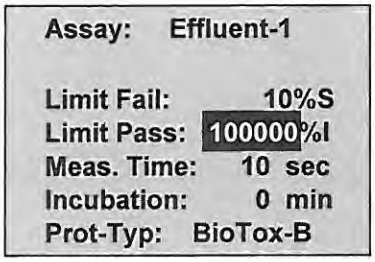
- Now move the cursor to the **< S / I >** option with the aid of the **[↓]** cursor key and confirm the selection mode with the **[↵]** key.



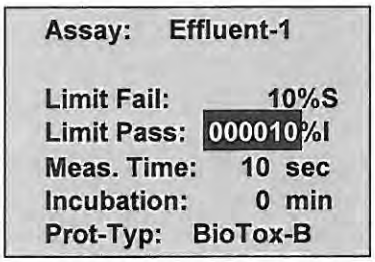
- You can now stipulate in the selection mode with the **[↑] / [↓]** cursor keys whether the limit fail should refer to the % inhibition (**< I >**) or % stimulation (**< S >**), e.g. "S". Confirm the entry by pressing the **[↵]** key.



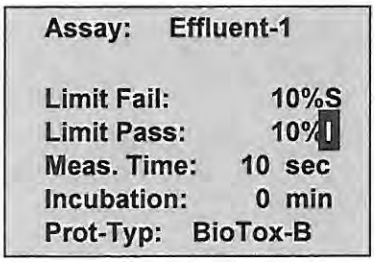
- You can now move the cursor to the **<Limit Pass>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



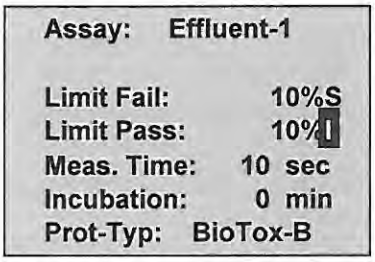
- In the selection mode enter a lower **limit pass** e.g. "000010" with the **[↑] / [↓] / [←] / [→]** keys.
  Confirm the limit pass entered by pressing the **[↵]** key.



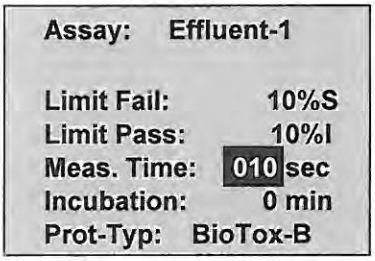
- Now move the cursor to the **< S / I >** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



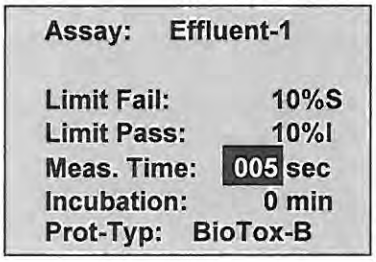
- You can now stipulate in the selection mode with the **[↑] / [↓]** cursor keys whether the limit pass should refer to the % inhibition (**< I >**) or % stimulation (**< S >**), e.g. "I".
  Confirm the entry by pressing the **[↵]** key.



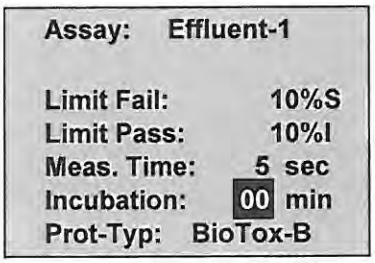
- Now move the cursor to the **<Meas.Time>** option with the aid of the **[↓]** cursor key and activate selection mode with the **[↵]** key.



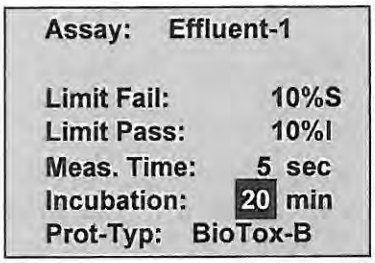
- Enter any **measuring time** between 0 and 999 sec with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "005".
  Confirm the measuring time entered by pressing the **[↵]** key.



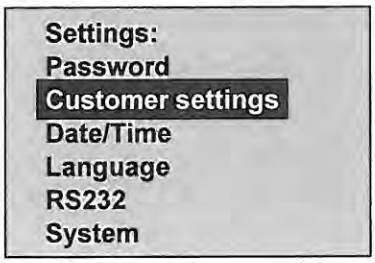
- Now move the cursor to the **<Incubation>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



- Select any **incubation time** between 0 and 39 min with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "20".
  Confirm the incubation time entered by pressing the the **[↵]** key.



- If all the parameters have been set then press the **[ESC]** key and you return to the **<Settings>** submenu.



Setting of <Limit Fail> and <Limit Pass> :

The entry of defined limit values in this as already stated in the case of the **<BioTox-S>** protocol type serves to classify and evaluation the measurement results displayed. Individual measuring ranges can also be defined by the entry of 2 limit values in the case of the **<BioTox-B>** protocol type on the basis of which the samples can be classified as unsafe or safe in terms of their toxicity against luminous bacteria. It is possible by means of the variable entry of limit values depending on the sampling location to take account of changing basic levels of toxicity or to compensate for them during the results classification.

In the **<BioTox-B>** protocol type the measurement results are evaluated as follows:

Measurement result	Evaluation	Signal light
Measurement value between limit fail and limit pass	O.K.	Green
Measurement value outside of the limit fail and limit pass boundaries	Warning	Red

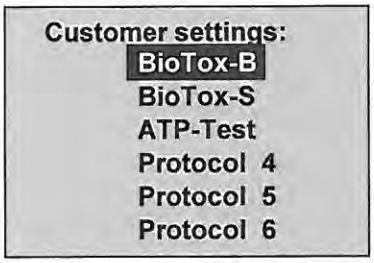
The yellow signal light is only active when using the **<RLU>** protocol type.

5.2.2.3 <RLU> protocol type

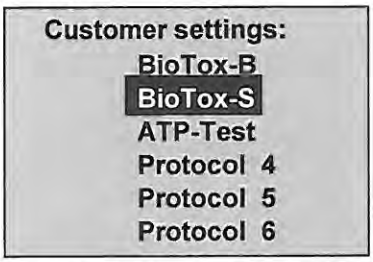
The **<RLU>** protocol type serves to measure and evaluate luminescence from the fields of hygiene monitoring (ATP and biological mass analyses), molecular-biological diagnostics (Reporter gene assays, DNA sample assays, luminescence immunoassays etc) and the mutagenicity tests (Mutatox® gene toxicity test). By contrast with the **<BioTox-S>** and **<BioTox-B>** protocol types the parameter **<Incubation>** ceases to apply in the case of the **<RLU>** protocol type.
When using the **<RLU>** protocol type the results are stated as "relative light units" (RLU).

Setting of the measurement parameters in the <RLU> protocol type:

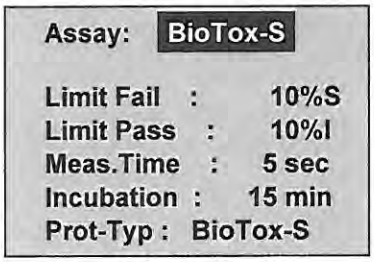
- Call up the **<Customer Settings>** submenu with the list of the stored measurement protocols.



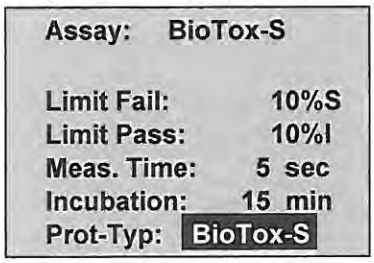
- Select any program space and confirm with the [↵] key.



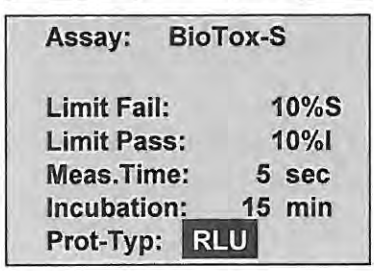
- Now the submenu for the setting of the measurement parameters appears.



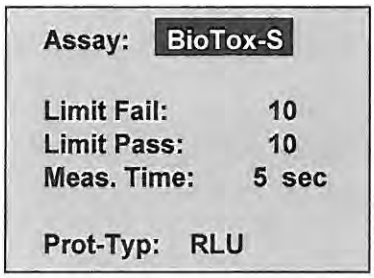
- To begin with select the **<Prot-Typ>** option with the aid of the [↑] / [↓] cursor keys and activate selection mode with the [↵] key.



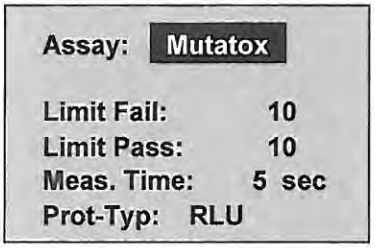
- Select the **<RLU>** option in the selection mode with the [↑] / [↓] keys and confirm with the the [↵] key.



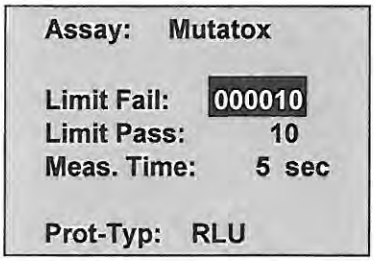
- Now move the cursor bar back to the **<Assay>** option with the aid of the **[↑]** cursor key and activate the selection mode with the **[↵]** key.



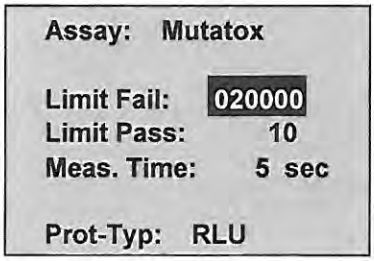
- In the selection mode any **name for the measurement protocol** can now be entered in the selection mode (max. 10 figures) with the **[↑] / [↓] / [←] / [→]** keys, e. g. "Mutatox". Confirm the name that has been entered by pressing the **[↵]** key.



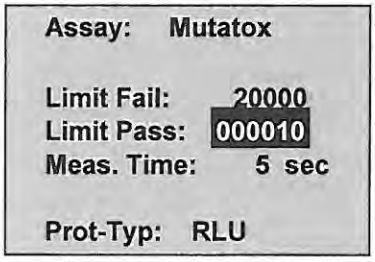
- Now move the cursor to the **<Limit Fail>** option with the aid of the **[↓]** option and activate the selection mode with the **[↵]** key.



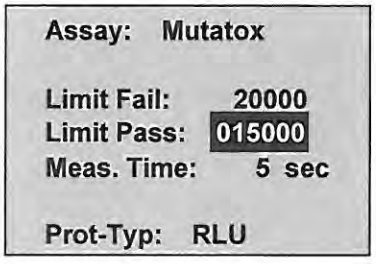
- Enter an <u>upper</u> **limit fail** with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "020000". Confirm the limit fail entered by pressing the **[↵]** key.



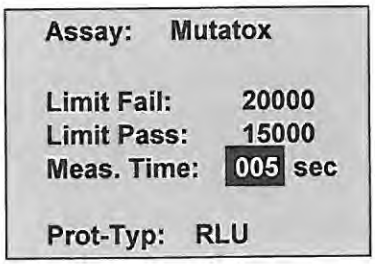
- Now move the cursor to the **<Limit Pass>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



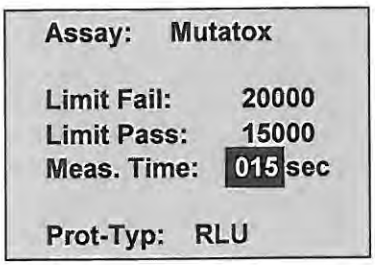
- Enter an <u>lower</u> **limit pass** with the **[↑] / [↓] / [←] / [→]** keys in the selection mode, e.g. "015000". Confirm the limit pass entered by pressing the **[↵]** key.



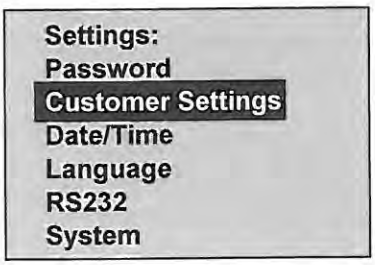
- Now move the cursor to the **<Meas.Time>** option with the aid of the **[↓]** cursor key and activate the selection mode with the **[↵]** key.



- In the selection mode enter any **measuring time** between 0 and 999, e.g. "015".
Confirm the measuring time entered by pressing the **[↵]** key.



- If all the parameters have now been set press the **[ESC]** key and you return to the **<Settings>** submenu.



Setting of <Limit Fail> and <Limit Pass> :

In accordance with the defined boundary value the measurement results will also be displayed and classified in the **<RLU>** protocol type. However, by contrast with the **<BioTox-S>** and **<BioTox-B>** protocol types no measurement areas are defined within which results are described as O.K. and which, if they do not fall within the boundaries, are classified as serious (not O.K.). Instead the boundary values in the **<RLU>** protocol type serve to optionally categorise high or low measurement values as desirable (O.K.). Two differing evaluations of the measurement results emerge depending on the selection of the boundaries:

I. Limit Fail > Limit Pass

Measurement result	Evaluation	Signal light
Measurement value ≤ Limit Pass	O.K.	Green
Limit Pass < Measurement value < Limit Fail	Warning	Yellow
Measurement value ≥ Limit Fail	Alarm	Red flashing

II. Limit Fail < Limit Pass

Measurement result	Evaluation	Signal light
Measurement value ≥ Limit Pass	O.K.	Green
Limit Fail < Measurement value < Limit Pass	Warning	Yellow
Measurement value ≤ Limit Fail	Alarm	Red flashing

5.2.3 <Date/Time> option

When calling up this option the real time clock inside the instrument the date and time can be entered or modified. All the measurement values will be stored with the current time and date information. These serve as selection criteria for the representation of measurement results in the display, the transfer of measurement data to a PC and the deletion of measurement results. In addition the **<Date/Time>** option permits the selection of the format of the data and time display.

Setting of the date and time:

- Select the **<Date/Time>** option in the **<Settings>** submenu and confirm with the **[↵]** key.
- The **<Date/Time>** submenu appears with the following parameters that can be set: "**Date**", "**Time**", "**Format Date**" and "**Format Time**".
- Highlight the **<Date>** option with the **[↑]** / **[↓]** cursor keys, confirm with the **[↵]** key and enter the desired date with the entry mode.
- Highlight the **<Time>** option with the **[↑]** / **[↓]** cursor keys and confirm with the **[↵]** key and enter the desired time via the entry mode. Confirm the entry with the **[↵]** key.
- Highlight the **<Format / Date>** option with the **[↑]** / **[↓]** cursor keys confirm with the **[↵]** key and select the desired format for the representation of the date (**"Europe"** or **"USA"**) via the select mode. Confirm the entry with the **[↵]** key.
- Highlight the **<Format / Time>** option with the **[↑]** / **[↓]** cursor keys confirm with the **[↵]** key and select the desired format for the representation of the time (**"24h"** or **"12h am/pm"**) via the select mode. Confirm the entry with the **[↵]** key.
- Following the setting of all the parameters you return to the **<Settings>** submenu by pressing the **[ESC]** key.

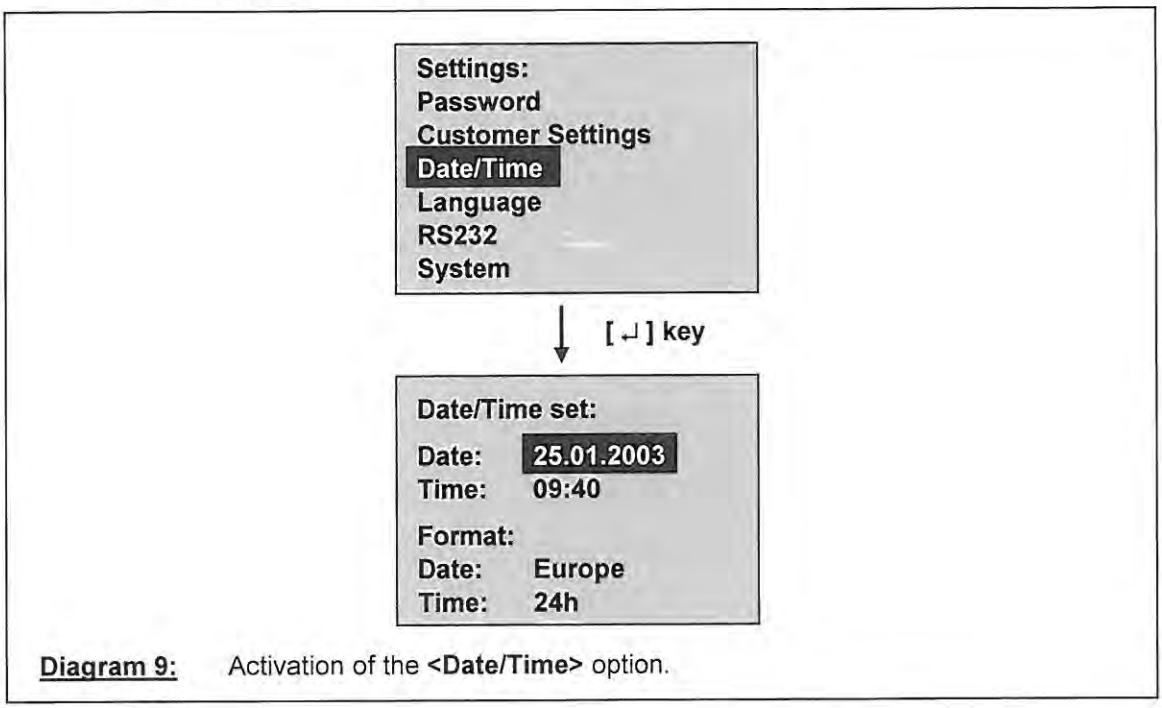


Diagram 9: Activation of the **<Date/Time>** option.

5.2.4 <Language> option

The language for the menu mode of the **BioFix® *Lumi-10*** software can be set using this option. You have a choice between German and English.

Setting of the language for the user interface:

- Select the **<language>** option in the **<Settings>** submenu and confirm it with the [↵] key.
- The **<language>** submenu appears. You have a choice between German and English.
- Select the desired language with the [↑] / [↓] cursor keys.
- After confirming the selected language with the [↵] key you automatically return to the **<Settings>** submenu.

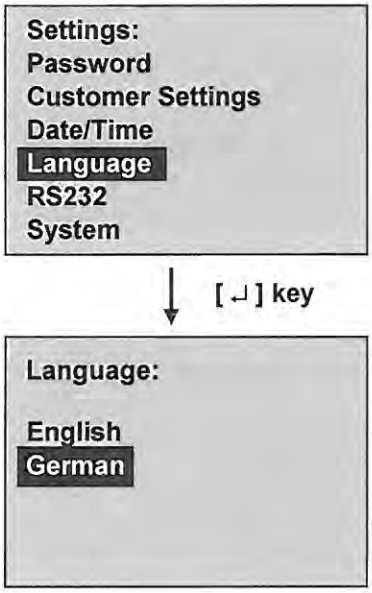


Diagram 10: Activation of the **<Language>** option.

5.2.5 <RS232> Option

The **<RS232>** option is used to configure the RS232 interface on the rear side of the **BioFix®** ***Lumi-10*** luminometer. The stored measurement data (Data of max. 2000 measurements) can be transferred to a standard PC via this interface. To this end a standard null modem cable is necessary.

All the transmission parameters that are necessary for the computer configuration are set in the **<RS232>** submenu. In general the preset parameters can be retained. However, the possible settings should be examined in the terminal program of the PC used. However, if necessary the transmission parameters of the **BioFix®** ***Lumi-10*** luminometer and the terminal program of the PC must be adapted to one another. Otherwise the data transmission is not possible.

Setting of the transmission parameters:

- Select the **<RS232>** option in the **<Settings>** submenu and confirm with the **[↵]** key.
- The **<RS232>** submenu appears with the following parameters that can be set: **"Baudrate"**, **"Databits"**, **"Parity"**, **"Stopbits"**, **"Flowcontrol"**.
- Select the desired parameters with the **[↑]** / **[↓]** cursor keys and activate it by pressing the **[↵]** key. Following this call up the desired values in the respective selection mode with the **[↑]** / **[↓]** cursor keys and confirm with the **[↵]** key.
- You return to the **<Settings>** submenu by pressing the **[ESC]** key following the setting of all the parameters.

Overview of the transmission parameters that can be set and selection of the possible settings that go with them:

Transmission parameters	Selection of the settings
<Baudrate>	300, 600, 1200, 2400, 4800, 9600, 19200, 38400
<Databits>	7 or 8
<Parity>	none, odd, even
<Stopbits>	1 or 2
<Flowcontrol>	XON/XOFF, Rts/Cts, none

Select the **<Results>** submenu in the main menu and select the **<PC>** option there in order to transfer the desired measurement data to the PC. In this case it is possible to select the measurement data to be transferred in accordance with the date, time, location and series identity number (see chapter 5.4.2).

Please note:

A Terminal program (Windows® 3.1) or Hyper terminal program (Windows® 95/98/NT) is necessary on the PC used in order to be able to process the data to be transferred.

Alternatively, the stored measurement data of the **BioFix®** ***Lumi-10*** luminometer can also be transferred to a standard PC by using the software NANOCOLOR® Data Export (Cat. No. 919 02.1). There you have the choice between a selective data transfer to MS Excel or MS Access.

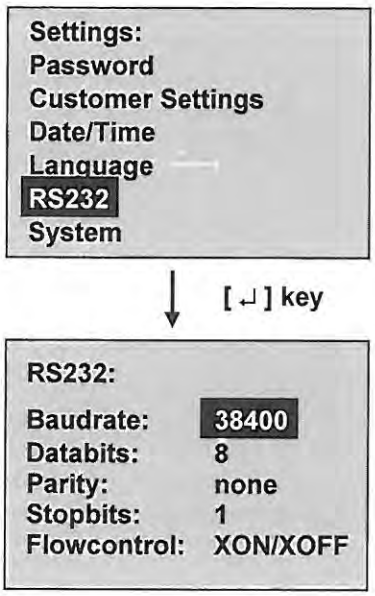


Diagram 11: Activation of the **<RS232>** option.

5.2.6 <System> option

In this submenu the "**Brightness**" and "**Contrast**" of the LCD display can be changed.

Setting of the transmission parameters:

- Select the **<System>** option in the **<Settings>** submenu and confirm with the **[↵]** key.
- The **<System>** submenu appears with the following parameters that can be set: **"Brightness"**, **"Contrast"**.
- Highlight the **<Brightness>** option with the **[↑]** / **[↓]** cursor keys, activate it with the **[↵]** key and set the desired brightness in the selection mode (selection from level 1 to 4). Confirm the entry with the **[↵]** key.
- Highlight the **<Contrast>** option with the **[↑]** / **[↓]** cursor keys, activate it with the **[↵]** key and set the desired contrast (selection from level 1 to 10). Confirm the entry with the **[↵]** key.
- You return to the **<Settings>** submenu by pressing the **[ESC]** key following the setting of both parameters.

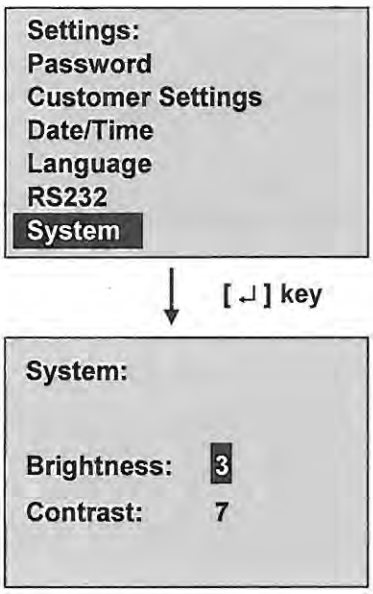


Diagram 12: Activation of the **<System>** option.

5.3 Setting and implementation of measurements (menu <Measure>)

In the **<Measure>** submenu a measurement is started by selecting the **<Measurement Start>** option and pressing the **[↵]** key.

The measurement menu is called up by initially highlighting the **<Measure>** option with the aid of the **[↑] / [↓]** cursor keys and following that confirmed with the **[↵]** key (see diagram 13). The **<Measurement Start>** menu appears in the display.

The following options appear on the screen, which can be individually selected and modified once more prior to the start of measurement, after calling the **<Measurement Start>** menu:

Protocol type	Options in the <Measurement Start> menu
<BioTox-S> and **<BioTox-B>**	Assay, Incubation, Location, Series, SampleNo
<RLU>	Assay, Location, Test

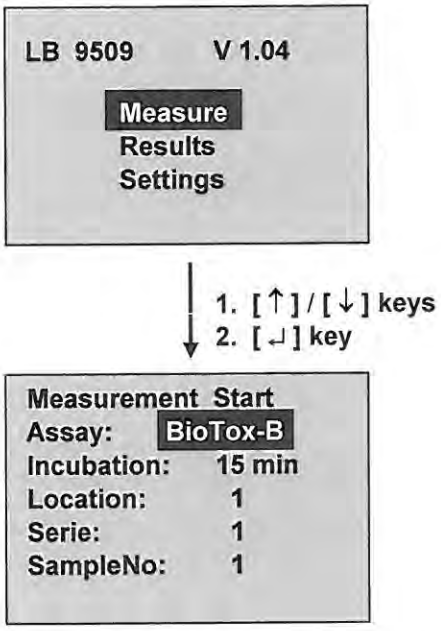


Diagram 13: Calling up the **<Measure>** submenu. Example of measurement menu with the **<BioTox-B>** protocol type.

Setting options in the <Measurement Start> menu

All the options in the measurement menu can be selected with the **[↑] / [↓]** cursor keys prior to starting the measurement and activated with the **[↵]** key. The desired parameters can be modified once more prior to carrying out the measurement with the aid of the four cursor keys in the respective selection ("Assay" option) or entry mode ("Incubation", "Location", "Serie", "SampleNo", "Test" option).

<Assay> option: The desired protocol can be selected from the 6 measurement protocols stored in the <Customer Settings> submenu. See chapter 5.2.2 with respect to the setting up of these measurement protocols.

<Incubation> option: Here the specified incubation time can be modified once more in the measurement protocol called up. However, this modification only applies for the current measurement and is not adopted within the stored measurement protocol.

<Location> option: Here an identification number for the location of the sampling can be entered.

<Serie> option: This option permits the entry of an identification number for the measurement series. In the case of each additional measurement series this value increases by 1, but can be changed at will by the user.

<SampleNo> option: In this case an identification number can be entered for the first sample of a measurement series measured. In the case of each additional measurement in the course of a measurement series this value is increased automatically by 1.

<Test> option: Within this option an identification number can be entered for each individual measurement. This is automatically increased by 1 following each measurement but can be changed at will by the user.

Implementation of measurements

Please obtain detailed instructions and additional details with respect to the implementation of the various different bioluminescence tests from chapters 6, 7 and 8.

Automatic storage of measurement results

All the measurement results are automatically stored and attached to the last measurement. If the memory is full (following 2000 measurements), the oldest measurement respectively will be deleted when storing.

Cancelling measurements

A current measurement of the light intensity can be cancelled at any time by pressing the **[ESC]** key:

- Countdown of the measurement time is running.
- Press **[ESC]** key. .
- The **< Cancel Measurement? YES / NO >** safety enquiry appears.
- Select **<YES>** or **<NO>** with the aid of the **[←] / [→]** cursor keys and confirm with the **[↵]** key. The measurement is cancelled after selecting **<YES>** and the programme returns to the main menu. If you select **<NO>** the measurement continues and there is the option of storing the result.

Termination of measurement series

<BioTox-S> protocol type: The measurement series can be terminated following any number of samples by pressing the **[ESC]** key. Following this the safety enquiry **< Cancel Measurement? YES / NO >** appears to begin with. Select **<YES>** or **<NO>** with the aid of the **[←] / [→]** cursor keys and confirm with the **[↵]** key. After selecting **<YES>** the measurement series is terminated and program returns to the **<Measurement Start>** menu. When confirming with **<NO>** the measurement program continues and additional samples can be measured.

<BioTox-B> protocol type: Both when measuring the initial light intensity and also when measuring the final light intensity, the measurement series can be aborted by pressing the **[ESC]** key following any number of samples. Following this the safety query **< Cancel Measurement? YES / NO >** appears to begin with. Select **<YES>** or **<NO>** with the aid of the **[←] / [→]** cursor keys and confirm with the **[↵]** key. After selecting **<YES>** the measurement series is terminated and program returns to the **<Measurement Start>** menu. When confirming with **<NO>** the measurement program continues and additional samples can be measured.

<RLU> protocol type: The measurement series can be terminated and you can return to the main menu following each individual measurement by pressing the **[ESC]** key.

Return to the <Measurement Start> menu and restart a measurement

Following the termination of a measurement series in the **<BioTox-S>** protocol type the program automatically returns to the **<Measurement Start>** menu.

During the measurement of the initial light intensity the incubation time that is set in the **<BioTox-B>** protocol type starts following the termination of a measurement series. Upon the termination of a measurement series during the measurement of the final light intensity the program automatically returns to the **<Measurement Start>** menu.

When using the **<RLU>** protocol type you can return to the **<Measurement Start>** menu by pressing the **[↵]** key following each measurement.

Back in the **<Measurement Start>** menu any new measurement series can immediately be started or individual parameters can be reset or modified to begin with.

5.4 Data administration (<Results> menu)

Several options are available in the **<Results>** submenu for the administration and processing of the stored measurement results:

<Screen>: The measured values can be displayed in the LCD display selected according to the date, time, location and series digit.

<PC> option: The measured values can transferred to a PC selected on the basis of the date, time, location and series identification digit.

<delete> option: The measured values can be deleted on the basis of the date, time, location and series identification digit. This option can only be called up by means of the entry of a password.

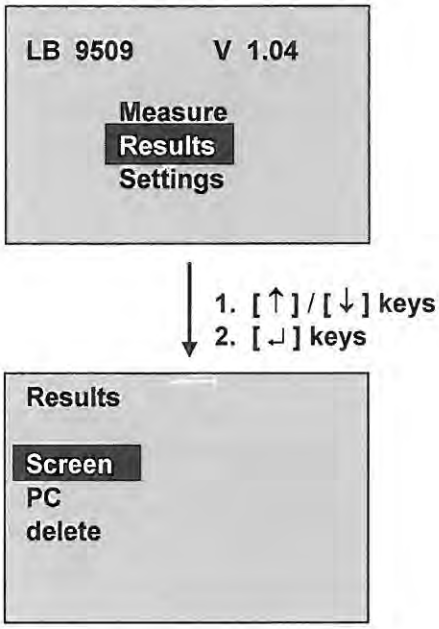


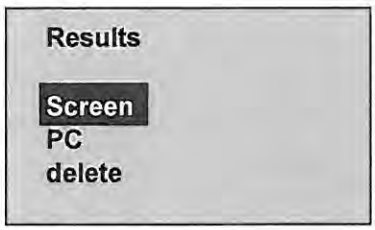
Diagram 14: Calling up the **<Results>** submenu.

5.4.1 Displaying results on the screen (<Screen> option)

If stored measured results should be displayed in the LCD display of the **BioFix® *Lumi-10*** luminometer then select the **<Screen>** option in the **<Results>** submenu. In this case the results can be call up selected on the basis of the date, time, location and series identification digits.
You call up the individual selection options with the aid of the **[↑] / [↓]** cursor keys and confirm it with the **[↵]** key. In this way the respective entry mode is activated and the desired characteristics can be entered with the aid of the 4 cursor keys and confirmed for their part with the **[↵]** button.

Calling up the stored measurement values on the LCD display:

- Call up the **<Results>** submenu and highlight the **<Screen>** option. Confirm it with the **[↵]** key.



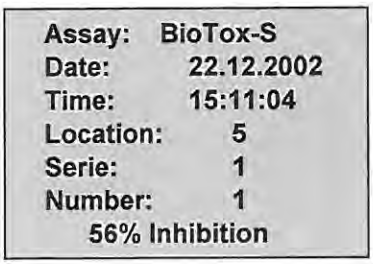
- The display **<Start Display at>** with the selection criteria **"Date"**, **"Time"**, **"Location"** and **"Serie"** appears.
The current data, the current time as well the location identification digit **< 0 >** and the series identification digit **< 0 >** are displayed. If nothing is altered a search is carried out through all the results and the oldest result is displayed.

- Enter **<Date>** and **<Time>** as the starting point. In this way all the measurements carried out between this point in time and the current date are selected for display on the LCD display.

- Enter the identification digits for the **<Location>** of the sampling as well as the measurement **<Serie>**, the measurement results of which should be displayed, as additional selection criteria.
If the measurement results should be displayed irrespective of the location and series labelling, then please enter the **< 0 >** digit respectively.

- Highlight the **<start search>** option following the entry of all the selection characteristics with the aid of the **[↑] / [↓]** cursor keys and press the **[↵]** key.

- Following this the first, earliest measured result is displayed that corresponds to the selection criteria, e. g.



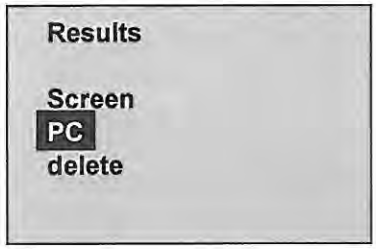
- You scroll backwards and forwards with the [↑] / [↓] keys to display additional results.
- You return to the **<Results>** submenu by pressing the **[ESC]** key.

5.4.2 Data transfer to the PC (<PC> option)

The stored measured results can only be transferred to a standard PC in accordance with the date, time, location and series identification units. To this end one selects the **<PC>** option in the **<Results>** submenu.
You can call up the individual selection options with the aid of the cursor keys [↑] / [↓] and confirmation with the [↵] key. In this way the respective entry mode is activated and the desired characteristics can be entered with the aid of the 4 cursor keys and confirmed for their part with the [↵] key.

Transfer of stored memory results to a PC:

- To begin with the RS232 interface on the rear of the instrument of the **BioFix® *Lumi-10*** luminometer with the serial interface of the PC with the standard null modem cable.
- Start the Terminal (Windows® 3.1) or Hyperterminal-program (Windows® 95/98/NT).
- Highlight the **<PC>** option in the **<Results>** submenu of the **BioFix® *Lumi-10*** luminometer and confirm it with the [↵] key.



- The **<Start Transfer at>** display appears with the selection criteria **"Date"**, **"Time"**, **"Location"** and **"Serie"**.
The current date and the current time as well as the location characteristic **< 0 >** and the series characteristic **< 0 >** are displayed. If nothing is changed, all the results are searched through and the oldest result is displayed.

- Enter the **<Date>** and **<Time>** as the starting point. In this way all the measurements carried out between this point in time and the current date for transmission to the PC are selected.

- As additional selection criteria enter the identification digits for the **<Location>** of the sampling as well as the measurement **<Series>**, the measuring results of which should be transferred. If the measuring results should be transferred irrespective of the location and series label, then please enter the digit **< 0 >** respectively.

- Highlight the **<start transfer>** option following the entry of all the selection characteristics with the aid of the cursor keys **[↑] / [↓]** and press the **[↵]** key.

- Following this all the measuring results which correspond to the selection criteria are transferred to the PC. The message **<transfer data>** appears.

- You return to the **<Results>** submenu by pressing the **[ESC]** key.

Format of the data transfer

a) Data transfer of the measuring results to a PC

The data are transferred in the following sequence:

Date
Time
Protocol name (assay)
Boundary 1 (limit fail)
Boundary 2 (limit pass)
Measured time
Incubation time
Location
Series
Number
Integral value or **"Overload"**
<CR> (= carriage return)
<LF> (= line feed)

If an overload results during the measurement the text **"Overload"** will be outputted instead of the integral value.
All the values are separated from one another by **<TAB>**.

Example:

11.01.2003<TAB>11:32:54<TAB>BioTox-B<TAB>10%S<TAB>10%H<TAB>5<TAB>15 <TAB>5<TAB>1<TAB>1<TAB>1556<TAB><CR><LF>

b) Online-Date transfer to a PC

The test results can also be transferred directly online from the **BioFix® *Lumi-10*** luminometer to a connected PC. In this case the data are outputted in the same form and sequence as already described under a), yet additionally with 100 individual value which are positioned in front of the integral value. The individual values are also separated by **<TAB>**.
If the measurement is aborted the values are transferred until the point of the abortion and following that the **<CANCEL>** text.

Special remark:

Alternatively, the stored measurement results of the **BioFix® *Lumi-10*** luminometer can also be transferred to a standard PC by using the **software NANOCOLOR® Data Export** (Cat. No. 919 02 1). There you have the choice between a selective data transfer to MS Excel or MS Access.

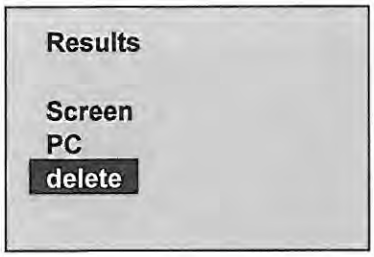
Minimum system requirements:
Pentium I (> 100 MHz) processor, 8 MB free hard disc memory, 64 MB RAM, Windows® 95, 98, NT4 or 2000, 1 free serial interface, CD-ROM drive, MS Office 97 or Office 2000.

5.4.3 Deletion of data (<delete> option)

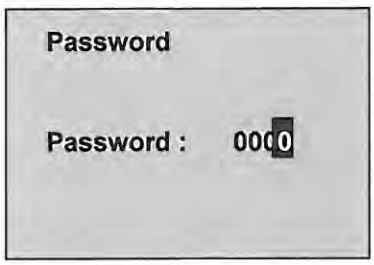
The measurement results in the data storage of the **BioFix® *Lumi-10*** luminometer can be displayed selectively in the display via the selection criteria date, time, location and the series identification digit and following this they can be deleted. The access to the **<delete>** option is only possible via the **<Password>** option, in order to prevent the unauthorised deletion of data.
The calling up of the individual selection options is carried out with the aid of the cursor keys [↑] / [↓] and confirmation with the [↵] key. In this way the respective entry mode is activated and the selected characteristics can be entered with the aid of the [↵] key.

Delete the stored test results:

☛ Call up the **<Results>** submenu and highlight the **<delete>** option. Confirm with the [↵] key.



☛ To begin with the display to enter the password.



☛ Enter the password via the entry mode with the aid 4 cursor keys and confirm with the [↵] key, e. g.

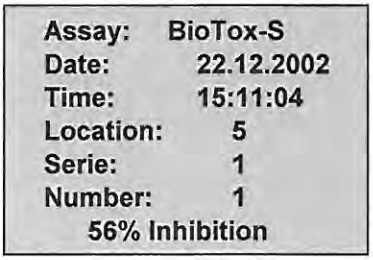
☛ The display **<Start Display at>** appears with the selection criteria **"Date"**, **"Time"**, **"Location"** and **"Serie"**.
The current date and the current time as well as the location identification digit **< 0 >** and the series identification digit **< 0 >** are displayed. If nothing is changed then all the results are searched through and the oldest result is displayed.

- Enter the **<Date>** and **<Time>** as the starting point. In this way all the measurements carried out between this point in time and the current date are selected for display on the LCD display.

- Enter the identification digits for the **<Location>** of the sampling as well as the **<Series>** identification digit the measurement results of which should be displayed.
If the measurement results should be displayed Irrespective of the location and series labelling, then please enter the **< 0 >** digit respectively.

- Highlight the **<start search>** option following the entry of all the selection characteristics with the aid of the **[↑] / [↓]** cursor keys and press the **[↵]** key.

- Following this the first, earliest measured result is displayed that corresponds to the selection criteria, e. g.



- If the displayed measurement result should be deleted press the **[↵]** key. The safety enquiry **< Delete Measurement? YES / NO >**.

- After selecting **<YES>** and pressing the **[↵]** key the displayed measurement result will be deleted and the next measurement is displayed. The deletion operation is aborted by pressing the **[ESC]** key or alternatively by selecting **<NO>** and pressing the **[↵]** key, and it is possible to continue scrolling through the list of stored measurement results, which fulfil the selection criteria.

- In this way any measurements can be deleted from the list.

- You can return to the **<results>** submenu by pressing the **[ESC]** key.

6. Implementation of BioFix® *Lumi* luminous bacteria tests

6.1 General test conditions

Time and temperature

Various different chemicals influence living organisms in differing ways, amongst other things due to the differing actions. In the case of some chemicals the influence upon the light intensity of the luminous bacteria is already evident after 5 minutes, in the case of other chemicals more reliable results are only achieved after a contact period of 15 minutes. There is the option with the **BioFix® *Lumi-10*** luminometer of setting the incubation times variably in minute steps of 0 to 39 minutes.

The **BioFix® *Lumi-10*** test system very reliably works in a broad temperature range (+15 °C to +30 °C) and thus permits the flexible testing of environmental samples under variable environmental conditions. Certain fluctuations with respect to the data and measurement results obtained can be observed, which can be put down to different temperatures during the text execution and in conjunction with a variable initial light intensity of the luminous bacteria.

The documentation of the incubation time that arose and the ambient temperature at the point in time of the measurement is necessary in order to be able to reliably interpret the measurement results.

Reagents blank value (control)

During the execution of the luminous bacteria tests a reagent blank value (control) for each test series is required, which is registered in parallel with the sample preparations. This is necessary as the light intensity of the luminous bacteria can change slightly when stored for a longer period of time and also depending on the incubation time during a test. Therefore during the calculation of the results the altered light intensity in a test preparation is compared with the light intensity of the reagent blank value (control). The results in the case of the luminous bacteria protocol types **<BioTox-S>** und **<BioTox-B>** are therefore stated as the % inhibition or % stimulation of the light intensity of the sample preparations compared to the light intensity in an unhindered control.

Interferences

Samples that contain large quantities of solid particles can disrupt the luminous bacteria tests. In this case the sample must be filtered, centrifuged or diluted prior to the test execution. Samples that are greatly coloured (in particular coloured red or brown) also disrupt the test and must if necessary be diluted accordingly prior to the test. Samples that contain chlorine must also be dechlorinated, e.g. by means of the addition of 1 % sodium thiosulphate solution. The optimum ph range for the implementation of luminous bacteria tests ranges between pH 6 and 8. Samples with a pH-value that falls outside of this range can have a negative influence upon the toxicity measurements and should be corrected accordingly with the aid of 1 N NaOH or 1 N HCl sample prior to the start of the test.

Due to the marine origins of the luminous bacteria the salination of the samples to a salt content of approx. 2 % is recommended prior to the implementation of the tests. This can, for example, be carried out by means of the addition of 2.0 g of sodium chloride to a 100 ml sample solution.

6.2 Sampling and sample preparation

Sample types

A large number of differing environmental samples can be tested with the **BioFix® *Lumi-10*** luminometer for evidence of luminous bacteria toxicity and mutagenicity/genotoxicity. This comprises surface stretches of water, groundwater, local authority and industry waste water, sewage wastewater treatment plant inlets and outlets, sewage wastewater treatment plant partial currents, disposal site and water seeping through the ground, soil extracts, abandoned polluted areas and sediment and much more besides.

Sampling

Ideally environmental samples should be collected in new 30 – 50 ml borosilicate laboratory flasks with Teflon seals. The sample vessel must be filled and sealed free of bubbles. In this way slightly volatile contents are retained in the sample.

Sample storage

The samples should be tested as soon as possible following the sampling. If this is not possible then the samples will be stored in a standard commercially available refridgerator at +2 °C to +8 °C until they are tested.

In an optimum case the testing of the samples within 2 to 4 hours following the sampling is recommended as the samples can alter during the storage.

Prior treatment of the samples

The majority of the samples do not require a special prior treatment and can be tested immediately. Despite this it can be necessary in individual cases to carry out the special prior treatment of the samples before the actual tests.

a) Turbid samples:

In the case of turbid samples or samples which contain solid particles that cannot be separated the turbid particles must be removed to begin with. This is best achieved by means of centrifugation or filtration.

It must be observed that the clouding of a sample can cause an unspecific increase or decrease of the light intensity and thus lead to incorrect results.

b) Coloured samples:

Strongly coloured samples (specially coloured red, brown or black) can reduce the light signal of the bacteria and disrupt the measurement due to absorption. Such samples should be diluted to 25 % or 50 % with distilled or deionised water prior to the testing.

c) Samples that contain chlorine:

Samples that contain chlorine (e.g. as a disinfectant) due to chlorination processes disrupt the test as the chlorine has a negative influence on the vitality of the luminous bacteria. Such samples must be dechlorinated prior to the testing, e.g. by means of the addition of a 1 % sodium thiosulphate solution ($Na_2S_2O_3$).

When using a 1 % sodium thiosulfate solution 1 part of this solution is added to 100 parts of the sample solution for the purposes of dechlorination (e.g. 1% sodium thiosulphate solution to 100 ml of the sample) and well mixed. Following this the solution is used in the test as a sample.

Setting of the pH-value of the sample

The optimum pH-range of the luminous bacteria toxicity tests ranges between pH 6.0 and 8.0. Samples with a pH-value outside of this range can negatively influence the toxicity measurement. Such samples should be adjusted with 1 N NaOH or 1 N HCl so that they have a pH-value between 6 and 8.

Salination of the samples

The luminous bacteria used for the implementation of the toxicity tests are of marine origins and require a certain salt content in their environment in order to be able to survive. The optimum salt concentration in the test preparation amounts to 2 %, the tolerance range of the bacteria amounts to between 1.5 % and 3.5 %. It is recommended that the samples are salinated when preparing them so that they have a salt concentration of approx. 2 %. This is best achieved by using the "BioFix® *Lumi* Osmotic Adjusting Solution" (OAS) of **MACHEREY-NAGEL** (Cat. no. 945 602). 10 parts of the sample are mixed with 1 part of the BioFix® *Lumi* OAS for the setting of the osmotic pressure in order to salinate the sample, e.g.10 ml sample + 1 ml BioFix® *Lumi* OAS. Alternatively the salination of the sample can also be achieved by means of the addition of 2.0 g of sodium chloride to 100 ml of the sample solution.

A salt burden that already exists (e.g. brackish water or seawater samples) must be taken into account accordingly in both cases.

6.3 BioFix® *Lumi* reagents and accessories

BioFix® *Lumi* luminous bacteria for toxicity measurements

Luminous bacteria of the species *Vibrio fischeri* NRRL B-11177 are used as test organisms. These are provided by **MACHEREY-NAGEL** in a freeze-dried form that is immediately ready for use. Appropriate packaging sizes are available for both users who only want to examine individual or a few samples with respect to their toxicity and also for users who have to carry out comprehensive series of measurements:

- **BioFix® *Lumi* "Single-Shot" luminous bacteria** (Cat. no. 945 021):
  - Packaging unit: 20 vials
  - 1 vial is sufficient for 1 control + 1 sample
  - Packaging unit is sufficient for 20 toxicity tests
  - Range of application: individual measurements during the course of the activity analysis and in-house control
- **BioFix® *Lumi* "Multi-Shot" luminous bacteria** (Cat. no. 945 022):
  - Packaging unit: 10 vials
  - 1 vial is sufficient for 1 control + max. 9 samples
  - Packaging unit is sufficient for max. 100 measurements
  - Range of application: multiple measurements during the course of the activity analysis and in-house control
- **BioFix® *Lumi* luminous bacteria (20 determinations/vial)**:
  - Packaging units: 10 vials (Cat. no. 945 007) or 20 vials (Cat. no. 945 006)
  - 1 vial is sufficient for max. 20 measurements (1 control + max 19 samples)
  - Packaging unit sufficient for max. 200 measurements (Cat. no.945 007) or 400 measurements (Cat. no.945 006)
  - Range of application: multiple measurements during the course of the activity analysis and in-house control

- **BioFix® *Lumi* luminous bacteria (100 determinations/vial)**:
  - Packaging unit: 10 vials (Cat. no. 945 003) or 20 vials (Cat. no. 945 002)
  - 1 vial is sufficient for max. 100 measurements (1 control + max. 99 samples)
  - Packaging unit sufficient for max. 1000 measurements (Cat. no.945 003) or 2000 measurements (Cat. no.945 002)
  - Range of application: comprehensive screening measurements

All the test kits with freeze-dried BioFix® *Lumi* luminous bacteria include the necessary reactivation solutions and a test certificate in accordance with DIN EN ISO 11348-3. The reagent sets of BioFix® *Lumi* "Single-Shot" luminous bacteria and BioFix® *Lumi* "Multi-Shot" luminous bacteria additionally contain a BioFix® *Lumi* Control solution.

The freeze-dried BioFix® *Lumi* luminous bacteria are treated with special additives in order to guarantee high stability and the ability to reproduce. They must be stored at temperatures of between -15 °C and -25 °C in a freezer compartment or in a freezer until they are used. The BioFix® *Lumi* luminous bacteria can be kept until at least the expiry data printed. The freeze-dried BioFix® *Lumi* luminous bacteria have a shelf life of at least 18 months from the date of manufacture onwards.

Important notices:

In the case of on-site measurements it is possible to remove a vial with freeze-dried, non-reactivated BioFix® Lumi luminous bacteria from the freezer and to hold it in intermediate storage at room or ambient temperatures.

One and the same vial can be subject to these temperature transitions from approx. -20 °C to ambient temperature a maximum number of three times without any losses in stability.

As soon as a vial is reactivated with freeze-dried BioFix® Lumi luminous bacteria then this solution must be used within 4 to 6 hours in order to prevent the negative influences of the results due to the declining stability of the reactivated luminous bacteria.

Mutatox® luminous bacteria for mutagenicity and genotoxicity measurements

With the aid of a dark mutant of the *Vibrio fischeri* luminous bacteria (strain M169, "Light⁻-mutant") samples can be detected that have the potential of changing the genotypes. Agents that have a gene toxic or mutagenic effect cause a remutation (reversion) of the "Light⁻-mutant" to the luminous variant of the luminous bacteria ("Light⁺") and thus the restoration of the bioluminescence.

These luminous bacteria are provided by **MACHEREY-NAGEL** in a freeze-dried form that is immediately ready for use. The following packaging sizes are available:

- **Mutatox® luminous bacteria**:
  - Packaging unit: 5 vials à 1 ml (Cat. no. 945 501.1) or 10 vials à 1 ml (Cat. no. 945 501)
  - 1 vial is sufficient for a max. of 100 mutagenicity measurements
  - Packaging unit sufficient for 500 mutagenicity measurements (Cat. no. 945 501.1) or 1000 mutagenicity measurements (Cat. no. 945 501)
  - Range of application: investigation of the potential of environmental samples to change the genotypes, chemical pure substances or substance mixtures in the areas of pharmacy, plant protection, foods etc.

BioFix® *Lumi* Medium for freeze dried luminous bacteria (Cat. no. 945 608)

This medium is only required when using the BioFix® *Lumi* luminous bacteria, 100 determinations/vial (Cat. no.945 002 / 945 003). It is not enclosed with these test kits and must be ordered separately!

This medium serves to prepare the luminous bacteria test series. It is a defined compound mineral salt medium in accordance with DIN EN ISO 11348-3. The shelf life of the medium amounts to 2 years at a storage temperature of + 2 °C to + 8 °C.

BioFix® *Lumi* Osmotic Adjusting Solution (OAS) (Cat. no. 945 602)

This solution is a highly concentrated sodium chloride solution which serves to set the osmotic pressure of a sample (salination). It is not enclosed in any BioFix® *Lumi* luminous bacteria test kit and must always be ordered separately! The solution has a shelf life of 2 years at a storage temperature of + 2 °C to + 8 °C.

10 parts of the sample are mixed with 1 part of the BioFix® *Lumi* OAS for the setting of the osmotic pressure", e.g. 10 ml sample solution + 1 ml BioFix® *Lumi* OAS in order to salinate a sample to a salt content of approx. 2 %.

BioFix® *Lumi* Diluent (Cat. no. 945 601)

This solution is used to dilute samples. In this case it is a specially pre-treated, weakly concentrated NaCl solution. It is not enclosed in any BioFix® *Lumi* luminous bacteria test kits and must always be ordered separately! The shelf life of the medium amounts to 2 years at a storage temperature of + 2 °C to + 8 °C.

Glass cuvettes (Cat. no. 916 912)

50 x 12 mm Glass cuvettes are used as test vessels. These should only be used once and discarded following use. The cleaning and reuse of the glass cuvettes is not recommended as potential residues of detergents or of previous samples could falsify the test results.

A sufficient number of glass cuvettes are only enclosed in the BioFix® *Lumi* "Single-Shot" luminous bacteria test kit (Cat. no. 945 021). In all the other cases the glass cuvettes are not a component part of the test kit and must not be ordered separately.

Cuvette rack (Cat. no. 945 013)

The use of an appropriate cuvette rack is advisable for the simplification and facilitation of the test implementation. The cuvette rack that can be obtained from **MACHEREY-NAGEL** under the cat. no. 945 013 has a total of 50 wells subdivided into 5 with 10 wells respectively.

Pipettes and pipette tips

Piston pipettes that can be set variably by means of which volumes of 500 µl to 2 ml can be pipetted as well as the pipette tips that go with them are advantageous for the implementation of the tests with the **BioFix® *Lumi-10*** luminometer. A range of appropriate piston pipettes is available from **MACHEREY-NAGEL**.

6.4 Principle implementation of BioFix® *Lumi* luminous bacteria tests

BioFix® *Lumi* luminous bacteria toxicity tests generally run in accordance with the same basic pattern. Smaller variations to the test implementation generally result according to whether the tests were carried out with the measurement of the initial light intensity or whether only the measurement of the final light intensity is used for the evaluation. It is possible with the aid of the **BioFix® *Lumi-10*** luminometer to select between these two basic luminous bacteria test variants. The two **<BioTox-S>** and **<BioTox-B>** protocol types are available to this end.

The **<BioTox-S>** protocol type is advisable for rapid screening measurements in the case of which a rough estimate of the luminous bacteria toxicity is sufficient. In the case of this test program only the final light intensity of the test preparations following an incubation time t will be used for the estimate of the toxicity.

The selection of the **<BioTox-B>** basic test is advisable if more precise measurement results are desired. This is achieved by ensuring that also the initial light intensity of the luminous bacteria inserted at the beginning of the test is measured in addition to the final light intensity of test preparations. In this way the naturally existing drift in the bacteria light intensity depending on the incubation time t is also registered and taken into account during the determination of the results.

Various different packaging sizes are provided by **MACHEREY-NAGEL** as reagents in order to implement the BioFix® *Lumi* luminous bacteria toxicity tests:

- **BioFix® *Lumi* "Single-Shot" luminous bacteria** (Cat. no. 945 021) for individual measurements during the course of the activity analysis and in-house control.
- **BioFix® *Lumi* „Multi-Shot" luminous bacteria** (Cat. no. 945 022) for multiple measurements during the course of the activity analysis and in-house control.
- **BioFix® *Lumi* luminous bacteria , 20 determinations/vial** (Cat. no. 945 006 / 945 007) for multiple measurements during the course of the activity analysis and in-house control.
- **BioFix® *Lumi* luminous bacteria , 100 determinations/vial** (Cat. no. 945 002 / 945 003) for the implementation of comprehensive screening measurements.

All the freeze-dried BioFix® *Lumi* luminous bacteria test kits that can be obtained from **MACHEREY-NAGEL** can optionally be carried out with the **<BioTox-S>** or **<BioTox-B>** measuring protocol.

It should be observed prior to the implementation of the BioFix® *Lumi* luminous bacteria tests that the samples or test preparations as well as the **BioFix® *Lumi-10*** luminometer are adapted to the ambient temperature. This should amount to between +15 °C and +30 °C. The **BioFix® *Lumi-10*** no longer works reliably beyond this range.

To begin with the principal implementation of luminous bacteria tests in diagram 15 is represented as a summary before a detailed description of the various different BioFix® *Lumi* luminous bacteria tests is carried out, which can be implemented on the **BioFix® *Lumi-10*** luminometer with the reagents of **MACHEREY-NAGEL**.

1. **Sample preparation:**
   - Remove turbidity
   - Set pH 6 to 8
   - Salination of the sample to a salt content of approx. 2 %
   - If necessary prepare sample dilutions (dilute with "BioFix® *Lumi* Diluent ")

↓

2. **Reactivation** of the freeze-dried BioFix® *Lumi* luminous bacteria.

↓

3. Pipette the reactivated **BioFix® *Lumi* luminous bacteria into cuvettes**.

↓

4. **Switch on the BioFix® *Lumi-10* luminometer**.
   - Call up the <Measurement Start> menu and in the <Assay> option call up the desired measurement protocol with the desired protocol type e.g. "BioTox-B" (initial and final light intensity) or "BioTox-S" (only final light intensity)
   - If necessary modify the preset incubation time
   - Start measurement

↓

5. Measure **initial light intensity** of the test preparations (only in the case of the "BioTox-B" protocol type).

↓

6. **Addition of sample**(dilutions) to the submitted luminous bacteria.

↓

7. Wait for the **Incubation time**.

↓

8. Measure the **final light intensity** of the test preparations.

↓

9. **Statement of the results** in the display of the luminometer.
   Differentiated statement of results as **% inhibition** or **% stimulation** of the light intensity of the sample compared to the control and automatic storage of the measurement results in the instrument memory.

↓

10. Option of **data administration** (representation in the display, transfer to the PC, delete).

Diagram 15: Principle implementation of BioFix® *Lumi* luminous bacteria tests.

6.5 <BioTox-S> test mode

The **<BioTox-S>** protocol type is suitable as a rapid test (rapid = german: schnell) for screening measurments, during which a rough estimate of the degree of toxicity of the samples is sufficient. Therefore during this test mode only the final light intensity of the test preparation following the incubation time t is used to estimate the toxicity.

The statement of the results is carried out in a differentiated manner as the % inhibition or % stimulation of the luminescence of the sample preparations compared to the luminescence in the uninhibited control.

All the BioFix® *Lumi* luminous bacteria, freeze-dried test kits that can be obtained from **MACHEREY-NAGEL** can be carried out with the **<BioTox-S>** test protocol.

6.5.1 BioFix® *Lumi* "Single-Shot" luminous bacteria test

BioFix® *Lumi* "Single-Shot" luminous bacteria (Cat. no. 945 021) are very well suited for individual measurements in the course of the manufacturing control and in-house monitoring. A vial of BioFix® *Lumi* "Single-Shot" luminous bacteria is sufficient for the preparation of one control and one sample.

Test protocol:

Importance notice:

The reactivation of BioFix® Lumi "Single-Shot" luminous bacteria is carried out with BioFix® Lumi „Single-Shot" Reactivation solution kept in cold storage in the refridgerator at +2 °C to +8 °C.

Working step 1: **Preparation of the samples**

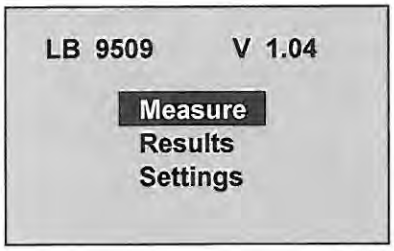
Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. the pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by additing 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* "Single-Shot" luminous bacteria**

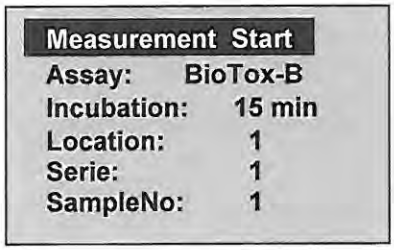
1. Remove a vial with BioFix® *Lumi* "Single-Shot" luminous bacteria from the refrigerator section, open it carefully and add **1.0 ml BioFix® *Lumi* "Single-Shot" Reactivation solution**.
2. Carefully mix the vial with reactivated luminous bacteria and label it as a "control".
3. Provide an empty glass cuvettel and label it as a "sample". Following this pipette **0.5 ml** of the **reactivated luminous bacteria solution** into the glass cuvette labelled as the "sample". Leave the "sample" and the "control" cuvette to stand on their own for approx. 10 minutes without moving them.

Working step 3: **Test preparation and measurement**

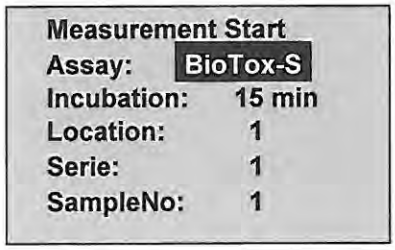
1. Switch on the luminometer **BioFix® *Lumi-10*** by pressing the **[ON]** key. The main menu appears. The **<Measure>** option has already been automatically selected



2. Confirm the **<Measure>** option by pressing the **[↵]** key. The submenu **<Measurement Start>** appears.

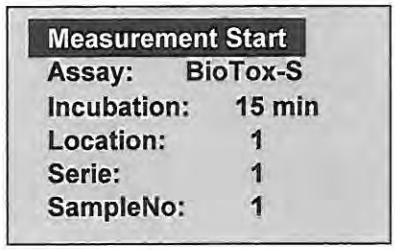


3. Select the **<Assay>** option with the aid of the **[↑] / [↓]** keys and activate it with the **[↵]** key. Select the desired measurement protocol in the selection mode with the aid of the arrow keys **[↑] / [↓]** with the protocol type **<BioTox-S>** and confirm with the **[↵]** key.



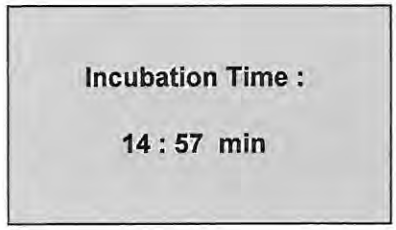
4. Depending on your personal preference the parameters **<Incubation>**, **<Location>**, **<Serie>** and **<SampleNo>** can be individually selected and modified. (See chapter 5.3 with respect to the details concerning the setting of the different parameters.)

5. Return to the **<Measurement Start>** option following the setting of all the desired parameters with the aid of the **[↑] / [↓]** keys.

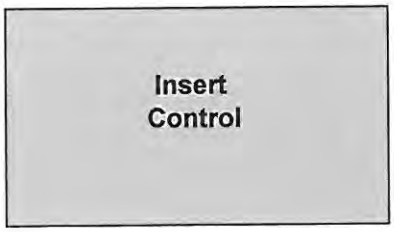


6. Addition of **500 µl BioFix® *Lumi* "Single-Shot" Control solution** to the control cuvette and addition of **500 µl sample solution** to the sample cuvette.

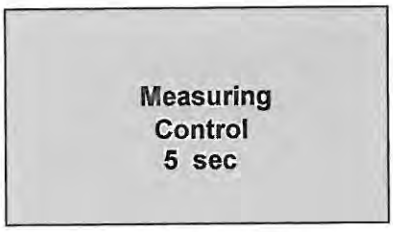
7. Following this immediately press **[↵]** key <u>without</u> placing a cuvette in the measurement shaft of the luminometer whilst the measurement shaft is <u>closed</u>. In the display **starts** the countdown of the set **incubation time** following this.



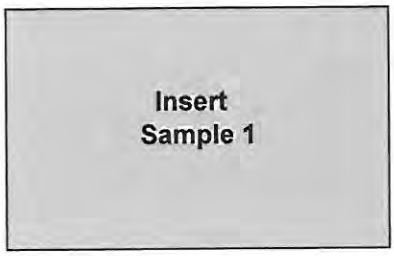
8. A tone rings out following the expiry of the incubation time and the demand to **insert the control cuvette in the measurement shaft** appears.



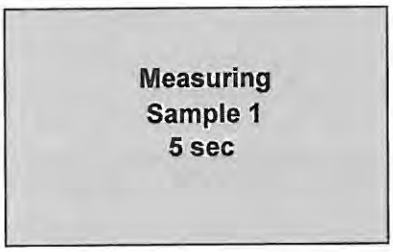
9. Open the measurement shaft, place the control cuvette in the cuvette shaft, close the measurement shaft once more and press the **[↵]** key. The **final light intensity of the control preparation is** measured.



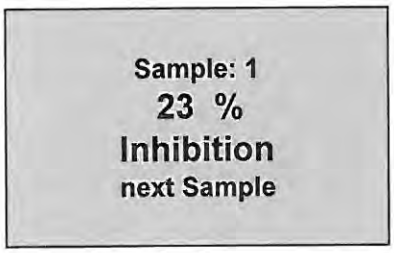
10. Following this you are demanded to **insert sample 1 in the measurement shaft**.



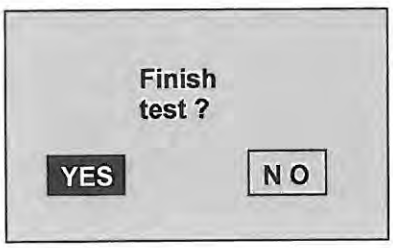
11. Open the measurement shaft, insert the sample cuvette 1 in the cuvette shaft, close the measurement shaft and press the **[↵]** key. The **final light intensity of the sample cuvette 1** is measured.



12. The result of **sample 1** is stated as **% inhibition or % stimulation** and at the same time the demand to measure the next sample.



13. Terminate the test by pressing the **[ESC]** key. The safety enquiry **< Finish test ? YES / NO >** appears in the display.

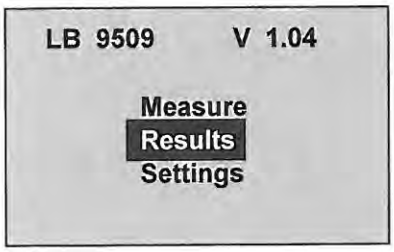


14. Answer the safety enquiry with **<YES>**. The program automatically returns to the **<Measurement Start>** submenu.

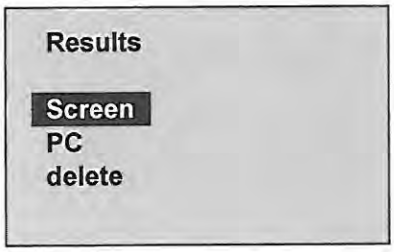
Working step 4: **Data processing**

1. You return to main menu from the submenu **<Measurement Start>** by pressing the **[ESC]** key once more.

2. Select the **<Results>** option with the aid of the **[↑] / [↓]** keys and confirm it with the **[↵]** key.



3. The submenu <Results> appears with the the **<Screen>**, **<PC>** and **<delete>** option. (See chapter 5.4 for further details with respect to the data administration)



6.5.2 BioFix® *Lumi* "Multi-Shot" luminous bacteria test

BioFix® *Lumi* "Multi-Shot" luminous bacteria (Cat. no. 945 022) are very well suited for routine measurements during the manufacturing control und in-house monitoring or if a low number of samples or samples or sample dilutions should be measured at the same time. One BioFix® *Lumi* "Multi-Shot" luminous bacteria vial is sufficient for a maximum of one control and 9 samples (dilutions).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi "Multi-Shot" luminous bacteria is carried out with BioFix® Lumi "Multi-Shot" Reactivation solution that was in cold storage in the refridgerator at temperatures of between +2 °C to +8 °C.

Working step 1: **Sample preparation**

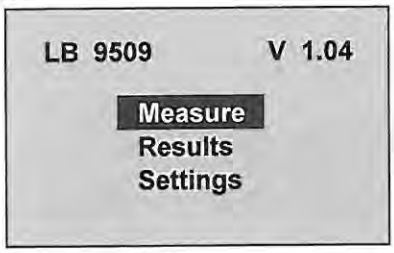
Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by adding 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* "Multi-Shot" luminous bacteria**

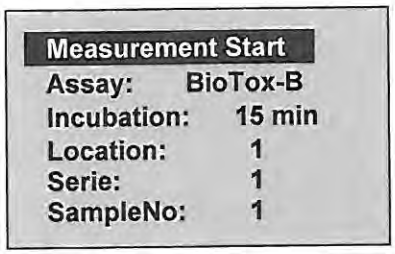
1. Remove a deep frozen **vial** with **BioFix® *Lumi* "Multi-Shot" luminous bacteria** from the freezer compartment and the pre-cooled bottle with **BioFix® *Lumi* "Multi Shot" Reactivation solution** from the refridgerator.
2. Add **6 ml BioFix® *Lumi* "Multi-Shot" Reactivation solution** ("shock thawing") as quickly as possible.
3. Carefully mix the vial with reactivated luminous bacteria.
4. Provide the necessary number of glass cuvettes in a cuvette rack and label it (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** into each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes in order to stabilise them.

Working step 3: **Test preparation und measurement**

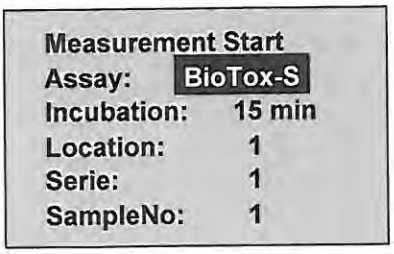
1. Switch on the **BioFix® *Lumi-10*** luminometer by pressing the **[ON]** key. The main menu appears. The **<Measure>** option has already been automatically selected.



2. Confirm the **<Measure>** option by pressing the **[↵]** key. The **<Measurement Start>** submenu appears.

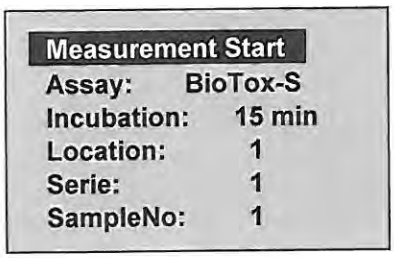


3. Select the **<Assay>** option with the aid of the **[↑] / [↓]** keys and activate it with the **[↵]** key. Select the desired measurement protocol in the selection mode with the aid of the **[↑] / [↓]** keys with the protocol type **<BioTox-S>** and confirm it with the **[↵]** key.



4. Depending on your personal preference the parameters **<Incubation>**, **<Location>**, **<Serie>** and **<SampleNo>** can now be individually selected and modified. (See chapter 5.3. with respect to the details concerning the setting of the different parameters.)

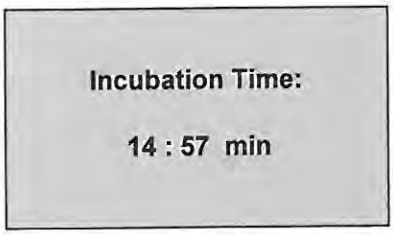
5. Return to the **<Measurement Start>** option with the aid of the **[↑] / < ↓ >** keys following the setting of all the desired parameters.



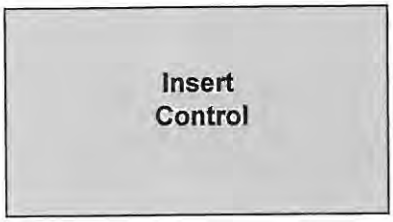
6. Addition of **500 µl BioFix® *Lumi* "Multi-Shot" Control solution** to the control cuvette.

7. Addition of **500 µl sample solution** respectively to the individual sample cuvette.

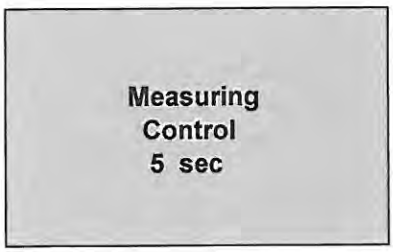
8. Following this immediately press **[↵]** key <u>without</u> placing a cuvette in the measurement shaft of the luminometer whilst the measurement shaft is <u>closed</u>. In the display **starts** the countdown of the set **incubation time** following this.



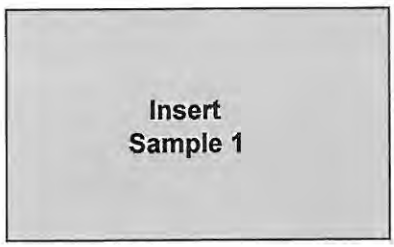
9. A tone rings out following the expiry of the incubation time and the demand to **insert the control cuvette in the measurement shaft** appears.



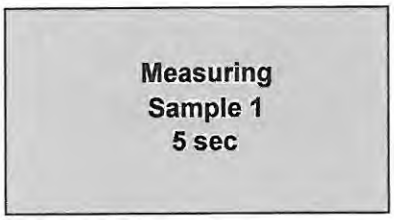
10. Open the measurement shaft, place the control cuvette in the cuvette shaft, close the measurement shaft once more and press the [↵] key. The **final light intensity of the control** preparation is measured.



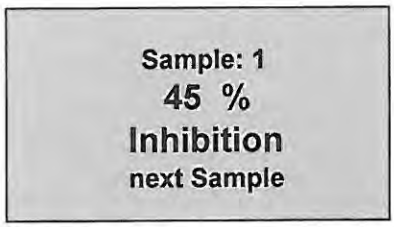
11. Following this you are demanded to **insert sample 1 into the measuring shaft**.



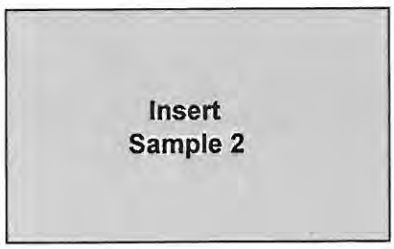
12. Open the measuring shaft, insert the sample cuvette 1 in the cuvette shaft, close the measuring shaft once again and press the [↵] key. The **final light intensity of the sample cuvette 1** is measured.



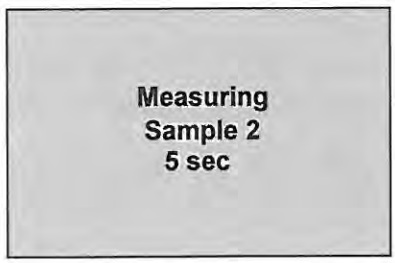
13. The result of **sample 1** is stated as **% inhibition or % stimulation** and at the same time the demand to measure the next sample appears



14. Press the [↵] button. The demand to **insert sample 2 in the measuring shaft** appears.

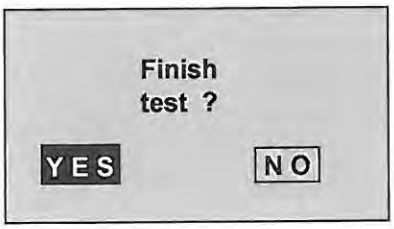


15. Open the measurement shaft, insert the sample cuvette 2 in the cuvette shaft, close the measuring shaft again and press the [↵] key. The **final light intensity of the sample cuvette 2** is measured.



16. The result of **sample 2** is stated in **% inhibition or % stimulation** and at the same time the demand to measure the next sample appears.

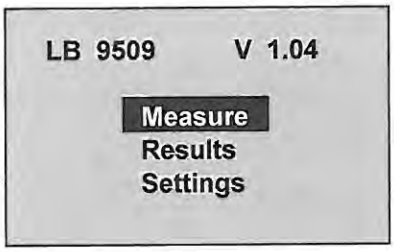
17. Terminate the test by pressing the **[ESC]** key following the measurement of the last sample. The safety enquiry **< Finish test ? YES / NO >** appears in the display.



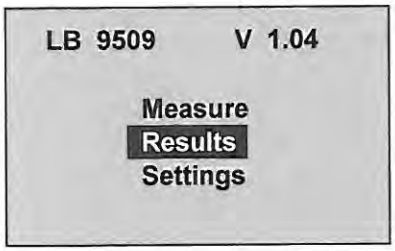
18. Answer safety enquiry with **<YES>**. The program automatically returns to the **<Measurement Start>** submenu.

Working step 4: **Data processing**

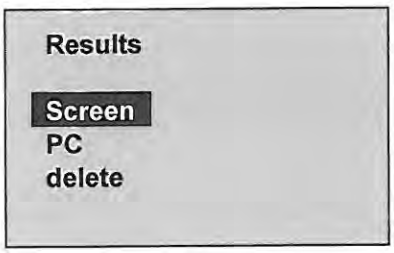
1. You return back to the main menu from the **<Measurement Start>** submenu by pressing the **[ESC]** key.



2. Select the **<Results>** option with the aid of the **[↑] / [↓]** keys and confirm with the **[↵]** key.



3. The submenu <Results> appears with the options **<Screen>**, **<PC>** and **<delete>**. (See chapter 5.4 for further details with respect to the data administration.)



6.5.3 BioFix® *Lumi* luminous bacteria test (20 determinations/vial)

BioFix® *Lumi* luminous bacteria, 20 determinations/vial (Cat. no. 945 006 / 945 007) are very well suited for comprehensive routine measurements during the course of the manufacturing control and in-house monitoring if a large number of samples or sample dilutions are measured at the same time. One vial of this BioFix® *Lumi* luminous bacteria is sufficient for a maximum of one control and 19 samples (dilutions).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi luminous bacteria, 20 determinations/vial is carried out with "BioFix® Lumi Medium for freeze-dried luminous bacteria" that is held in cold storage in the refridgerator at +2 °C to +8 °C. This medium is enclosed in each test kit.

Working step 1: **Sample preparation**

Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by adding 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* luminous bacteria (20 determinations/vial)**

1. Remove a frozen **vial** with **BioFix® *Lumi* luminous bacteria** from the freezer compartment and the pre-cooled bottle with "**BioFix® *Lumi* Medium for freeze-dried luminous bacteria**" from the refridgerator.
2. Add **11.0 ml "BioFix® *Lumi* Medium for freeze-dried luminous bacteria"** ("Shock thawing") as quickliy as possible.
3. Carefully mix the vial with reactivated luminous bacteria.
4. Provide the required number of glass cuvettes in a cuvette rack and label them (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** in each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes without moving them in order to stabilise them.

Test preparation, **measurement** and **data processing** are carried out following this in exactly the same manner as already described in detail in chapter 6.5.2 "BioFix® *Lumi* "Multi-Shot" luminous bacteria test"! "BioFix® *Lumi* Diluent" (Cat. no. 945 601) is used instead of the BioFix® *Lumi* "Multi-Shot" Control solution.

6.5.4 BioFix® *Lumi* luminous bacteria test (100 determinations/vial)

The BioFix® *Lumi* luminous bacteria, 100 determinations/vial (Cat. no. 945 002 / 945 003) is very well suited for comprehensive screening measurements if a large number of samples or sample dilutions should be measured. One vial of this BioFix® *Lumi* luminous bacteria is sufficient for a maximum number of 100 test preparations (control and sample preparations).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi luminous bacteria, 100 determinations/vial is carried out with "BioFix® Lumi Reconstitution solution" that is held in cold storage in the refridgerator at +2 °C to +8 °C. This solution is enclosed with each test kit.
The additionally required "BioFix® Lumi Medium for freeze-dried luminous bacteria" (Cat. no. 945 608) must be ordered separately and must be stored at +2 °C bis +8 °C in the refridgerator until it is used.

Working step 1: **Sample preparation**

Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by additing 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* luminous bacteria (100 determinations/vial)**

1. Remove a frozen **vial** with **BioFix® *Lumi* luminous bacteria** from the freezer compartment, carefully open it, add **1 ml "BioFix® *Lumi* Reconstitution solution"** (+2 °C to +8 °C) and mix well.
2. Transfer **50 ml** of refrigerated (+2 °C bis +8 °C) **"BioFix® BioFix® *Lumi* Medium for freeze-dried luminous bacteria"** in a vessel of a suitable size (e.g. 100 ml beaker).
3. Following that **immediately** add the entire reactivated BioFix® *Lumi* luminous bacteria solution to **50 ml of "BioFix® *Lumi* Medium for freeze-dried luminous bacteria"**.
4. Provide the required number of glass cuvettes in a cuvette rack and label them (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** diluted in in BioFix® *Lumi* Medium in each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes without moving them.

Test preparation, **measurement** and **data processing** are carried out following in exactly the same manner as already described in detail in chapter 6.5.2 "BioFix® *Lumi* „Multi-Shot" luminous bacteria test"! "BioFix® *Lumi* Diluent" (Cat. no. 945 601) is used instead of the BioFix® *Lumi* "Multi-Shot" Control solution.

6.6 <BioTox-B> test mode

The **<BioTox-B>** protocol type is suited as a basic test for BioFix® *Lumi* luminous bacteria toxicity tests, if precise measurement results are desired. This is achieved because the initial light intensity of the luminous bacteria at the beginning of the test is also measured in addition to the measurement of the final light intensity of the test preparations. As a result, the naturally existing drift of the bacterial light intensity is also measured in connection with the incubation time t and is also taken into consideration during the determination of the measurement results.

The results are indicated differentially as % inhibiton or % stimulation of the light intensity of the sample assays in comparison to the light intensity in the uninhibited control.

All of the freeze-dried BioFix® *Lumi* luminous bacteria test kits available at **MACHEREY-NAGEL** can be carried out with the test procedure **<BioTox-B>**.

6.6.1 BioFix® *Lumi* "Single-Shot" luminous bacteria test

BioFix® *Lumi* "Single-Shot" luminous bacteria (Cat. no. 945 021) are very well suited for individual measurements in the course of the manufacturing control and in-house monitoring. A vial of BioFix® *Lumi* "Single-Shot" luminous bacteria is sufficient for the preparation of one control and one sample.

Test protocol:

Importance notice:

The reactivation of BioFix® Lumi "Single-Shot" luminous bacteria is carried out with BioFix® Lumi „Single-Shot" Reactivation solution kept in cold storage in the refridgerator at +2 °C to +8 °C.

Working step 1: **Preparation of the samples**

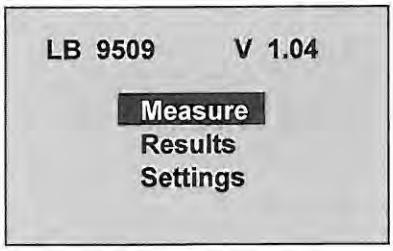
Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. the pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by additing 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* "Single-Shot" luminous bacteria**

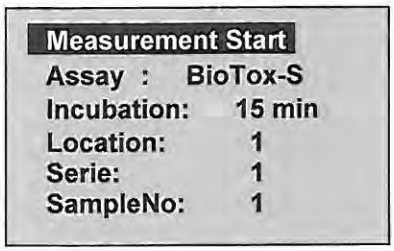
1. Remove a vial with BioFix® *Lumi* "Single-Shot" luminous bacteria from the refrigerator section, open it carefully and add **1.0 ml BioFix® *Lumi* "Single-Shot" Reactivation solution**.
2. Carefully mix the vial with reactivated luminous bacteria and label it as a "control".
3. Provide an empty glass cuvettel and label it as a "sample". Following this pipette **0.5 ml** of the **reactivated luminous bacteria solution** into the glass cuvette labelled as the "sample". Leave the "sample" and the "control" cuvette to stand on their own for approx. 10 minutes without moving them.

Working step 3: **Test preparation and measurement**

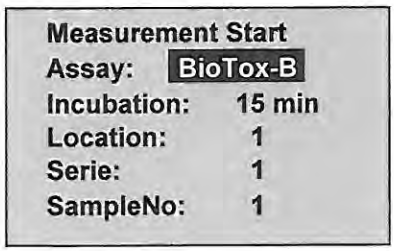
1. Switch on the luminometer **BioFix® *Lumi-10*** by pressing the **[ON]** key. The main menu appears. The **<Measure>** option has already been automatically selected.



2. Confirm the **<Measure>** option by pressing the **[↵]** key. The submenu **<Measurement Start>** appears.

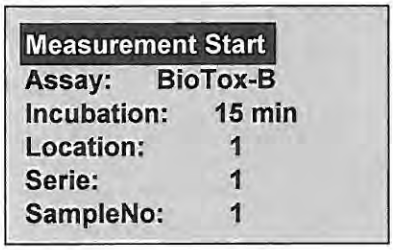


3. Using the cursor keys **[↑] / [↓]** select the **<Assay>** option and activate with the **[↵]** key. Using the cursor keys **[↑] / [↓]** in the selection mode, select the desired measurement procedure with the protocol type **<BioTox-B>** and confirm with the **[↵]** key.



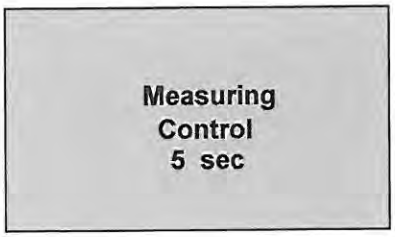
4. Depending on your personal preference, the parameters **<Incubation>**, **<Location>**, **<Serie>** and **<SampleNo>** can now be selected and modified. (For details concerning adjustment of the different parameters, see chapter 5.3.)

5. After adjusting all of the desired parameters by using the cursor keys **[↑] / [↓]** return to the option **<Measurement Start>**.

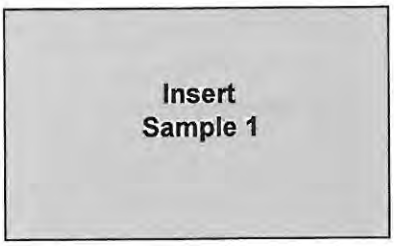


6. Start the **measurement of the initial light intensity** by pressing the **[↵]** key. The demand to insert the control cuvette in the measurement shaft appears.

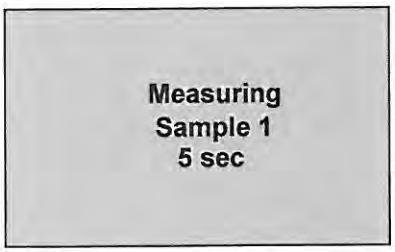
7. Open the measurement shaft, place the control cuvette in the cuvette shaft, close the measurement shaft once more and press the [↵] key. The **initial light intensity of the control preparation** is measured.



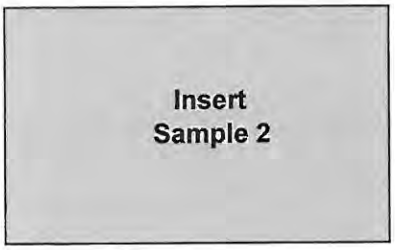
8. Following this you are demanded to **insert sample 1 in the measurement shaft.**



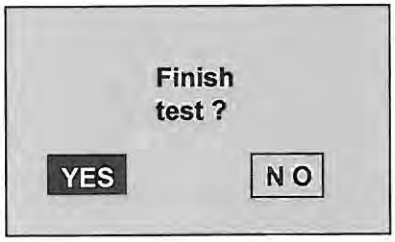
9. Open the measurement shaft, insert the sample cuvette 1 in the cuvette shaft, close the measurement shaft and press the [↵] key. The **initial light intensity of the sample cuvette 1** is measured.



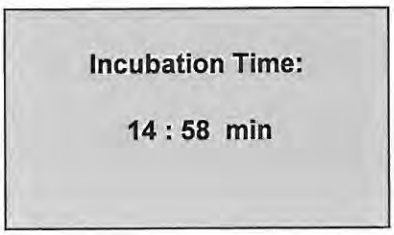
10. The demand to insert the next sample cuvette now appears.



11. By pressing the **[ESC]** key, the measuring series is terminated. The safety enquiry **< Finish test ? YES / NO >** appears in the display.

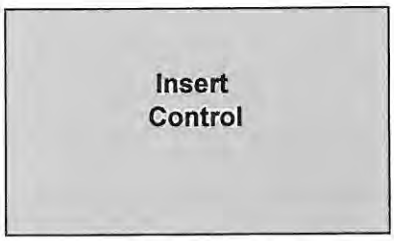


12. Answer the safety enquiry with **<YES>** .In the display, the countdown of the adjusted **incubation time starts** automatically.

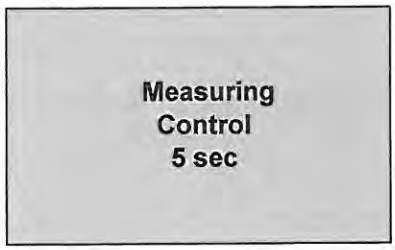


13. Now **immediately** add **500 µl BioFix® *Lumi* "Single-Shot" Control solution** to the control cuvette and add **500 µl sample solution** to the sample cuvette.

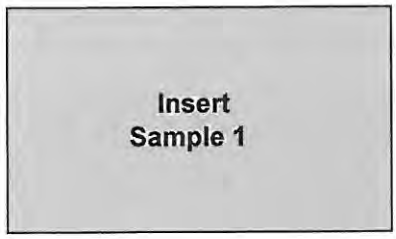
14. After lapse of the incubation time, a signal sounds and the demand to **insert the control cuvette in the measurement shaft** appears in the the display.



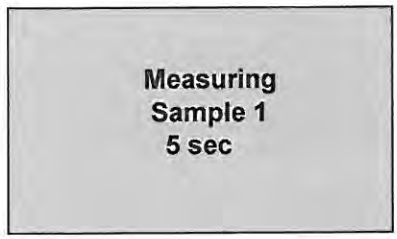
15. Open the measurement shaft, place control cuvette in the cuvette shaft, close the measurement shaft once more and press the **[↵]** key. The **final light intensity of the control preparations** is measured.



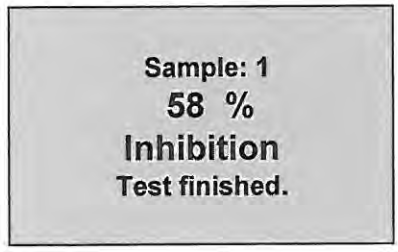
16. Following this you are demanded to **insert sample 1 in the measurement shaft**.



17. Open the measurement shaft, place sample cuvette 1 in the cuvette shaft, close the measurement shaft once again and press the **[↵]** key. The **final light intensity of the sample cuvette 1** is measured.
5 sec



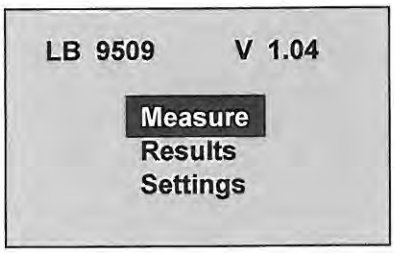
18. The result of **sample 1** is stated as **% inhibition or % Stimulation** and simultaneously the notice that the measurement series has ended.



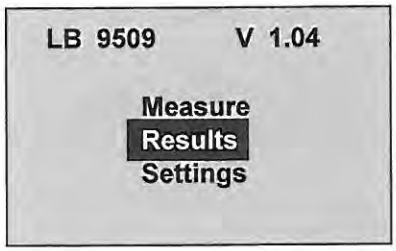
19. By pressing the **[↵]** key, the program automatically returns to the submenu **<Measurement Start>**.

Working step 4: **Data processing**

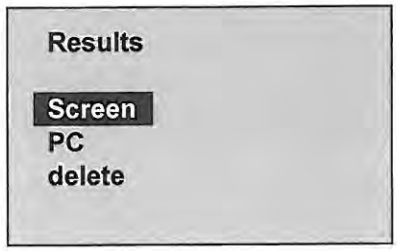
1. You return to main menu from the submenu **<Measurement Start>** by pressing the **[ESC]** key once more.



2. Select the **<Results>** option with the aid of the **[↑] / [↓]** keys and confirm it with the **[↵]** key.



3. The submenu <Results> appears with the the **<Screen>**, **<PC>** and **<delete>** option. (See chapter 5.4 for further details with respect to the data administration)



6.6.2 BioFix® *Lumi* "Multi-Shot" luminous bacteria test

BioFix® *Lumi* "Multi-Shot" luminous bacteria (Cat. no. 945 022) are very well suited for routine measurements during the manufacturing control und in-house monitoring or if a low number of samples or samples or sample dilutions should be measured at the same time. One BioFix® *Lumi* "Multi-Shot" luminous bacteria vial is sufficient for a maximum of one control and 9 samples (dilutions).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi "Multi-Shot" luminous bacteria is carried out with BioFix® Lumi "Multi-Shot" Reactivation solution that was in cold storage in the refridgerator at temperatures of between +2 °C to +8 °C.

Working step 1: **Sample preparation**

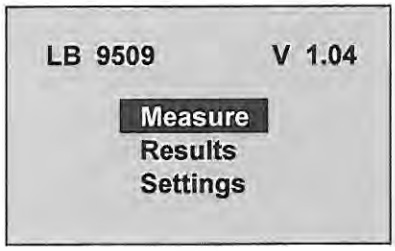
Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by adding 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* "Multi-Shot" luminous bacteria**

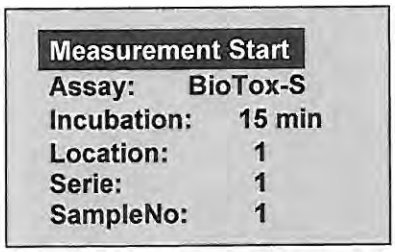
1. Remove a deep frozen **vial** with **BioFix® *Lumi* "Multi-Shot" luminous bacteria** from the freezer compartment and the pre-cooled bottle with **BioFix® *Lumi* "Multi Shot" Reactivation solution** from the refridgerator.
2. Add **6 ml BioFix® *Lumi* "Multi-Shot" Reactivation solution** ("shock thawing") as quickly as possible.
3. Carefully mix the vial with reactivated luminous bacteria.
4. Provide the necessary number of glass cuvettes in a cuvette rack and label it (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** into each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes in order to stabilise them.

Working step 3: **Test preparation and measurement**

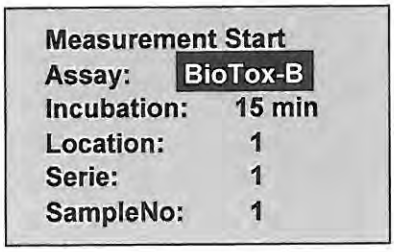
1. Switch on the **BioFix® *Lumi-10*** luminometer by pressing the **[ON]** key. The main menu appears. The **<Measure>** option has already been automatically selected.



2. Confirm the **<Measure>** option by pressing the **[↵]** key. The submenu **<Measurement Start>** will appear.

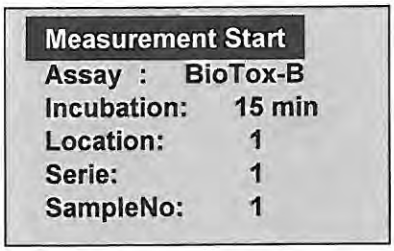


3. Using the cursor keys **[↑] / [↓]** select the **<Assay>** option and activate with the **[↵]** key. In the select mode use the cursor keys **[↑] / [↓]** to select the desired measurement procedure with the protocol type **<BioTox-B>** and confirm with the **[↵]** key.

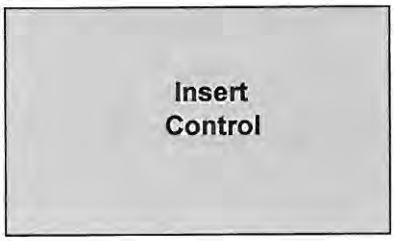


4. Depending on your personal preference, the parameters **<Incubation>**, **<Location>**, **<Serie>** and **<SampleNo>** can also be individually selected and modified. (For more details on adjusting different parameters, see chapter 5.3.)

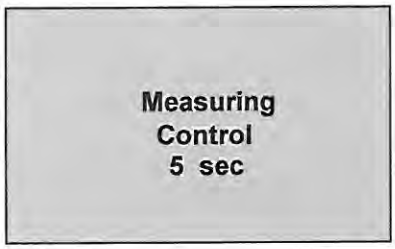
5. After modifying all of the required parameters, use the cursor keys **[↑] / [↓]** to return to the **<Measurement Start>** option.



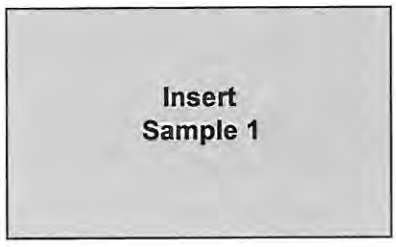
6. Start the **measurement of the initial light intensity** by pressing the **[↵]** key. The demand to insert the control cuvette in the measurement shaft appears.



7. Open the measurement shaft, place the control cuvette in the cuvette shaft, close the measurement shaft once more and press the **[↵]** key. The **initial light intensity of the control preparation** is measured.

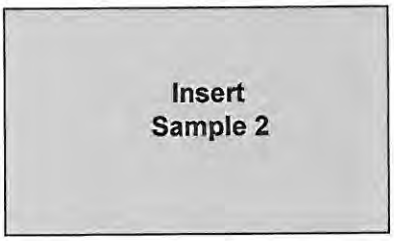


8. Following this you are demanded to **insert sample 1 in the measurement shaft.**

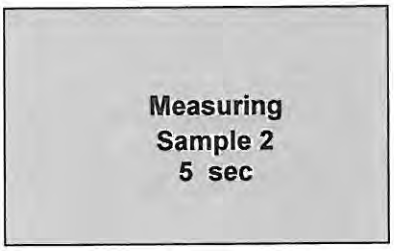


9. Open the measurement shaft, insert the sample cuvette 1 in the cuvette shaft, close the measurement shaft once more and press the **[↵]** key. The **initial light intensity of the sample cuvette 1** is measured.

10. Following this you are demanded to **insert sample 2 in the measurement shaft.**

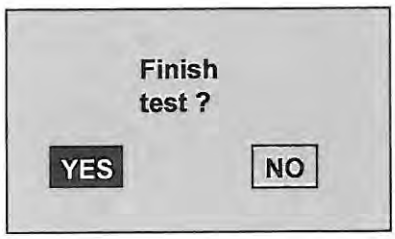


11. Open the measurement shaft, insert the sample cuvette 2 in the cuvette shaft, close the measurement shaft once again and press the **[↵]** key. The **initial light intensity of the sample cuvette 2** is measured.

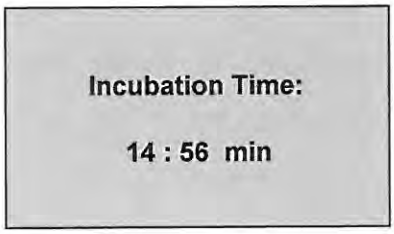


12. The demand to insert the next sample cuvette now appears.

13. After the initial light intensity of the last sample preparation has been measured, press the **[ESC]** key to end the measurement series. The safety enquiry **< Finish test ? YES / NO >** appears in the display.



14. Answer the safety enquiry with **<YES>**. In the display, the countdown of the adjusted **incubation time starts** automatically.

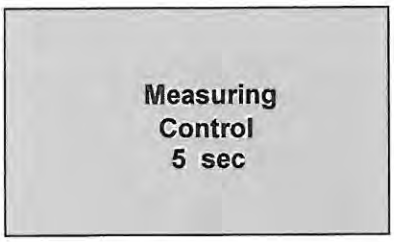


15. Then **immediately** add **500 µl BioFix® *Lumi* "Multi-Shot" Control solution"** to the control cuvette.

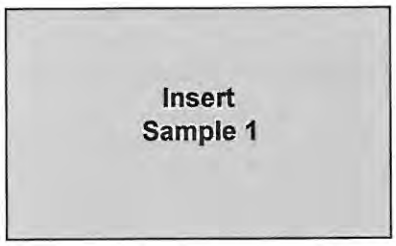
16. **Immediately** add **500 µl sample solution** to each of the sample cuvettes.

17. After the incubation time has lapsed, a signal tone sounds and the demand to **insert the control cuvette in the measurement shaft** appears in the display.

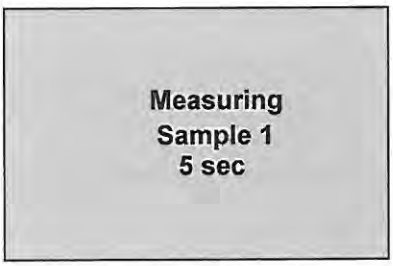
18. Open the measurement shaft, place the control cuvette in the cuvette shaft, close the measurement shaft once more and press the [↵] key. The **final light intensity of the control preparation** is measured.



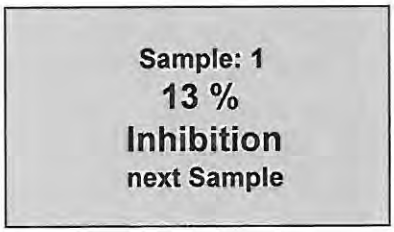
19. Following this you are demanded to **insert sample 1 in the measurement shaft.**



20. Open the measurement shaft, insert the sample cuvette 1 in the cuvette shaft, close the measurement shaft once more and press the [↵] key. The **final light intensity of the sample cuvette 1** is measured.

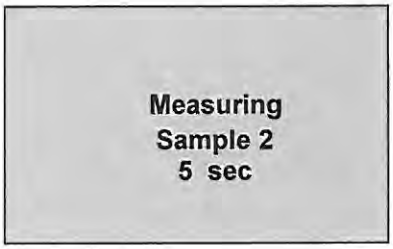


21. The result of **sample 1** is stated as **% inhibition or % stimulation** and at the same time the demand to measure the next sample appears.



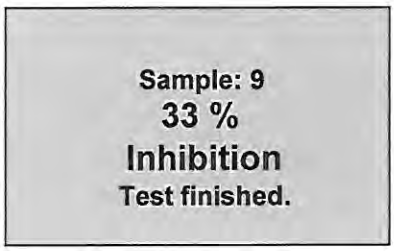
22. Press the [↵] key. You are demanded to **insert sample 2 in the measurement shaft.**

23. Open the measurement shaft, insert the sample cuvette 2 in the cuvette shaft, close the measurement shaft once again and press the **[↵]** key. The **final light intensity of the sample cuvette 2** is measured.



24. The result of **sample 2** is stated as **% inhibition or % stimulation** and at the same time the demand to measure the next sample appears.

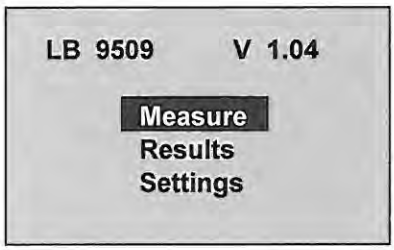
25. After the measurement of the final light intensity of the last sample preparation, the result in **% inhibition or % stimulation** is displayed and simultaneously the note that the measurement series has ended appears.



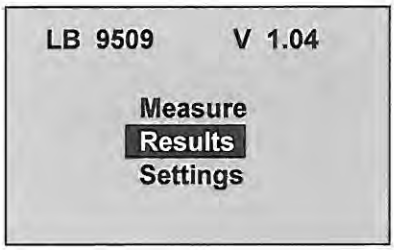
26. By pressing the **[↵]** key, the program automatically returns to the submenu **< Measurement Start >**.

Working step 4: **Data processing**

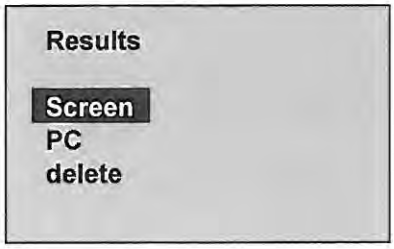
1. You return back to the main menu from the **<Measurement Start>** submenu by pressing the **[ESC]** key.



2. Select the **<Results>** option with the aid of the **[↑] / [↓]** keys and confirm with the **[↵]** key.



3. The submenu <Results> appears with the options **<Screen>**, **<PC>** and **<delete>**. (See chapter 5.4 for further details with respect to the data administration.)



6.6.3 BioFix® *Lumi* luminous bacteria test (20 determinations/vial)

BioFix® *Lumi* luminous bacteria, 20 determinations/vial (Cat. no. 945 006 / 945 007) are very well suited for comprehensive routine measurements during the course of the manufacturing control and in-house monitoring if a large number of samples or sample dilutions are measured at the same time. One vial of this BioFix® *Lumi* luminous bacteria is sufficient for a maximum of one control and 19 samples (dilutions).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi luminous bacteria, 20 determinations/vial is carried out with "BioFix® Lumi Medium for freeze-dried luminous bacteria" that is held in cold storage in the refridgerator at +2 °C to +8 °C. This medium is enclosed in each test kit.

Working step 1: **Sample preparation**

Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by adding 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* luminous bacteria (20 determinations/vial)**

1. Remove a frozen **vial** with **BioFix® *Lumi* luminous bacteria** from the freezer compartment and the pre-cooled bottle with **"BioFix® *Lumi* Medium for freeze-dried luminous bacteria"** from the refridgerator.
2. Add **11.0 ml "BioFix® *Lumi* Medium for freeze-dried luminous bacteria"** ("Shock thawing") as quickliy as possible.
3. Carefully mix the vial with reactivated luminous bacteria.
4. Provide the required number of glass cuvettes in a cuvette rack and label them (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** in each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes without moving them in order to stabilise them.

Test preparation, **measurement** and **data processing** are carried out following this in exactly the same manner as already described in detail in chapter 6.6.2 "BioFix® *Lumi* "Multi-Shot" luminous bacteria test"! "BioFix® *Lumi* Diluent" (Cat. no. 945 601) is used instead of the BioFix® *Lumi* "Multi-Shot" Control solution.

6.6.4 BioFix® *Lumi* luminous bacteria test (100 determinations/vial)

The BioFix® *Lumi* luminous bacteria, 100 determinations/vial (Cat. no. 945 002 / 945 003) is very well suited for comprehensive screening measurements if a large number of samples or sample dilutions should be measured. One vial of this BioFix® *Lumi* luminous bacteria is sufficient for a maximum number of 100 test preparations (control and sample preparations).

Test protocol:

Important notice:

The reactivation of the BioFix® Lumi luminous bacteria, 100 determinations/vial is carried out with "BioFix® Lumi Reconstitution solution" that is held in cold storage in the refridgerator at +2 °C to +8 °C. This solution is enclosed with each test kit.
The additionally required "BioFix® Lumi Medium for freeze-dried luminous bacteria" (Cat. no. 945 608) must be ordered separately and must be stored at +2 °C bis +8 °C in the refridgerator until it is used.

Working step 1: **Sample preparation**

Remove **turbidity particles** in the event of turbid samples by means of centrifugation or filtration. The pH-value of the sample should range between **pH 6 and 8** and if necessary must be corrected accordingly with the aid of 1 N NaOH or 1 N HCl.
The **salt concentration** of the sample should amount to **2 %**. This is best achieved by additing 1 portion of "BioFix® *Lumi* Osmotic Adjusting Solution" (Cat. no. 945 602) for 10 portions of the sample solution. By way of an alternative the salification can also be achieved by means of the addition of 0.2 grams of sodium chloride (NaCl) per 10 ml sample. An already existent salt load (e.g. brackish or seawater) should be taken into account accordingly.

Working step 2: **Reactivation of the BioFix® *Lumi* luminous bacteria (100 determinations/vial)**

1. Remove a frozen **vial** with **BioFix® *Lumi* luminous bacteria** from the freezer compartment, carefully open it, add **1 ml "BioFix® *Lumi* Reconstitution solution"** (+2 °C to +8 °C) and mix well.
2. Transfer **50 ml** of refrigerated (+2 °C bis +8 °C) **"BioFix® BioFix® *Lumi* Medium for freeze-dried luminous bacteria"** in a vessel of a suitable size (e.g. 100 ml beaker).
3. Following that **immediately** add the entire reactivated BioFix® *Lumi* luminous bacteria solution to **50 ml of "BioFix® *Lumi* Medium for freeze-dried luminous bacteria"**.
4. Provide the required number of glass cuvettes in a cuvette rack and label them (1 cuvette for control preparation and 1 cuvette respectively for each sample).
5. Following this pipette **0.5 ml respectively** of the **reactivated luminous bacteria solution** diluted in in BioFix® *Lumi* Medium in each glass cuvette that has been provided. Leave these preparations to stand on their own for approx. 10 minutes without moving them.

Test preparation, **measurement** and **data processing** are carried out following in exactly the same manner as already described in detail in chapter 6.6.2 "BioFix® *Lumi* „Multi-Shot" luminous bacteria test"! "BioFix® *Lumi* Diluent" (Cat. no. 945 601) is used instead of the BioFix® *Lumi* "Multi-Shot" Control solution.

6.7 Colour correction procedure for coloured samples

Method: **Four-Cuvettes-Procedure** with adsorption correction cuvettes in accordance with B.Klein, Z. Wasser-Abwasser-Forsch. **23**, 70-74, 1990

Fundamentals of the colour correction procedure:

Spatial separation of luminous bacteria and sample by means of the use of special adsorption correction cuvettes. In this process the narrow, central interior cylinder of these cuvettes contains the luminous bacteria suspension as a source of light. The transmission reduction is analysed by means of colours due to the alternative filling of the external chamber of the adsorption correction cuvettes with optically empty dilution water or a coloured sample.

Special notices :

- *To begin with the desired luminous bacteria test will also be carried out in the conventional manner precisely in accordance with the regulations for the testing of a colour sample. A potential* ***colour correction*** *will be carried* ***only following the*** *normal luminous bacteria toxicity testing.*
- *It is recommended that the tests are carried out in the form of* ***repeat analyses*** *(doublefold determinations) to order to ensure better statistical results.*
- *The colour correction procedure should be carried out at the same ambient temperatures or incubation temperatures as the preceding luminous bacteria toxicity test that goes with it.*

Required accessories:

4 Adsorptions correction cuvettes (Cat. no. 940 006), BioFix® *Lumi-10* luminometer (Cat. no. 940 008), BioFix® Diluent (Cat. no. 945 601), 0.2 – 1.0 ml piston pipette with tips, Pasteur pipettes

Test procedure:

1. Prepare four adsorption correction cuvettes (cuvettes 1, 2, 3, 4) in an appropriate cuvette rack.
2. Add **1 ml BioFix® *Lumi* Diluent** respectively to the external chamber of the cuvettes 1 and 2, following this add **1 ml of the colour sample** into the cuvettes 3 and 4.
3. Following this you fill the interior cylinder of the cuvettes 1 to 4 with reactivated luminous bacteria suspension up to the same level of the filling height of the external chamber with the aid of Pasteur pipettes in a **1 minute tact**.

   Notice: *The remaining reactivated luminous bacteria solution of the preceding luminous bacteria test that goes with it should be used as the luminous bacteria suspension.*
4. Leave these preparations to stand on their own for **5 minutes** respectively (if possible at the same ambient and incubation temperatures as with the preceding luminous bacteria toxicity test that goes with it).

5. Measure the light intensity with the aid of the **<RLU> protocol type** of the BioFix® *Lumi-10* luminometer and note them down after 5 minutes respectively (in 1 minute tact).

   Recommended luminometer settings:

   Protocol type: RLU

   Meas. Time: 10 sec

   Limit Fail: 900 000

   Limit Pass: 800 000

6. Calculate the average values of both control measurements (I_o) and sample measurements (I_f), respectively.
   Then insert both values together with the inhibition value (in %) obtained from the normal luminous bacteria test (in %) in the equation listed below and **calculate the colour-corrected inhibition.**

7. Evaluation:

Due to the differing geometry of the light source in normal cuvettes and adsorption correction cuvettes the luminous power I_o/I_f (I_o = light intensity behind an optically empty medium; I_f = light intensity behind a coloured content) should not be directly used as a factor for the correction of an inhibition value that was obtained before but instead converted by means of the two following relations:

[1] corrected transmission $Tc = \dfrac{1 - e^{-3,1 \ln (Io/If)}}{3,1 \ln (I_o/I_f)}$

[2] **colour corrected inhibition $I_c\%$** $= \dfrac{\text{Inhibition \%} - 100 (1 - T_c)}{T_c}$

6.8 Testing of soil samples

The analysis of soil samples or other sample substances present in a solid form with the aid of BioFix® *Lumi* luminous bacteria toxicity tests from **MACHEREY-NAGEL** differs from the tests of conventional, liquid environmental samples only in terms of the type of sample preparation. The implementation of the actual toxicity test itself does not change. When using the **BioFix® *Lumi-10*** luminometer you can also choose between the protocol types **<BioTox-S>** (only measurement of final light intensity) and **<BioTox-B>** (measurement of both, initial and final light intensity).

The following accessories are required to prepare the soil samples:

- scales
- 100 ml beaker
- Magnet agitator or shaker
- 50 ml measuring cylinder
- 2 % sodium chloride solution
- Membrane filtration set 1.2 µm (Cat. no. 916 511), consisting of 2 syringes, vol. 20 ml and 25 CHROMAFIL® membrane filters, pore size 1.2 µm
- Membrane filtration set 0.45 µm (Cat. no. 916 50), consisting of 2 syringes, vol. 20 ml and 25 CHROMAFIL® membrane filters, pore size 0.45 µm

Preparation of soil samples

* Weigh a **10 g soil sample**, place it in a 100 ml beaker and add **40 ml 2 % sodium chloride solution**.
* Shake or agitate robustly of a shaker or magnet agitator for 20 minutes.
* Following this allow the suspension to stand on its own without being moved for approx. 10 minutes.
* Remove 10 ml supernatant with a membrane filtration syringe and filter it with the aid of a membrane filter, pore size 1.2 µm.
* If the sample is clearly turbid following this then a filtration system with a membrane filter, pore size 0.45 µm should be connected.
* The eluate that is obtained is inserted as a sample in the luminous bacteria test without further salting.
  See chapter 6.5 and 6.6 for further proceeding of the luminous bacteria tests.

Special notice:

Organic solvents *(ethanol, methanol, DMSO etc.) can also be used for the elution of soil samples. However, in this process it must be ensured that a 1:50 dilution (1 ml eluate + 49 ml 2% sodium chloride solution) of the eluate that is obtained containing solvent is produced, so that the solvent share amounts to a maximum of 2 %! This dilution is then used as a sample in the luminous bacteria test.*

6.9 Mutatox® Genotoxicity test

General information

With the aid of a dark mutant of the *Vibrio fischeri* luminous bacteria (strain M169, "Light⁻-mutant") samples can be detected that have the potential of changing the genotypes. Such agents that have a gene toxic or mutagenic effect cause a re-mutation (reversion) of the "Light⁻-mutant" to the luminous variant of the luminous bacteria ("Light⁺") and thus the restoration of the bioluminescence.

The intensity of the luminescence in the sample preparation in comparison with a non-luminous control is deemed as being the measure for the genotoxicity/mutagenicity or for the potential of a sample to change genotypes.

Prinicipally – as already seen in the case of the Ames test – two parallel preparations are mixed per sample: one test series with S9-mix and one test series without S9-mix.
The so-called S9-mix is a microsome fraction consisting of rat liver homogenate and simulates in the broader sense the liver function of higher organisms, e.g. humans. In this way compounds can also be detected that are not originally mutagenic and which are only activated to become effective mutagenic substances in the course of their metabolisation by the enzymes provided by the liver.
A test preparation covers the media control (Mutatox®-medium and Mutatox®-S9-medium), solvent control (if a sample contains solvent, e.g. eluate of soil samples, pure substances dissolved in solvents) as well as the sample and their dilutions. In addition to carrying out positive controls like phenol (variant without S9-mix) and benzo[a]pyrene (variant with S9-mix), it is recommended that each sample should be diluted 9 times on the basis of a 1 : 2 ratio per test preparation. The incubation of the test preparation is carried out at 27 °C and evaluated following 16, 20 and 24 hours.
The measurement of the light intensities of the test preparations is carried out with the **BioFix® *Lumi-10*** luminometer in the **<RLU>** protocol type. The light intensities that are measured are best entered in the enclosed evaluation sheets (see pages 83 to 85), which you certainly may copy.

By way of a conclusion the Mutatox® Genotoxicity test is compared with the well known test for mutagenicity analyses, the Ames test:

Criteria	Mutatox® test	Ames test
Test organism	Luminous bacteria *Vibrio fischeri*, mutant M 169	Various strains of *Salmonella typhimurium*
Number of test strains	1	2 – 5
Mutation types that can be detected	Base exchange (point mutation), Frame-Shift-Mutation, Induction of the SOS-System, DNA Intercalation	*Strain TA 98:* Frame-Shift-Mutation *Strain TA 100:* Base exchange (point mutation)
Production of the bacterial cell suspension	Rehydration	Microbiological cultivation
Result	Light intensity (Luminescence)	Colony formation/growth
Test duration	16 - 24 hours	48 - 72 hours
Incubation temperature	+27 °C	+37 °C
Sterile test conditions	No	Yes
Test implementation	Simple	Complex
Equipment	**BioFix® *Lumi-10*** luminometer; water-bath or incubator	Complete microbiology laboratory
Disposal (volume)	Non-pathogen (low)	Potentially pathogen (high)
Metabolic activation	S9-Mix	S9-Mix
Data recording	Automatic	Manual
Evaluation	Manual	Manual

Mutatox® test protocoll for aqueous environmental samples
(Example: 1 medium control, 2 positive controls, 3 samples in 9 dilutions)

Working step 1: **Measuring area and sample preparation**

1. Heat up a separate water bath or an incubator to 35° C.
2. Store the BioFix® *Lumi* Reconstitution solution at a cold temperature of +2 °C to +8 °C in the refridgerator.
3. Set pH-value of the sample to pH 7.0 ± 0.3:
   - If the pH-value is below pH 6.7 then set it to pH 6.7 with 1 N NaOH
   - If the pH-value is above pH 7,3, then set it to pH 7.3 with 1 N HCl
4. Produce stock solutions of the positive controls:
   a) **Phenol standard** (positive control for Mutatox® test preparation without S9-mix):
   0.2 g phenol + 20 ml BioFix® *Lumi* Reconstitution solution
   b) **Benzo[a]pyrene standard** (positive control for Mutatox® test preparation with S9-mix):
   0,01 g benzo[a]pyrene + 20 ml DMSO
5. Provide 1 cuvette rack each for test preparations with or without S9-mix.
6. Fill the cuvette rack for the **test preparations without S9-mix** (Mutatox®-medium) as follows with empty glass cuvettes:

1	2	3	4	5	6	7	8	9	10		
x	x	x	x	x						A	**Medium control**
x	x	x	x	x	x	x	x	x	x	B	**Phenol standard**
x	x	x	x	x	x	x	x	x	x	C	**Sample 1**
x	x	x	x	x	x	x	x	x	x	D	**Sample 2**
x	x	x	x	x	x	x	x	x	x	E	**Sample 3**

7. Fill the cuvette rack for the **test preparations with S9-mix** (Mutatox®-S9-medium) as follows with empty glass cuvettes:

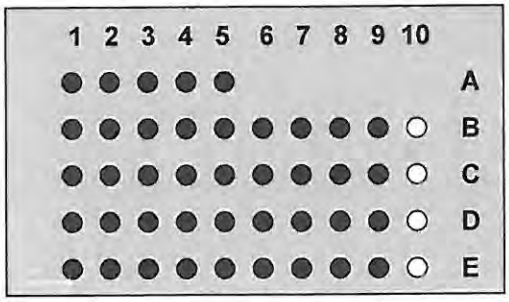
1	2	3	4	5	6	7	8	9	10		
x	x	x	x	x	x	x	x	x	x	A	**Medium/solvent control (DMSO)**
x	x	x	x	x	x	x	x	x	x	B	**Benzo[a]pyrene standard**
x	x	x	x	x	x	x	x	x	x	C	**Sample 1**
x	x	x	x	x	x	x	x	x	x	D	**Sample 2**
x	x	x	x	x	x	x	x	x	x	E	**Sample 3**

Working step 2: **Preparation of the Mutatox® test media and Mutatox® luminous bacteria**

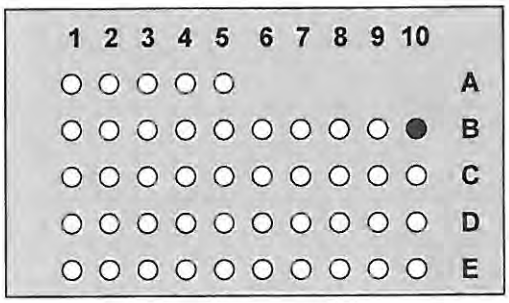
1. Add 15 ml of cooled BioFix® *Lumi* Reconstitution solution to a vial with **Mutatox®-medium** (without S9-Mix) and mix it well.
   ***Important:* Use Mutatox®-medium immediately after dissolving.**

2. **Pipette 250 µl of Mutatox®-medium** respectively into the following cuvettes of the cuvette rack for test preparations without S9-medium:

A1 to A5
B1 to B9
C1 to C9
D1 to D9
E1 to E9



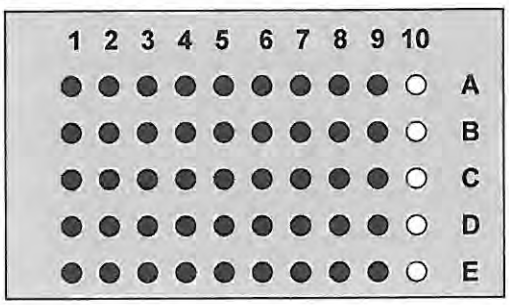
3. Addition of **500 µl Mutatox®-medium** to cuvette **B10** of the cuvette rack for test preparations without S9-medium:



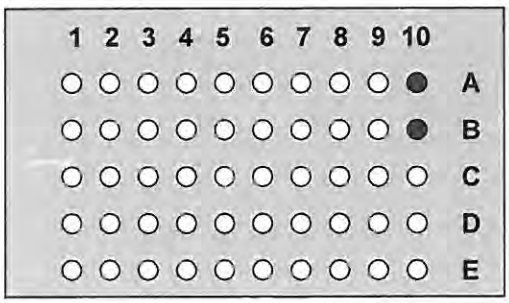
4. Addition of 15 ml of cooled BioFix® *Lumi* Reconstitution solution to a vial with **Mutatox®-S9-medium** and mix it well.
   ***Important:* Use Mutatox®-S9-medium immediately after dissolving.**

5. Pipette **250 µl of Mutatox®-S9-medium** respectively into the following cuvettes of the cuvette rack for test preparation with S9-medium:

A1 to A9
B1 to B9
C1 to C9
D1 to D9
E1 to E9



6. Add **500 µl of Mutatox®-S9-medium** to the cuvettes **A10 and B10** of the cuvette rack for test preparations with S9-medium:



Working step 3: **Addition of control and sample solutions as well as the preparation of serial dilution series (1 : 2 dilutions)**
(See pipette schemes 1 and 2 for an overview)

1. Addition of **control solutions** in accordance with the following scheme:
   - **10 µl DMSO** (solvent control) to cuvete **A 10** of the cuvette rack for test preparations **with S9-mix**.
   - **10 µl phenol standard** to cuvette **B10** of the cuvette rack for test preparations **without S9-mix**.
   - **10 µl benzo[a]pyrene standard** to cuvette **B10** of the cuvette rack for test preparations **with S9-mix**.

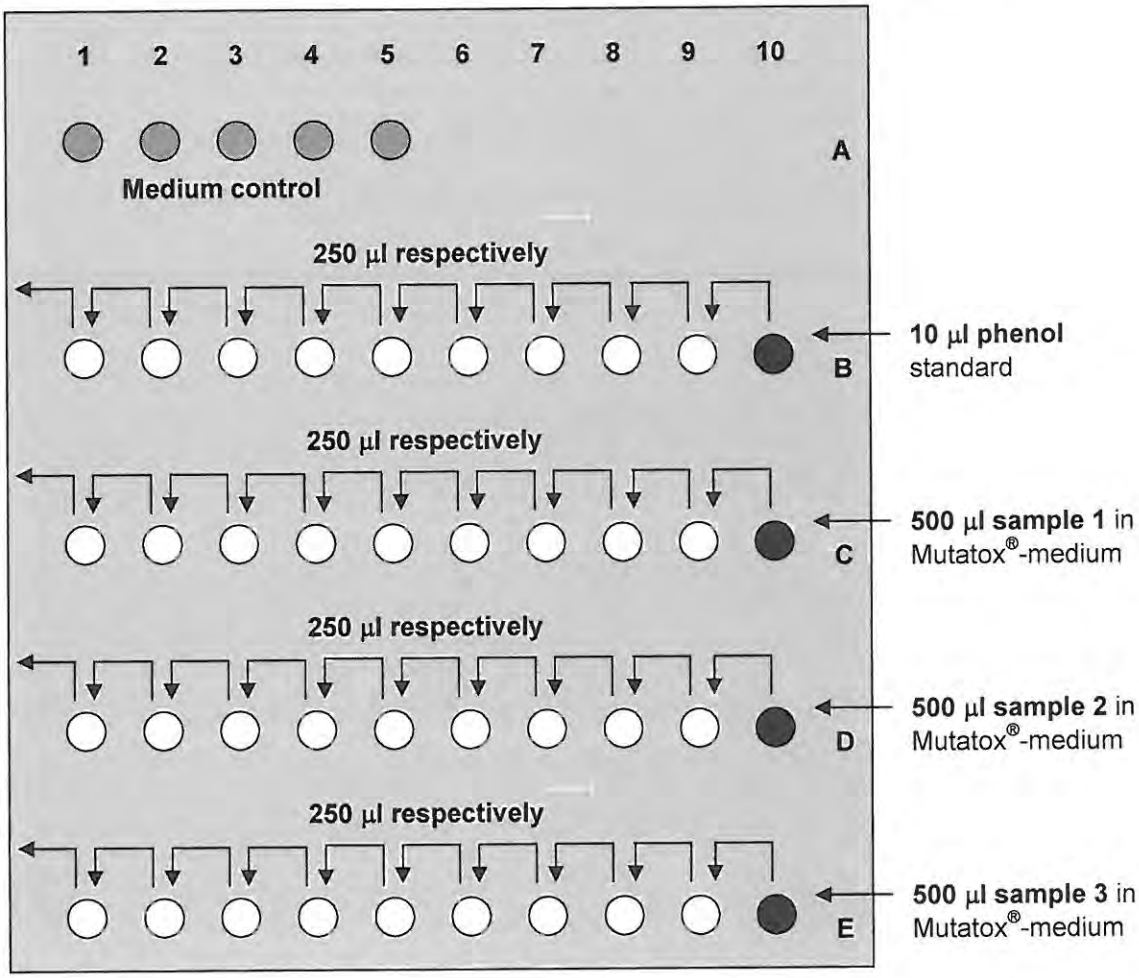
2. Preparation and addition of **sample 1**:
   a) Add 15 ml sample 1 in a vial with Mutatox®-medium (without S9-mix) and mix the solution well.
   b) Pipette 500 µl of the sample 1 / Mutatox®-medium mixture in cuvette C10 of cuvette rack for test preparations without S9-mix.
   c) Add 15 ml of sample 1 in a vial with Mutatox®-S9-medium and mix the solution well.
   d) Pipette 500 µl of the sample 1 / Mutatox®-S9-medium mixture in cuvette C10 of the cuvette rack for test preparations with S9-mix.

3. Preparation and addition of **sample 2**:
   a) Add 15 ml sample 2 in a vial with Mutatox®-medium (without S9-mix) and mix the solution well.
   b) Pipette 500 µl of the sample 2 / Mutatox®-medium mixture in cuvette D10 of the cuvette rack for test preparations without S9-mix.
   c) Add 15 ml sample 2 in a vial with Mutatox®-S9-medium and mix the solution well.
   d) Pipette 500 µl of the sample 2 / Mutatox®-S9-medium mixture in cuvette D10 of the cuvette rack for test preparations with S9-mix.

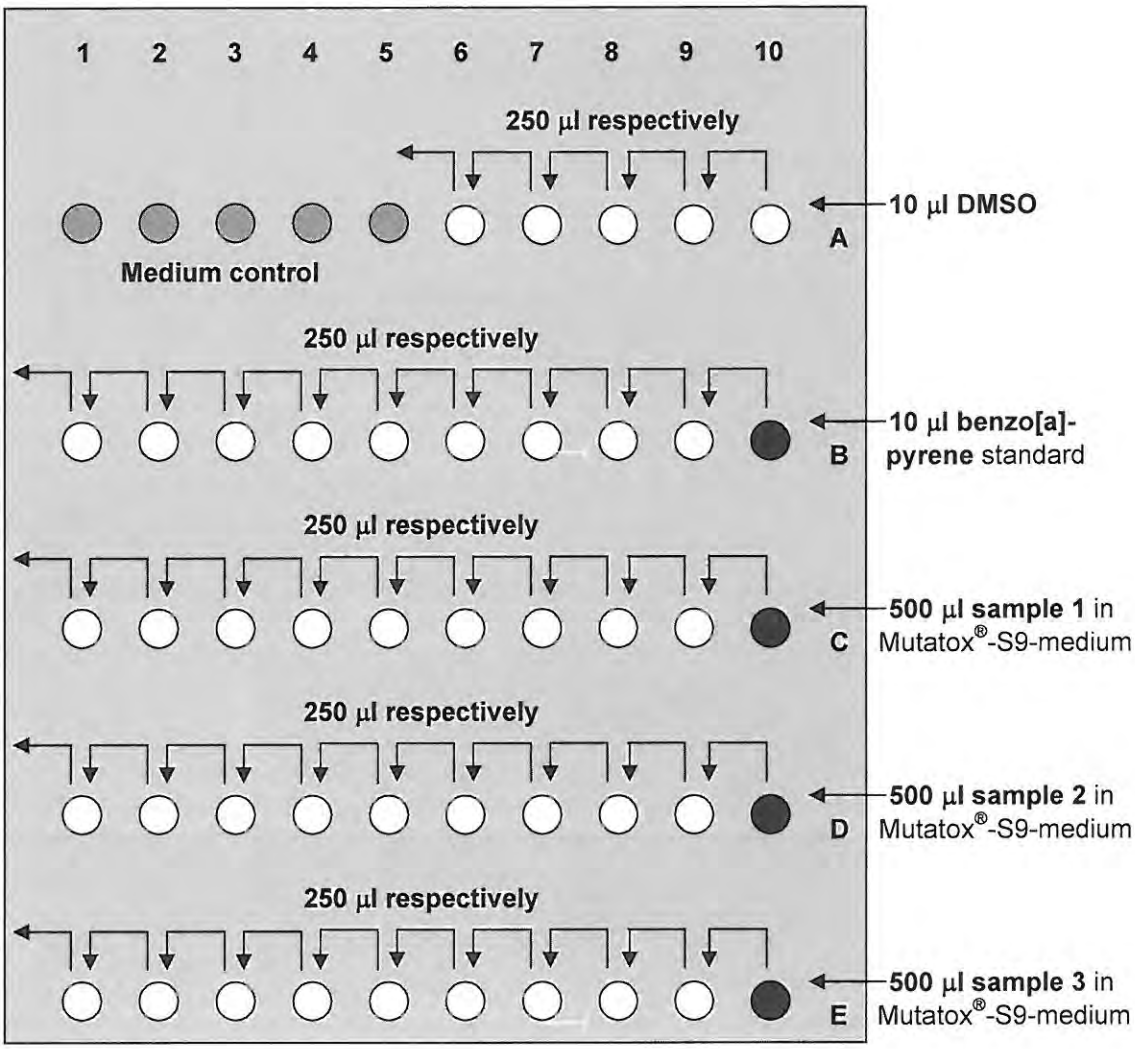
4. Preparation and addition of **sample 3**:
   a) Add 15 ml of sample 3 in a vial with Mutatox®-medium (without S9-Mix) and mix the solution well.
   b) Pipette 500 µl of the sample 3 / Mutatox®-medium mixture in cuvette E10 of the cuvette rack for test preparations without S9-mix.
   c) Add 15 ml of sample 3 in a vial with Mutatox®-S9-medium and mix the solution well.
   d) Pipette 500 µl of the sample 3 / Mutatox®-S9-medium mixture in cuvette E10 of the cuvette rack for test preparations with S9-mix.

5. Preparation of the **serial dilution series** (1 : 2 dilutions):
   The production of 1 : 2 dilutions of the test preparations is carried out by means of the **transfer of 250 µl of solution respectively from cuvette to cuvette** in accordance with the following pipette schemes 1 and 2. Following each a three time mixing is carried out with the aid of the piston pipette employed.
   Following the production of the smallest dilution level of a control or sample 250 µl of the test preparation of this smallest dilution level are discarded.

Pipette scheme 1: **Test preparations with Mutatox®-medium (without S9-Mix)**



Pipette scheme 2: **Test preparations with Mutatox®-S9-medium**



Working step 4: **Preparation of the Mutatox® luminous bacteria**

1. Remove a deep frozen vial with freeze-dried Mutatox® luminous bacteria from the refridgerator and open it carefully.
2. Pour **1.1 ml of cooled BioFix® *Lumi* Reconstitution solution** as quickly as possible into the vial with Mutatox® luminous bacteria, dissolve the luminous bacteria and mix well by shaking the vial several times.
3. Transfer the entire reactivated Mutatox® luminous bacteria solution into an empty cuvette and store it in a cool place until the luminous bacteria are used again (refridgerator or ice bath).

Working step 5: **Addition of Mutatox® luminous bacteria and incubation of the test preparations**

1. Immediately add 10 µl of reconstituted Mutatox® luminous bacteria solution to all the test preparations with and without S9-mix in the following sequence:

   Test preparations with Mutatox®-medium (without S9-mix):

   A1 to A5
   B1 to B10
   C1 to C10
   D1 to D10
   E1 to E10

   Test preparations with Mutatox®-S9-medium:

   A1 to A10
   B1 to B10
   C1 to C10
   D1 to D10
   E1 to E10

2. Note down the start time of the incubation phase that now follows.
3. Mix all the test preparations well by shaking them.
4. Place the **test preparations with Mutatox®-S9-medium** in a water bath or incubator and incubate them for **45 min at +35 ± 0,5 °C** .
   During this time store the test preparations with Mutatox®-medium (without S9-mix) at room termperature on the laboratory bench.
5. Folllowing a 45 minute incubation time at +35 ± 0,5 °C, leave the test preparations with Mutatox®-S9-medium in the water bath or incubator and reduce the incubation temperature to +27 °C.

   ***Important:* It should not take longer than 30 minutes before the water bath or the incubator has reached the new incubation temperature of +27 ± 0,5 °C.**
6. As soon as the temperature of the water bath or incubator has reached the temperature of **+27 ± 0,5 °C**, **insert the test preparations with Mutatox®-medium (without S9-mix)**.
7. The **measurement of the test preparations** is carried out after **16, 20 and 24 hours**.

Working step 6: **Measurement of the test preparations and evaluation of the measurement results**

1. On the **BioFix®** ***Lumi-10*** luminometer call up the **<Customer Settings>** option via the **<Settings>** menu and draft a measurement program.

   ***Important:* The measurement of the test preparations of the Mutatox® Genotoxicity test is only possible with the <RLU> protocol type!**

   For first time performance of the Mutatox® Genotoxicity test the following **parameter settings are recommended**:

<Prot.-Typ>:	**RLU**	**<Limit Fail>:**	**990000**
<Meas. Time>:	**10 sec**	**<Limit Pass>:**	**950000**
<Assay>:	Enter any name (max. 10 figures), e.g. **"Mutatox-1"**		

   Notice: See chapter 5.2.2.3 for the drafting of a measurement program with the <RLU> protocol type and entry of the measurement parameters.

2. After setting up the measurement program call up the **<Measure>** submenu in the main menu of the **BioFix® *Lumi-10*** luminometer.

3. In the **<Measurement Start>** menu call up the measurement program established for the measurement of the Mutatox® test preparations via the **<Assay>** option.

4. If you wish you also can enter figures for the **<Location>** and **<Test>** (Start number for first measurement; subsequent measurements will be counted continually).

5. Following this measure all the test preparations with and without S9-mix in accordance with the sequence:

   To begin with select the **<Measurement Start>** option, then place the cuvette in the cuvette shaft and close the black measurement shaft lid. The measurement of the light intensity is triggered off by pressing the **[↵]** key. The light units of the measured test preparation appear as **"RLU"** (relative light units) as the **result.**

6. Enter the measurement results on evaluation sheets (see pages 83-85).

Notice:

The transfer of the measurement value from the memory of the luminometer to a standard PC is possible (see chapter 5.4.2 "Data transfer to the PC"). There the data can be further processed with MS Excel for example.

7. **Evaluation:**

   Those agent or sample concentrations are defined as potentially genotoxic/mutagenic/ potentially genotype transforming that in the case of the **test preparation without S9-mix** cause at least a **fourfold** increase of light intensity and in the case of the **test preparation with S9-mix** at least a **twofold** increase *vis-à-vis* the average light intensity of the control preparations in at least two successive dilutions.

Practical tip:

Following the measurement of the control preparations and **prior** to the measurement of the standard and sample preparations you should initially calculate the average value of the light intensity in the control. Following that calculate the fourfold figure of this value (in the event of control with S9-mix) as the treshold value for the evaluation of a sample as potentially mutagenic. Following that go into the **<Settings / Customer Settings>** submenu, call up the measurement protocol for the Mutatox® measurement and modify the **<Limit Fail / Limit Pass>** values in accordance with the calculated treshold value for the estimation of the mutagenicity of the samples. In this way during the measurement of the sample preparation it is already displayed upon the display of the measurement results by means of the lighting up of the green, yellow or red warning lights whether the corresponding treshold value has been exceeded and the sample is evaluated as being potentially genotype transforming in the measured concentration.

Return to the **<Measurement Start>** menu following the setting of the treshold values and now measure the light intensities of the standard and sample preparations as already described in accordance with the sequence.

Evaluation sheet for Mutatox® Genotoxicity test on BioFix® *Lumi-10*

Date test preparation: _____ **Date test evaluation:** _____

I. Results Mutatox® without S9-mix:

Medium control			Standard: Phenol				Sample 1:				Sample 2:				Sample 3:			
RLU			Conc. [µg/ml]	RLU			Conc.	RLU			Conc.	RLU			Conc.	RLU		
16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h

Evaluation:

Medium control average value:

16 hours: _____ RLU 20 hours: _____ RLU 24 hours: _____ RLU

Treshold value for potential mutagenicity = Average value medium control x 4 =

16 hours: _____ **RLU** **20 hours:** _____ **RLU** **24 hours:** _____ **RLU**

Phenol standard potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 1 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 2 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 3 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

II. Results Mutatox® with S9-mix:

Medium control / Solvent control			Standard: Benzo[a]pyrene				Sample 1:				Sample 2:				Sample 3:			
RLU			Conc. [µg/ml]	RLU			Conc.	RLU			Conc.	RLU			Conc.	RLU		
16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h		16 h	20 h	24 h

Evaluation:

Medium control average value:

16 hours: _____ RLU 20 hours: _____ RLU 24 hours: _____ RLU

Solvent control average value (Solvent .....) :

16 hours: _____ RLU 20 hours: _____ RLU 24 hours: _____ RLU

Treshold value for potential mutagenicity = Average value of medium control x 2 =

16 hours: _____ RLU 20 hours: _____ RLU 24 hours: _____ RLU

Benzo[a]pyrene standard potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 1 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 2 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

Sample 3 _____ potentially mutagenic:

No ☐ Yes ☐, as of a concentration of _____

III. Evaluation of the Mutatox® results:

Phenol standard: .....

.....

.....

.....

Benzoa[a]pyrene standard: .....

.....

.....

.....

Sample 1: .....

.....

.....

.....

Sample 2: .....

.....

.....

.....

Sample 3: .....

.....

.....

.....

8. ATP measurements and other bioluminescence tests

In addition to the bioluminescence procedures based on luminous bacteria the luminometer **BioFix® *Lumi-10*** is also suitable for a wide range of luminometric tests from the areas of hygiene monitoring and molecular biological/biochemicals diagnostics, e.g.:

- **Determination of ATP and biomasses**
- **Reporter gene assays**
- **DNA probe assays**
- **NADP(H) measurements**
- **Luminescence immunoassays**

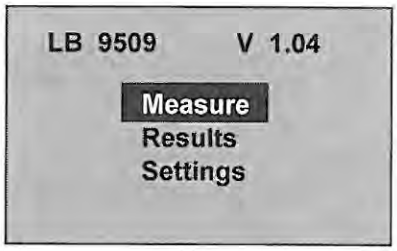
This universal applicability of the **BioFix® *Lumi-10*** luminometer is also aided by the fact that the **cuvette formats of 12 - 15 mm diameter and a height of 47 - 75 mm** fit into the cuvette shaft and can thus be measured.
All the commercially available test kits or reagent sets of the widely known suppliers can be used in order to implement the aforementioned tests. The implementation of the tests is carried out in accordance with the respective working regulations, which are generally enclosed with the reagent packaging in the form of instruction leaflets. Special applications can also be requested from MACHEREY-NAGEL especially for ATP measurements.

The measurement of the luminescent intensities of the test preparations is always carried out in the case of all the tests in accordance with the same pattern and is described in detail below. These measurements are always carried out whilst using the **<RLU>** protocol type.

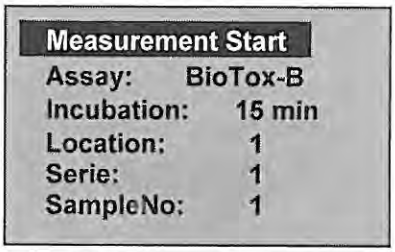
Measurement of the light intensity in the case of the other bioluminescence tests:

1. Prepare the test series (controls, standards, samples) in accordance with the instructions of the test kit or reagent manufacturer.

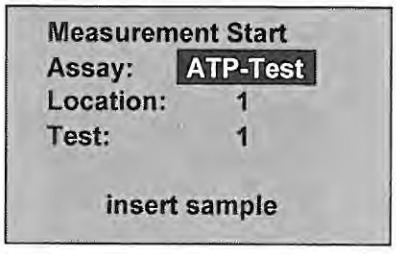
2. Switch on the **BioFix® *Lumi-10*** luminometer by pressing the **[ON]** key. The main menu appears. The **<Measure>** option has already been automatically selected.



3. Confirm the **<Measure>** option by pressing the **[↵]** key. The submenu **<Measurement Start>** appears.

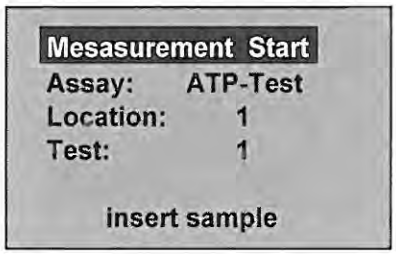


4. Select the **<Assay>** option with the aid of the **[↑] / [↓]** keys and activate it with the **[↵]** key. Select the desired measurement protocol in the selection mode with the aid of the **[↑] / [↓]** keys with protocol type **<RLU>** and confirm with the **[↵]** key. (See chapter 5.2.2.3 with respect to the details for the drafting of measurement protocols.)



5. Depending on your personal wish you can additionally enter identification digits for the parameters **<Location>** and **<Test >**. (See chapter 5.3 for further details of setting the different parameters.)

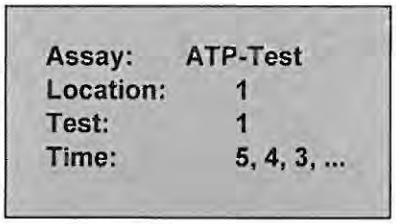
6. Return to the **<Measurement Start>** option with the aid of the **[↑] / [↓]** keys after setting the desired parameters.



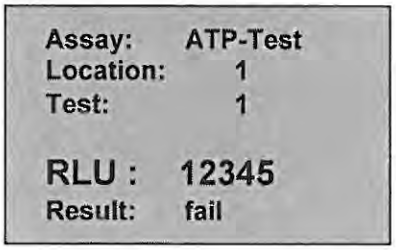
7. Open the measurement shaft and insert the cuvette to be measured, then close the measurement shaft once again. The sealing cover must audibly lock into place. You can now start the measurement.

8. Ensure that the **<Measurement Start>** option is highlighted with the cursor bar and then press the **[↵]** key.

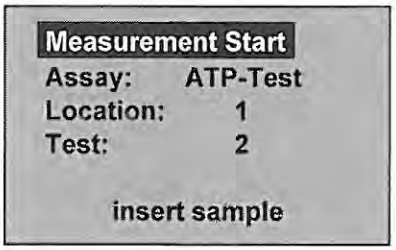
9. The measurement started and the countdown of the set measurement time runs.



10. The measured light intensity is stated as relative light units **[RLU]** as the result. In addition the measurement value is evaluated based on the treshold value settings (see chapter 5.2.2.3 for the details). The evaluation is displayed in an information line ("**fail**" / "**pass** " / "**warn**") and by the lighting of the corresponding signal light.



11. You return to the **<Measurement Start>** menu by pressing the **[↵]** key, the identification digit for the **<Test>** parameter is increased by 1 and the next test series can be measured.



12. You can return to the main menu at any time by pressing the **[ESC]** key.

13. It is possible to further process the measuring results at any time via the **<Results>** data administration option. Data can be called up in a targeted manner, transferred to a PC (e.g. for further processing in MS Excel) or deleted. See chapter 5.4 for the details.

9. Cleaning and maintenance

The **BioFix®** ***Lumi-10*** luminometer is extremely easy to maintain, but should as a security measure be regularly cleaned and maintained at least once a month. The cleaning and maintenance are easy to carry out and generally do not take more than 10 minutes.

Measurement unit (cuvette shaft and photomultiplier)

- The measurement unit must always stored in a clean and dust free state in order to ensure that the measurement results are not negatively influenced!
- Liquid in the cuvette shaft leads to instrument damage!
- If liquid has entered the measurement units then immediately remove the cuvette from the cuvette shaft and turn the instrument upside down so that the liquid runs out and cannot damage the photomultiplier! Following this clean the measurement unit.

Wipe down the instrument external side with a sponge that should ideally have been dipped in lukewarm water. Do not use any caustic washing-up liquids or emery paper.
Carefully clean the interior of the measurement unit and the cuvette shaft with a cotton wool pad soaked in alcohol and following this wipe it several times with dry cotton wool pads.

Rechargeable battery maintenance

The rechargeable batteries should be regularly discharged and then recharged again at regular intervals (every 2-3 months) in order to prevent the capacity of the rechargeable batteries from deteriorating.

Duration of the discharging: approx. 6 hours.

Duration of the recharging: approx. 4 - 5 hours

Remove the **BioFix®** ***Lumi-10*** luminometer from the mains for the discharging and leave the instrument for instance switched on over night. Connect it to the mains once more in order to recharge it.

10. Error messages

Error message	Meaning	Resolution
<-10>	HV part is possibly damp.	1. Switch off the instrument and let it dry. Then switch it on once more. 2. If the above action is not successful then please contact **MACHEREY-NAGEL**.
<Overload>	The light intensity of the test preparation exceeds the measuring range.	1. Dilute the luminous bacteria reagent during the implementation of the luminous bacteria tests. 2. Dilute the sample during the implementation of other bioluminescence tests. 3. If the above action is not successful then please contact **MACHEREY-NAGEL**.
<Batt empty >	Error message which appears approximately a quarter of an hour before the battery is completely run down.	1. Connect the instrument to the mains and recharge it. 2. If the above action is not successful then please contact **MACHEREY-NAGEL**.
<STOP: Open Door, Measurement stopped>	The sealing cap of the measurement unit has been jolted open during a measurement or it was open or not properly closed right from the start.	1. Inspect the sealing cap and ensure that it was properly closed and audibly clicks into place. 2. If the above action is not successful then please contact **MACHEREY-NAGEL**.
Other error messages		Contact **MACHEREY-NAGEL**. Do not under any circumstances open the appliance and attempt to repair it yourself. This would result in you forfeiting all the guarantee claims that you potentially still have!
Flashing of the red warning light	Additional notice of all types of errors, e.g. **<Overload>**, **<Batt empty>**, **<STOP Open Door>**.	1. Register the error message in the display and rectify the error in accordance with the instructions in this table. 2. If the above action is not successful then please contact **MACHEREY-NAGEL**.

11. Technical specifications

Dimensions (H x W x D)	170 x 150 x 280 mm
Weight	2 kg (incl. batteries)
Mains power supply	230 V / 50 Hz, 115 V / 60 Hz
Power consumption	max. 35 VA
Batteries (rechargeable batteries)	3 rechargeable batteries: NiCd R14/C/Baby/UM2 batteries; 1600 mAh
Detector	Ultra Fast Single Photon Counter, Spectral wave range 380 – 630 nm
Sensitivity	10 fmol ATP when using „ATP Bioluminescence Assay Kit CLS II" of the Roche Diagnostics GmbH company, Mannheim, Germany
Dynamic range	More than 6 decades
Display	Backlit graphic display (128 x 64 dots)
Interface	RS232 interface for data transfer to the PC or printer
Software	Microprocessor software, 6 user-specific measurement protocols can be stored
Protocol types	Optionally <BioTox-S>, <BioTox-B> or <RLU>
Statement of results	Optionally % inhibition, % stimulation or relative light units (RLU)
Data storage	max. 2000 measurements
Measuring time	Can be freely selected in steps of 1 second between 1 and 999 seconds
Incubation time	Can be freely selected in steps of 1 minute between 1 and 39 minutes (only in the case of protocol types <BioTox-S> and <BioTox-B>)
User languages	Optionally German or English
Humidity	10 % to 90 % no condensation
Temperature range	+15 °C to +30 °C

RS232 Interface: Pin assignment

Pin 1		nc.
Pin 2	I	RxD
Pin 3	O	TxD
Pin 4		nc.
Pin 5	O	GND
Pin 6		nc.
Pin 7	I	RTS
Pin 8	O	CTS
Pin 9		nc.

12. Order information

Description	Cat. no.
Luminometer BioFix® *Lumi-10* Mobile measuring instrument for the implementation of bioluminescence tests (BioFix® *Lumi* luminous bacteria toxicity tests, Mutatox® Genotoxicity tests, ATP measurements, Reporter gene assays, DNA probe assays, luminescence immunoassays etc.) with integrated software for variable test evaluation and with 6 memory locations for individually programmable, customised measurement protocols.	940 008
Software NANOCOLOR® Data Export for simplified, selective data transfer to MS Excel or MS Access	919 02
BioFix® *Lumi* luminous bacteria, freeze-dried, 100 determinations/vial, 20 vials sufficient for up to 2000 toxicity measurements, including reconstitution solution	945 002
BioFix® *Lum* luminous bacteria, freeze-dried, 100 determinations/vial, 10 vials sufficient for up to 1000 toxicity measurements, including reconstitution solution	945 003
BioFix® *Lumi* luminous bacteria, freeze-dried, 20 determinations/vial, 20 vials sufficient for up to 400 toxicity measurements, including reactivation solution	945 006
BioFix® *Lumi* luminous bacteria, 20 determinations/vial, 10 vials sufficient for up to 200 toxicity measurements, including reactivation solution	945 007
BioFix® *Lumi* "Single-Shot" luminous bacteria, freeze-dried, for single measurements 20 vials for 20 toxicity measurements, including control and reactivation solution	945 021
BioFix® *Lumi* „Multi-Shot" luminous bacteria, freeze-dried, 20 determinations/vial, 10 vials for up to 100 toxicity measurements, including control and reactivation solution	945 022
Mutatox® luminous bacteria for genotoxicity tests, 5 vials sufficient for up to 500 genotoxicity tests	945 501.1
Mutatox® luminous bacteria for genotoxicity tests, 10 vials sufficient for up to 1000 genotoxicity tests	945 501
Mutatox®-medium, 10 x 15 ml	945 502
Mutatox®-S9-medium, 10 x 15 ml	945 503
BioFix® *Lumi* Diluent, 1 litre	945 601
BioFix® *Lumi* Osmotic Adjusting Solution, 50 ml	945 602
BioFix® *Lumi* Reconstitution solution, 1 litre	945 603
BioFix® *Lumi* Medium for freeze-dried luminous bacteria, 1 litre	945 608
Adsorptions correction cuvettes (colour correction cuvettes), 4 pcs. with aspirators	940 006
Glass cuvettes 50 x 12 mm, 672 pcs. per package	916 912
Cuvette rack, 15 x 10 wells	945 013
200 – 1000 µl digital piston pipette, can be set variably	916 77
1 – 5 ml digital piston pipette, can be set variably	916 909
Plastic tips for 200 – 1000 µl digital piston pipette, 100 pcs. per package	916 76
Plastic tips for 1 – 5 ml digital piston pipette, 100 pcs. per package	916 916

Technical consultation:

Our experts at **MACHEREY-NAGEL** will be happy to help if you have any problems with the instruments or if you have any queries and would like to obtain some more information about our **BioFix® *Lumi-10*** luminometer. Please contact:

MACHEREY-NAGEL GmbH & Co. KG, P. O. box 10 13 52, D-52313 Dueren, Germany

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Fax: +49 / (0) 24 21 / 969 – 199
e-mail: sales-de@mn-net.com