BIOSIDE

TECHNICAL SHEET

qualyfast® DNA Extraction kit II

DNA Extraction kit from dirty water and sediment

Via Albert Einstein, snc
Località Cascina Codazza
c/o Parco Tecnologico Padano
26900 Lodi (LO) ITALY

www.bioside.it

INTENDED USE

***qualyfast*® DNA Extraction kit II** product is a DNA extraction kit for dirty water samples and sediments. This kit has been specifically designed for the extraction of DNA from bacteria (e.g. Legionellae), fungi and protozoa. Extracted DNA can be directly used in downstream applications, including PCR and qPCR, without the need for further purification. For guidelines to the sampling water procedure please refer to the relevant ISO norms.

PRINCIPLE

The kit starts with the filtration of a water sample onto a filter membrane. Bioside filter membranes are sterile, disposable, easy to use and very suitable for the recovery of Legionellae.
Fast and efficient lysis is performed by bead beating in a unique lysis buffer. The purification of DNA is performed by special filtration columns in order to obtain inhibitor-free (e.g. humic acid) DNA.

MATERIALS PROVIDED

- 0,4 µm – 47 mm Ø microporous filter membranes
- Bead beating lysis tubes
- Lysis solution
- Orange Spin Filter s
- Collection tubes
- DNA Binding Buffer
- Clear Columns and Clear Columns II
- Buffer I and Buffer II
- DNA Elution Buffer

The provided quantity of reagents allows to carry out 48 or 96 reactions as indicated in the table below.

Catalog Number	Number of reactions	Content
D2.11001	48	2 packs of 24 filter membranes 48 Bead beating lysis tubes 1 bottle Pre-Lysis Buffer (40 ml) 48 Orange Spin Filter 192 Collection tubes 1 bottle Lysis Buffer (100 ml) 48 Clear Columns 48 Clear Columns II 1 bottle Buffer I (15 ml) 1 bottle Buffer II (50 ml) 1 bottle DNA Elution Buffer (10 ml)

		1 bottle Prep Buffer (30 ml)
D3.11001	96	4 packs of 24 filter membranes 2 packs of 48 Bead beating lysis tubes 2 bottle Pre-Lysis Buffer (40 ml) 2 packs of 48 Orange Spin Filters 2 packs of 192 Collection tubes 1 bottle Lysis Buffer (100 ml) 2 packs of 48 Clear Columns 2 bottle Buffer I (15 ml) 2 bottle Buffer II (50 ml) 2 bottle DNA Elution Buffer (10 ml) 2 bottle Prep Buffer (30 ml) 2 packs of 48 Columns II

MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile forceps (rather disposable)
- 1,5 ml microcentrifuge capable of at least 16000 x g
- Nuclease-free 1,5 ml microcentrifuge tubes
- Vortex or Cell Disruptor mixer with a 2 ml tube holder
- Biological cabinet
- Filtered tips (aerosol barrier)
- Pipettor (capacity 100-1000 µl, 20-200 µl)
- Disposable gloves, powderless
- Disposable, reusable filter funnels
- Vacuum filtration systems
- Freezer

ANALYSIS PROCEDURE

Protocol:

1. Filter the water sample using filter membrane provided (0,4 µm) in a reusable or disposable filter funnels attached to a vacuum source; after filtering remove the filter from the adapter and place it in the **Bead beating lysis tube**;
2. Add 750 µl **Pre-Lysis Buffer** (mix before use) to the tube;
3. Vortex at maximum speed for 5 minutes;
4. Centrifuge at 10000 x g for 1 minute at room temperature;
5. Transfer from 200 to 300 µl of supernatant to the **Orange Spin Filter** in a Collection Tube and centrifuge at 8000 x g for 1 minute;

6. Add 600 µl of **Lysis Buffer** to the filtrate in the Collection Tube from Step 5;
7. Transfer 800 µl of the mixture from Step 6 to a **Clear Column** in a Collection Tube and centrifuge at 10000 x g for 1 minute:
8. Discard the flow through from the Collection Tube;
9. Add 200 µl **Buffer I** to the Clear Column in a new Collection Tube and centrifuge at 10000 x g for 1 minute;
10. Add 500 µl **Buffer II** to the Clear Column and centrifuge at 10000 x g for 1 minute;
11. Transfer the Clear Column to a clean 1.5 ml microcentrifuge tube and add 50 µl **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10000 x g for 30 seconds to elute the DNA;
12. Place **Clear Column II** into a Collection Tube. Add 600 µl of **Prep Buffer** and centrifuge at 8000 x g for 3 minutes;
13. Transfer the eluted DNA to a prepared **Clear Column II** in a clean 1.5 ml microcentrifuge tube and centrifuge at 16000 x g for 3 minutes. The filtered DNA is now suitable for PCR and other downstream applications.

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

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

In Inhibition case it is possible to dilute the samples 3, 10 or, at maximum 100 fold before the use in real time PCR.

WARNINGS AND PRECAUTIONS

For professional use only.

Products are not intended for human, animal or therapeutic use but for laboratory, diagnostic and/or research.

These products do not contain any dangerous substances in concentrations >1%.

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 his custumers technical support and training.

STORAGE AND SHELF-LIFE

The kit is ready-to-use.

Store the products in its box at room temperature until the expiry date indicate in the label.

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
	Consult instructions for use
	Caution, consult accompanying documents
	Temperature limitation
	Keep away from sunlight
	Keep dry, keep away from rain
	Use by date
	Manufacturer
	Do not use if package is damaged
	This way up