BACTERIAL CONTAMINATION SCREENING KIT

BENCH PROTOCOL

Principle and features

The Bacterial Contamination Screening Kit is a "field test kit" for ultra-rapid gross screening of water contamination by microbes and biological residues through measurement of the amount of ATP (Adenosine TriPhosphate) which is the energy source of all living organisms. ATP in water can be measured by chemical luminescence through a luciferin/luciferase reaction with a specific chemical reagent. The techniques are applicable to any kind of freshwater (and the membrane filter procedure can even be applied to seawater), but they do not give information on the presence or absence of pathogenic microbes.

The tests can be performed at ambient temperature within the range 15°C to 25°C.

The test is performed in tubes containing ATP reagents, with direct measurement of the number of Relative Light Units (RLU) in a portable luminometer.

Tubes with ATP reagents

The Bacterial Contamination Screening kit contains 10 Unit boxes with tubes with ATP reagents, for analysis of two water samples each. The tubes are composed of an upper compartment containing a liquid ATP extractant and a bottom compartment containing a solid ATP reagent. The tubes must be stored in the refrigerator (2°C-8°C) prior to use, and have a shelf life of 6 months to 1 year. They are used in conjunction with the equipment and materials contained in the "Luminescence Measurements Case" and allow to perform two distinct types of analyses :

- a direct test which measures the "total amount of ATP" in the water sample, i.e. the "intracellular" ATP contained in the living bacteria and other biota and biological residues present in the water sample, and the "extracellular" ATP from biological residues
- a "membrane filter" test which only measures the ATP from the living bacteria after prior elimination of all extracellular and intracellular ATP from other organisms and biological residues.

N.B. The Luminescence Measurement Case also contains specific items to be used with the "Toxi-Screening Kit" for rapid "in situ" evaluation of the toxicity of suspect waters.

Direct test

1. With the aid of the plastic pincette, tear open the top cover of the tube with reagents and remove this cover completely.
2. Put one of the 2 finntips on the 200 µl Finpipette, take a 200 µl sample of the suspect water and transfer it into the chamber containing the liquid ATP extractant.
3. Mix the water sample with the ATP extractant, by repeatedly sucking up and pushing back the solution in the top chamber, with the same Finpipette.
4. After about 1 minute, suck up again 200 µl of the mixture, and slowly push the tip of the finntip further down in the tube, through the partition separating the two compartments.
5. Empty the contents of the finntip into the bottom chamber which contains the solid ATP reagent, and transfer step by step (with the same Finpipette) the entire solution of the upper chamber into the lower chamber.

N.B. It is imperative for a reliable measurement that the total volume of water sample + extractant is transferred to the bottom compartment of the tube !

6. Adjust one of the two transparent holders tightly to the tube and swirl the holder + tube gently to mix the water + extractant with the solid ATP reagent, till the latter is dissolved completely .
7. Take the luminometer , switch it on by pushing the "power" button and wait till the instrument has calibrated itself (a 10 seconds countdown visible on the display, followed by a beep signal).
8. Open the lid of the luminometer and insert the holder + tube.

N.B. : The luminometer must always be kept vertically when containing a holder + tube !!

9. Close the lid of the luminometer, wait for about 2 minutes prior to push the "Enter" button and wait again for the 10 seconds countdown to see the luminescence score on the display.
10. Remove the holder + tube from the luminometer and switch it off by pushing the "power" button.

N.B.1. When a holder + tube is kept for more than 30 seconds in the luminometer, the instrument will give beep signals indicating that the holder + tube must be removed.

N.B.2. ATP degrades rapidly and luminescence scorings must always be performed within a few minutes after introduction of the water sample in the tube with ATP reagents !

A second water sample can be analysed the same way with the second tube with ATP reagents and the second finntip.

Evaluation of the degree of contamination of water samples by bacteria and biological residues

A gross estimation of the degree of contamination of the water sample can be made as follows :

< 50 RLU	: very low contamination
< 200 RLU	: low contamination
200-1000 RLU	: significant contamination
> 1000 RLU	: very high contamination

Membrane filter test

A second test procedure can be applied for specific analysis of the ATP from the bacteria present in the suspect sample, after elimination of all other (interfering) ATP. The procedure is based on the capturing of the bacteria on a small 0.20 µm membrane filter, prior to extraction of their intracellular ATP.

1. Unscrew the 2 parts of the two filter holders and place them in the holes on the top left side of the Luminescence Measurement Case.

2. With the aid of the plastic pincette, take a prefilter from the small box containing the (10 µm) nylon gauze prefilters, place it in the hollow compartment of the first filter holder and rescrew tightly the 2 parts of the filter holder.
3. Proceed the same way for placing a 0.2 µm membrane filter (from the small box with membrane filters) in the second filter holder.

N.B. Don't touch the membrane filter with the fingers during these operations to avoid contamination of the filter by ATP (always) present on our skin !

4 Connect the filter holder containing the prefilter to the filter holder containing the membrane filter, by inserting the tip of the former into the Luer-opening of the latter.
5. Take the 10 ml syringe and fill it with a (preselected) volume of water sample.

N.B. The volume of water to be used is dependent of two factors :

a) the amount of "suspended solids" in the water, which may clog the prefilter (even after filtration of only a few ml water sample)

b) the degree of microbial contamination of the water which, in case of very low bacterial numbers, will necessitate to flush more than 10 ml water sample through the membrane filter, to obtain "a meaningful" RLU luminescence figure.

Determination of the most appropriate volume of water sample may therefore necessitate a few consecutive trials and measurements.

6. Insert the tip of the syringe in the Luer opening of the filter holder with the prefilter and fit it tightly in the opening.
7. Flush the total volume of water in the syringe through the connected filter holders.
8. Repeat this operation with additional volumes of water sample if appropriate
9. Remove the syringe from the connected filter holders and pull the plunger upwards to fill the syringe with air.
10. Connect the syringe again to the filter holders and push the air through the filter holders to eliminate all remaining water.
11. With the aid of the pincette, tear the top cover of the tube with the ATP reagents open and remove this cover completely.
12. Disconnect the 2 filter holders, take the membrane filter from the first filter holder with the aid of the pincette and insert it in the upper compartment of the tube containing the ATP extractant.

Make sure that the entire surface of the membrane filter is in good contact with the liquid ATP extractant and move the filter around with the plastic pincette to ensure a maximum contact of the extractant with the bacteria on the membrane filter.

13. After about 1 minute, remove the membrane filter from the tube with the aid of the pincette, taking care during this operation not to eliminate part of the extractant.
14. Put a finntip on the 200 µl Finpipette and insert the tip into the compartment with the ATP extractant.
15. Proceed further as indicated in steps 4 to 10 of the "Direct test" outlined above.

At the end of the test, take out the prefilter from the second filter holder and discard it.

Evaluation of the degree of microbial contamination of water samples

The RLU scores are first recalculated on the basis of "1 ml" water sample, by dividing the RLU figure by the number of ml water flushed through the filter.

A gross estimation of the amount of bacteria present in 1 ml of the analysed water can be made as follows :

< 200 RLU :	only a few bacteria	-
< 1000 RLU :	relatively low numbers of bacteria	+
> 1000 RLU :	a significant number of bacteria	++
> 5000 RLU :	high microbial contamination	+++
> 10.000 RLU :	very high microbial contamination	++++

Determination of microbial contamination of marine waters

The Bacterial Contamination Screening Kit can also be used for the evaluation of the degree of microbial contamination of estuarine/marine waters by marine as well as by freshwater bacteria. Salinity, however, interferes with the ATP reactions, so only the membrane filter method can be applied in combination with an extra step to eliminate the salts remaining on the filter after flushing the water sample through the 2 filter holders..

The marine membrane filter procedure - like its freshwater counterpart - thus also only measures the "intracellular" microbial ATP from the contaminating bacteria, after elimination of all other "interfering" ATP.

The testing procedure is similar to that detailed above for the membrane filter method, and shall be applied by first following steps 1 to 10 of the latter procedure. The syringe is subsequently filled with "sterile" freshwater to be flushed through the filter holder containing the membrane filter to eliminate the salts. The procedure is then continued from steps 11 to 15 of the membrane filter method detailed above.

Verification step

The degree of microbial contamination of a water detected in a few minutes by the former two screening methods, can subsequently be verified by a simple "plate count " procedure on small discs containing a bacterial nutrient agar. The inoculation of a "Verification"disc is carried out in the field by simply hydrating the nutrient agar in the disc with 1 ml water sample, followed by incubation of the disc in the laboratory either at room temperature (several days) or at 35°C (24h). The growth of the bacteria eventually leads to tiny red colonies which are clearly visible on the disc. The number of microbial colonies (Colony Forming Units = CFU) is representative for the degree of bacterial contamination of the water.

Decontamination of the syringe and the membrane filter holder subsequent to application of the membrane filter procedure

In order to avoid cross-contamination from one water sample to the other after application of with the membrane filter method, the syringe and the filter holders must be "decontaminated" after each test. This decontamination can be carried out in the field with the aid of disinfecting alcohol and sterile water provided in 2 bottles.

First 5 ml disinfecting alcohol is sucked up in the syringe and flushed through the connected filter holders. This operation is then repeated with the sterile water to eliminate all traces of disinfecting alcohol.