

Microbiological diagnostics and luminous bacteria test systems

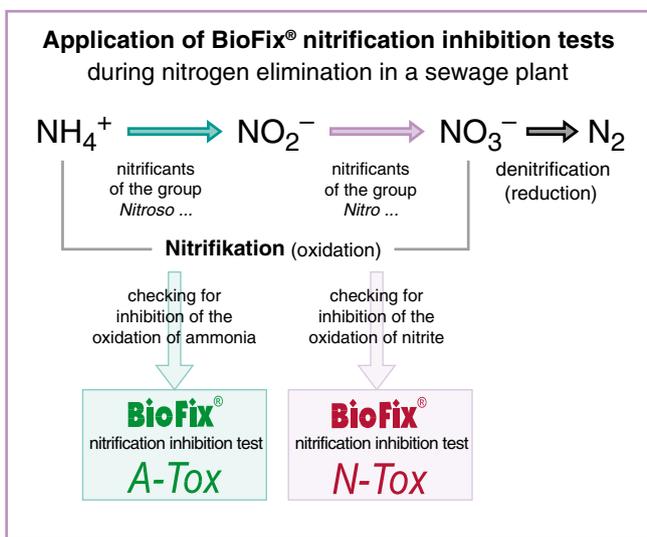
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Nitrification inhibition tests

BioFix® A-Tox / N-Tox

Does the biology in your sewage plant work properly?

During aerobic and anaerobic decomposition reactions nitrogen from nitrogen-containing organic substances is first converted to ammonium. The subsequent two-step microbial oxidation of ammonium to nitrate via nitrite is called nitrification. In soil as well as in water it is performed by nitrifying bacteria, a fact which is used for the purification of waste water in sewage plants. Nitrification is an important step during waste water purification in order to keep the concentration of ammonium ions in the effluents of the sewage plant as low as possible. Additionally, nitrification is prerequisite for the denitrification for complete nitrogen elimination, which is required for waste water treatment in many countries.



Proper nitrification and denitrification procedures are important for meeting governmental regulations concerning the nitrogen limits of a sewage plant. Nitrification is a very complex process which depends on many factors. Exceeding allowable limits may have drastic consequences on fees for waste water disposal.

Nitrificants or nitrifying bacteria belong to the group of gram-negative, chemolithotrophic, aerobic bacteria. One differentiates between the groups of ammonia oxidants and the group of nitrite oxidants. In the first step of the nitrification process the ammonia oxidants oxidize ammonium to nitrite in the presence of oxygen. In the second step the nitrite oxidants transform the nitrite to nitrate, again in the presence of oxygen.

Nitrifying bacteria are very sensitive to certain environmental influences. It is long known, that a number of substances can selectively inhibit nitrification. These substances can reach the sewage plant with the waste water from different sources and significantly, sometimes irreversibly, damage the population of nitrifying bacteria in the activated sludge.

Standard procedures for determination of the nitrification inhibition (e.g. DIN EN ISO 9509 – L38) are very time-consuming, and require lots of experience and work. For this reason it is seldom used in sewage plants, although knowledge about possible effects of sewage influx to the nitrifying bacteria of a sewage plant may help to maintain a proper cleaning process.

The solution for nitrification control: BioFix® nitrification inhibition A-Tox and N-Tox

With BioFix® nitrification inhibition tests you can now easily determine the nitrification inhibition in waste waters of all kinds as well as nitrification inhibition by individual substances or substance mixtures.

Principle: amperometric measurement of the oxygen consumption

As biomass these tests use nitrifying microorganisms which are typical for sewage plants, preferably *Nitrosomonas* and *Nitrobacter*. The bacterial strains are applied as inoculum for the test preparation in defined composition – concerning the precise bacterial strain as well as their concentration – either as pure cultures or as mixed cultures.

Measurement of the metabolic activity of the test organisms is performed with an apparatus for oxygen determination using a commercial oxygen electrode. The result is obtained as % inhibition of the oxygen consumption of the sample solution compared to a non-inhibited control.

BioFix® nitrification inhibition tests allow the following investigations:

- **BioFix® A-Tox:** direct test, whether the first step of the nitrification, the oxidation of ammonium, is inhibited by sample components.
- **BioFix® N-Tox:** direct test, whether the second step of the nitrification, the oxidation of nitrite, is inhibited by sample components.
- Undifferentiated screening test A/N-Tox using both BioFix® tests (A-Tox and N-Tox) to determine, whether nitrification in general is inhibited by sample components.

Please note: BioFix® A-Tox and N-Tox test kits require cooling during transport and storage!

Nitrification inhibition tests

BioFix® A-Tox / N-Tox

Advantages of BioFix® nitrification inhibition tests are:

- high sensitivity
- very good reproducibility due to defined bacterial strains used in defined concentrations
- easy procedure (by far less effort than for the DIN procedure)
- speed (considerable time-saving: test time 10 min; 4 hours for the DIN test)
- ready-to-use reagents
- reagents and preserved bacteria have a shelf life of at least 1 year under the temperatures indicated for storage
- free-of-charge disposal
- ability to differentiate between inhibition of the different steps of nitrification (ammonium and/or nitrite oxidation)
- Preserved nitrificants for application in accordance with DIN EN ISO 9509-L38



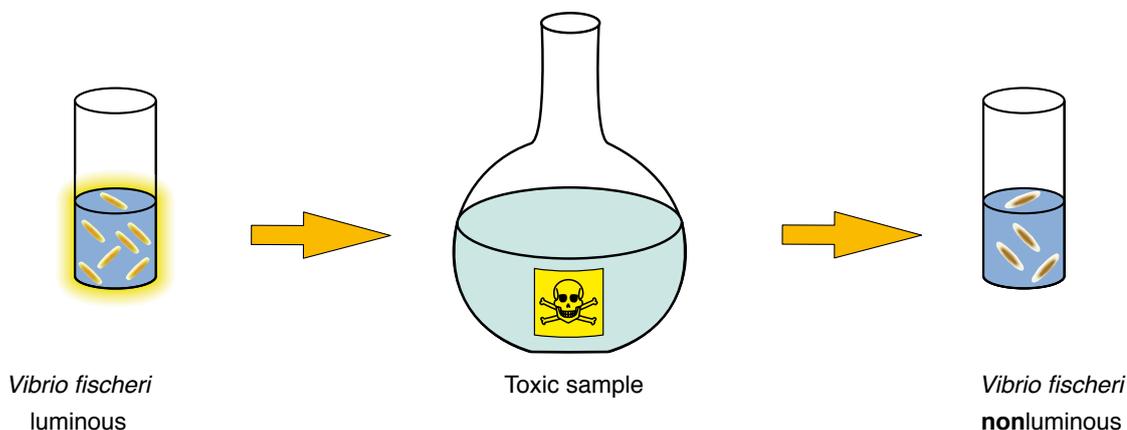
BioFix® nitrification inhibition tests · Ordering information

Type	Field of application	Evaluation of the biological conversion of	Number of tests / pack of	REF
A-Tox	1 st step of nitrification	ammonium to nitrite	10 – 19	970 001
N-Tox	2 nd step of nitrification	nitrite to nitrate	10 – 19	970 002
Starter kit for BioFix® nitrification inhibition tests :			1 Set	970 101
1 electrode adapter which holds the oxygen electrode, 3 x 2 seals for the electrode adapter, 2 mini-magnets for stirring, 1 micro syringe 100 µl, 1 filtration syringe 20 ml				
Preserved nitrificants for application in accordance with DIN EN ISO 9509 – L38				
Reagent BioFix® A-Tox R2, enriched nitrificants for oxidation of ammonia			10 x 2 ml	970 903
Reagent BioFix® N-Tox R2, enriched nitrificants for oxidation of nitrite			10 x 2 ml	970 902
Useful accessories				
CHROMAFIL® membrane filters, not sterilised, pore size 0.45 µ			50	916 52
Electrode adapter			1	970 111
Special adapter 12 mm for oxygen electrodes with membrane heads type WP3-ST			1	970 116
Seals for the electrode adapter			5 x 2	970 112
Reaction vessels			50	970 113
Magnetic stirring unit without heater			1	970 115
Mini-magnets for stirring			5	970 114

Luminous bacteria toxicity test systems

Determination of biotoxicity in accordance to EN ISO 11348

In contrast to chemical single parameter analysis, luminous bacteria tests are able to evaluate the toxicity of a sample. As an internationally standardised method (EN ISO 11348) it is one of the most important biological tests for toxicity analysis beside the also standardised fish-, daphnia- and algae-tests. For the performance of luminous bacteria tests a wide range of liquid- and freeze-dried products is available.



Reaction basis:

Luminous bacteria are used as test organisms because of their natural luminescence. In luminous bacteria tests, the marine bacterium *Vibrio fischeri* NRRL B-11177 is used, a halophile, facultative anaerobic, gram-negative rod with polar flagellum. Bioluminescence is a part of the metabolism of these bacteria. Toxic substances influence this sensitive metabolism and inhibit the bioluminescence.

The inhibition of the bacteria's bioluminescence is determined in a static test by mixing a defined amount of a sample with a luminous bacteria suspension in a cuvette. Subsequently, the inhibition of the luminescence in the sample is determined in comparison to an uninhibited control solution.

The evaluation of the toxicity analysis is performed with the BioFix® *Lumi-10*, a universal luminometer, which is suitable for portable use.

The test system BioFix® *Lumi* allows an easy and rapid toxicity control of environmental samples and offers significant advantages

- liquid and freeze dried luminous bacteria are available for determinations in accordance to DIN EN ISO 11348 part 2 and 3
- reliable and safe measurement results without high efforts
- trouble-free disposal
- suitable for ground-, surface-, seepage- and waste water
- determination of gene toxic substances

Luminous bacteria toxicity test systems

Luminometer BioFix® Lumi-10

Simple and mobile solution for bacteria toxicity tests, ATP- and biomass determinations, reporter gene assays, DNA probe assays

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

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

Modern and flexible

- Battery and mains operable
- High-resolution graphic display
- User language German or English
- Serial 9 PIN RS 232 interface for selective data transfer to a standard PC
- Variable measurement times in order to obtain optimum results even in case of weak luminescence

User-friendly

- Data memory for up to 2000 test results
- Selective data administration (calling/deleting) by means of the location identification digit, sample number, date, time parameters
- 6 individual programme places for user-defined measuring programmes
- Classification of test results by means of previously defined thresholds

Technical data:

Detector:	Ultra Fast Single Photon Counter, Spectral wave range 380 – 630 nm
Display:	illuminated graphic display (128 x 64 dots)
Data storage:	max. 2000 measurements
Humidity:	10 % to 90 % no condensation
Temperature range:	+15 °C to +30 °C
Software:	Microprocessor software, 6 user-specific measurement protocols can be stored
Interface:	RS 232 interface for data transfer to the PC or printer
Power supply:	3 rechargeable batteries: NiCd R14/C/ Baby/UM2 batteries; 2500 mAh or with mains power supply: 230 V / 50 Hz, 115 V / 60 Hz
Dimensions:	170 x 150 x 280 mm (H x W x D)
Weight:	2 kg (incl. batteries)
Warranty:	2 years
CE This device complies with the following directives: - 2006/95/EC - Low-Voltage Directive - 2004/108/EC - EMC Directive	



Test methods and measurement protocol:

A maximum of 6 different measurement protocols can be issued with an own name and stored with the protocol types and measurement parameters (incubation time, measuring time, limit values, test method, etc.).

The user can distinguish between the following 3 procedures:

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

Luminous bacteria tests with BioFix® Lumi-10:

Various different packaging sizes of liquid- and freeze-dried BioFix® Lumi are provided by MACHEREY-NAGEL. BioFix® Lumi Single-Shot luminous bacteria (REF 945 021) are especially suitable for single measurements. BioFix® Lumi Multi-Shot luminous bacteria (REF 945 022; freeze-dried or REF 945 025; liquid-dried) are especially suitable for multiple measurements during extensive routine control.

Luminous bacteria toxicity test systems

BioFix® Lumi

Ordering information

Description	REF
Luminometer	
BioFix® Lumi-10, mobile instrument for measuring BioFix® Lumi luminous bacteria toxicity tests, for ATP and biomass determinations, reporter gene assays, DNA probe assays and other bioluminescence tests, with integrated software for variable evaluation	940 008
BioFix® Lumi luminous bacteria, freeze-dried, in accordance with DIN EN ISO 11348-3*	
<i>All freeze-dried products are ready-to-use with a shelf life of at least 24 months after production when stored at -20 ± 2 °C. They are shipped in special cooling packages.</i>	
<i>All freeze-dried BioFix® Lumi luminous bacteria are supplied with a certificate of quality in accordance with DIN EN ISO 11348-3.</i>	
100 determinations per tube:	
20 tubes (1 ml), sufficient for up to 2000 toxicity tests, with reconstitution solution	945 002
10 tubes (1 ml), sufficient for up to 1000 toxicity tests, with reconstitution solution	945 003
20 determinations per tube:	
20 tubes, sufficient for up to 400 toxicity tests, with medium for freeze-dried luminescent bacteria	945 006
10 tubes, sufficient for up to 200 toxicity tests, with medium for freeze-dried luminescent bacteria	945 007
10 determinations per tube:	
BioFix® Lumi „Multi-Shot“ luminous bacteria, 10 tubes for up to 100 toxicity tests, with reactivation and control solution	945 022
2 determinations per tube:	
BioFix® Lumi „Single-Shot“ luminous bacteria, including the corresponding „Single-Shot“ reactivation and control solution; pack of 20 tubes for 40 toxicity tests	945 021
BioFix® Lumi luminous bacteria, liquid-dried, in accordance with DIN EN ISO 11348-2*	
<i>All liquid-dried products are ready-to-use with a shelf life of at least 24 months after production when stored at -20 ± 2 °C. They are shipped in special cooling packages.</i>	
<i>All liquid-dried BioFix® Lumi luminous bacteria are supplied with corresponding reactivation solution, sodium chloride standard solution and a certificate of quality in accordance with DIN EN ISO 11348-2.</i>	
20 determinations per tube:	
10 tubes, sufficient for up to 200 toxicity tests	945 023
20 tubes, sufficient for up to 400 toxicity tests	945 024
10 determinations per tube:	
10 tubes, sufficient for up to 100 toxicity tests	945 025
BioFix® Lumi auxiliary reagents	
BioFix® Lumi diluent, 1 x 1 litre	945 601
BioFix® Lumi osmotic adjusting solution, 1 x 50 ml	945 602
BioFix® Lumi reconstitution solution for REF 945 002 and 945 003, 1 x 1 litre	945 603
BioFix® Lumi diluent for solid phase test, 1 x 1 litre	945 604
BioFix® Lumi medium for freeze-dried luminous bacteria in accordance to DIN EN ISO 11348-3, 1 x 1 litre	945 608
Accessories for BioFix® Lumi luminous bacteria toxicity test systems	
Absorbance colour correction cuvettes (pack of 4) with 100 aspirators	940 006
Filter columns and filter tubes for solid phase test (100 each)	945 012
Glass cuvettes, 50 x 12 mm, pack of 672 916 912	916 912
Rack for glass cuvettes 12 mm diameter, 5 x 10 positions	945 013
Manual BioFix® Lumi-10, German	940 014
manual BioFix® Lumi-10, English	940 014.en
Other accessories on request	
*All freeze/liquid-dried BioFix® Lumi-10 luminous bacteria are also suited for luminometers of other manufacturers (e.g. LUMiStox, LUMiSmini of Dr. Lange, Germany)!	

Rapid tests for hygiene monitoring of surfaces and in liquid samples

With the BioFix® Lumi ATP tests and the Luminometer BioFix® Lumi-10 MACHEREY-NAGEL offers a complete system for professional hygienic control of surfaces and liquid samples.

Because of their high sensitivity and extreme easy handling, they are particularly suitable for hygiene monitoring within the scope of HACCP (Hazard Analysis and Critical Control Point) and IFS (International Food Standard) concepts.

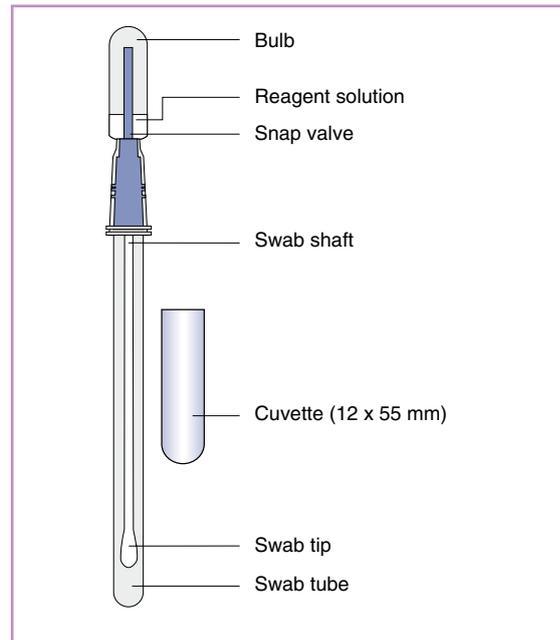
Furthermore cleaning processes can be controlled and optimised via the determination of adenosine triphosphate.

Milk, meat, fish, sausage, preserves, frozen foods, beverages etc: the production of food makes high demands on hygienic conditions. Each contamination leads to a loss of quality of the product or to a loss of time within the production. With the rapid determination of ATP, hygienic impurities on surfaces can easily be detected.

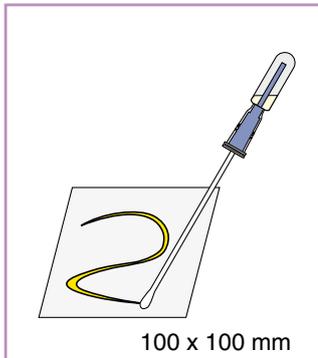
Impurities in boiler and cooling water circulations and other liquid samples can be monitored with BioFix® Lumi-10 ATP for liquid samples.

In addition, with the Luminometer BioFix® Lumi-10 the user can create individual measurement reports and define cut-off values for an optimal adaption to the requirements of the respective companies.

Specifications of a BioFix® ATP pen

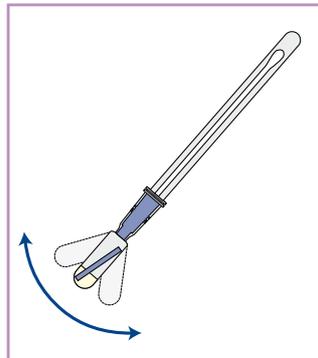


Procedure for hygiene monitoring of surfaces:



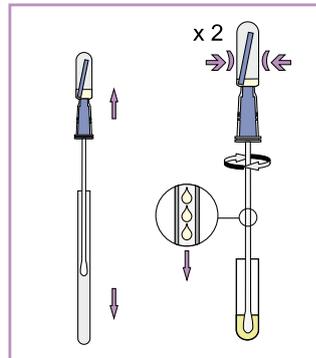
Sampling:

1. Remove swab tip from the swab tube.
2. Wipe over surface (approx. 10 x 10 cm).
3. Place swab tip back into the swab tube.



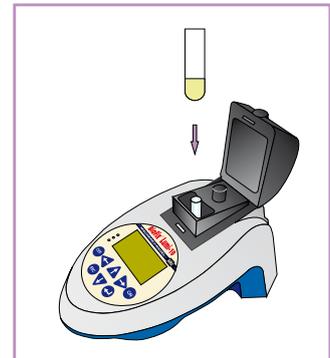
Test activation:

1. Turn BioFix® ATP pen on its head.
2. Bend the bulb of the ATP pen with the index finger and thumb until the blue valve pin breaks.



Reaction:

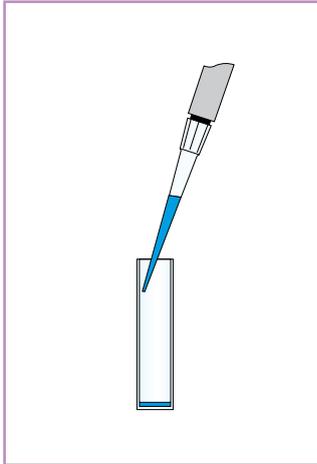
1. Carefully take the swab tip out of the swab tube and place at the bottom of a cuvette (12 x 55 mm).
2. Press the bulb of the ATP pen together.
3. Stir the swab tip in this solution for about 10 sec to release any remaining sample.
4. Squeeze the swab tip against the side of the cuvette and remove.



Measurement:

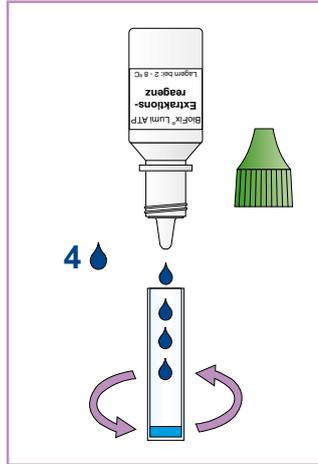
Put the cuvette in the cuvette shaft of a luminometer, close and start measurement within 2 min after test activation.

Procedure for monitoring of hygienic impurities and biological contaminations in liquid samples:



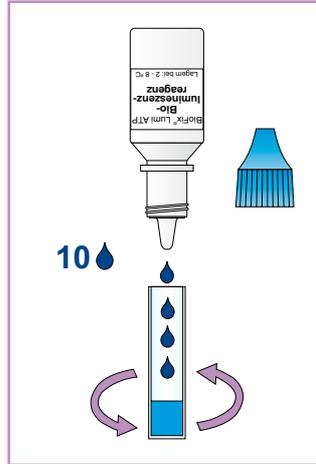
Sampling:

Add 0.1 ml sample solution to a clean disposable cuvette.



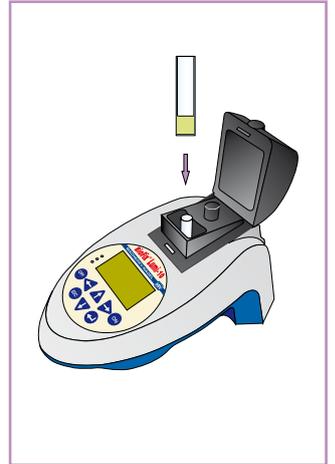
Extraction:

Add 4 drops BioFix® Lumi ATP extraction reagent and mix. Wait 60 sec.



Reaction:

Add 10 drops BioFix® Lumi ATP bioluminescent reagent and mix. Wait 30 sec.



Interpretation of the test:

- Sample clean
- Sample possibly contaminated
- Sample not clean

Ordering information

Type	Description	REF
Test kits for hygiene monitoring of surfaces with the luminometer BioFix® Lumi-10		
Content of these test kits: BioFix® ATP pens, disposable cuvettes 12 x 55 mm, instruction leaflet		
BioFix® Lumi ATP	rapid test for 25 determinations	946 001
BioFix® Lumi ATP	rapid test for 50 determinations	946 002
Test kits for hygiene monitoring of surfaces with the luminometer HY-LiTE® of Merck		
Content of these test kits: BioFix® ATP pens, disposable cuvettes 12 x 55 mm, instruction leaflet		
BioFix® Lumi ATP	rapid test for 25 determinations	946 011
BioFix® Lumi ATP	rapid test for 50 determinations	946 012
Test kits for monitoring of hygienic impurities and biological contaminations in liquid samples		
Content of this test kit: BioFix® Lumi ATP extraction reagent and bioluminescent reagent, disposable cuvettes 12 x 50 mm, instruction leaflet		
BioFix® Lumi ATP	rapid test for 100 determinations	946 006