

TECHNICAL SHEET

***qualyfast*[®] free DNA inactivator**

kit for elimination of free DNA contaminating water samples

INTENDED USE

qualyfast® free DNA inactivator kit is a fast product for a pre-treatment of samples in order to digest contaminating DNA from water samples. The kit is intended for use with DNA extraction kit I (Bioside) for the Legionella DNA extraction from clear water. During the process, a major problem is the potential presence of dead cells. Using **qualyfast® free DNA inactivator**, kit it enables the detection only of DNA present in intact living Legionella cells without the need of complicated instruments and/or protocols.

PRINCIPLE

The elimination of free DNA is performed by the combined action of a specific enzyme and optimized buffers. The kit starts with the filtration of a water sample onto a filter membrane. Bioside filter membranes (DNA extraction kit I) are sterile, disposable, easy to use and very suitable for the recovery of Legionellae. Bioside enzyme and buffers are then added to the membrane in order to remove free DNA and to maintain only viable cellular DNA.

MATERIALS PROVIDED

The provided quantity of reagents allows to carry out 200 reactions as indicated in the table below.

Catalog Number	Number of reactions	Content
D4.01009	200	1 DE amber vial (lyophilized) 1 DR tube 1 DBI bottle 1 DBII bottle

MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile forceps (rather disposable)
- 1,5 ml microcentrifuge capable of at least 10000 x g
- Nuclease-free 1,5 ml microcentrifuge tubes
- Drybath or water bath capable of 37 °C, 65 °C and 95 °C
- Vortex
- Biological cabinet
- Filtered tips (aerosol barrier)
- Pipettor (capacity 2-20 µl, 20-200 µl, 200-1000 µl)
- Disposable gloves, powderless
- Disposable, reusable filter funnels
- Vacuum filtration systems

- Freezer
- DNA Extraction kit I (Bioside)

ANALYSIS PROCEDURE

Before first use of the kit, add 550 µl of DR buffer to the DE vial and incubate for 1 minute at room temperature. Gently swirl the vial to completely dissolve the enzyme. Be careful not to mix DE vigorously. DE is sensitive to mechanical agitation. Dispense into aliquots and store at temperature equal or inferior of -20 °C.

1. Filter clear water sample using filter membrane provided with DNA extraction kit I in a reusable or disposable filter funnels attached to a vacuum source; after filtering remove the filter from the adapter and place it in a clean 1,5 ml centrifuge tube;
2. Add 2 µl of DNA Enzyme mix (DE), 20 µl of DNA Buffer I (DBI) and 200 µl of DNA Buffer II (DBII) to membrane filter;
3. Add 4 µl of Bioside Enzyme mix (BE) (DNA extraction kit I);
4. Vortex at maximum speed for 5 minutes;
5. Check that the membrane filter is totally immersed in solution and incubate 10 minutes at 37° C and 15 minutes at 65° C;
6. Add 200 µl of Bioside Buffer (BB) (DNA extraction kit I) (vortex 1 min. Bioside Buffer before the use);
7. Incubate 15 minutes at 95° C;
8. Centrifuge at 10000 x g for 1 minute at room temperature;
9. Transfer the supernatant into a clean 1.5 ml tube, leaving the membrane filter.

The DNA extracted can be stored at -20 °C.

It is recommended to use the DNA extracted immediately after thawing.

It is recommended to dilute the samples 3, 10 or 100 fold before the use in real time PCR. For clear water, we recommend a dilution 1:3 because the extracted DNA is very concentrated and using undiluted DNA, it could give inhibition. For more corpusculated waters, we recommend 1:10, while we recommend the dilution 1:100 only if there is inhibition referred to the previous cases.

WARNINGS AND PRECAUTIONS

For professional use only.

Product is not intended for human, animal or therapeutic use but for laboratory and/or research use.

DE amber vial contains hazardous contents. Wear gloves and goggles.

All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately.

Aseptic technique and usual precautions for handling the bacterial group studied should be observed.

Do not use the reagents behind expiry date.

No warranty is guaranteed for products beyond their listed expiry date. No warranty is applicable unless all product components are stored in accordance with instructions for use.

Bioside offers to customers technical support and training.

STORAGE AND SHELF-LIFE

Store the product in its box until the expiry date indicated in the label. Store the kit at 2-8 °C on arrival. The frozen working solutions of DE are stable for 6 months from the reconstitution date at temperature equal or inferior to -20 °C. Do not freeze/thaw the aliquots more than three times.

SPECIFICATIONS

qualyfast® free DNA inactivator kit reduces the amplification signal from free DNA of 3 log.

The kit contains high quality enzyme and buffers in order to remove even low traces of contaminating DNA.

An evaluation of the performance of the kit was done with samples spiked with different concentrations (high, medium and low) of free DNA and different concentrations (high, medium and low) of live *Legionella* spp. or *Legionella pneumophila* cells.

REFERENCES

- Anonymous. NF T90-471 Qualité de l'eau - Détection et quantification des Legionella et/ou Legionella pneumophila par concentration et amplification génique par réaction de polymérisation en chaîne en temps réel (RT - PCR). Association française de normalization; 2010.
- Anonymous. ISO/TS 12869, «Water quality e detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR)». International Organization for Standardization; 2012.
- Anonymous. NF148 - NF Mark VALIDATION Validation protocol for commercial methods of detection and quantification of Legionella and Legionella pneumophila by concentration and gene amplification by polymerase chain reaction (PCR). Revision 2 – Adopted by AFNOR Certification on 27 May; 2013.

PACKAGING SYMBOL



Catalogue number



Batch code



Temperature limitation



Use by date



Manufacturer