32-1_052012 (en)

Order No. 32-1003/-1010/-1050

AquaScreen® *FastExtract* (FX)

DNA Extraction Kit for Water Samples

version 1.0

Instructions for Use

Reagents for 3/10/50 extractions

Manufacturer:

Minerva Biolabs GmbH, Koepenicker Str. 325, 12555 Berlin, Germany

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Store at

Contains reagents for 3, 10 or 50 extractions

Manufacturer

INTENDED USE

AquaScreen® is an *in vitro* test system for quantitative detection of water pathogens. The AquaScreen® *Fast-Extract* (FX) kit can be used for the isolation of genomic DNA from water samples. The kit was especially designed to fit Minerva Biolabs AquaScreen® PCR kits and to provide best performance in means of sensitivity and robustness for the detection of legionella and other bacteria within the water sample.

PRINCIPLE OF THE METHODE

The flexibility of the test system allows for a variety of water sources to be tested. AquaScreen® utilizes standard 0.45 μm membranes used routinely with filtration systems, the lysis of the precipitated microorganisms directly on the filtration membrane, the DNA extraction as well as the purification and elution of the DNA in minimal volumes under conditions which are optimal for the subsequent PCR analysis. Cells are lysed by a combination of detergents and chaotropic salt. The lysate is directly applied onto the spin columns. The DNA is selectively bound to the highly specified silica membrane. Two subsequent washes remove residual contaminants, like proteins, metabolites, dyes, detergent etc. The purified DNA is eluted in TE buffer. The procedure is completed in 30 min. The DNA is ready-to-use for PCR.

REAGENTS

Each kit contains reagents for 3, 10 or 50 extractions. The expiry date of the unopened package is marked on the package label. The kit components are stored until use at room temperature (18 to 25 °C). The lot specific quality control certificate (*Certificate of Analysis*) can be downloaded from our website (www.minerva-biolabs.com).

Kit component Label information	3 extractions Order No. 32-1003	10 extractions Order Nr. 32-1010	50 extractions Order Nr. 32-1050
Membrane Filter	3	10	50
Incubation Dishes	3	10	50
Incubation Tubes	3	10	50
Spin Columns	3	10	50
Collection Tubes	6	20	100
Sample Storage Tubes	3	10	50
Lysis Buffer	6.5 ml	22 ml	110 ml
Wash Buffer 1*	3 ml add 1.7 ml ethanol	2.7 ml add 3.3 ml ethanol	13.5 ml add 16.5 ml ethanol
Wash Buffer 2†	3 ml add 2.2 ml ethanol	1.7 ml add 4.3 ml ethanol	8.0 ml add 21.5 ml ethanol
Elution Buffer	1x 1 ml	1x 1 ml	4x 1 ml

(*) Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach.

(†) Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Kit components are stored at room temperature. By following these recommendations, the kit is stable until the expiration date. Following the initial use, the reagents are to be used within 2 months. Prior to first use, add the above mentioned volumes of ethanol (96-100 %) to Wash Buffer 1 and 2. Bring the reagents to room temperature (+18 °C to +25 °C) prior to use. Dissolve possible precipitate in the Lysis Buffer by heating. Buffer may be heated up to 70 °C without loss of quality.

NEEDED, BUT NOT INCLUDED IN THE KIT

The AquaScreen® *FastExtract* (FX) kit contains special reagents for the extraction of genomic DNA from water samples. General industrial supplies and reagents, usually available in a molecular biology laboratory are not included:

- Equipment for water filtration with 47 or 55 mm filters (manifold, pump, frit, tubing, etc.; Please see our Technical Note for recommendations.)
- Ethanol > 96 % abs.
- Micro centrifuge and heat block (70 °C) for 1.5 ml reaction tubes
- Micro pipettes and filtered tips (10, 100 and 1000 µl)
- Incubator (37 °C for petri dishes)

PRECAUTIONS

MB DNA Extraction Kit is intended for research use only. Not for clinical diagnostics or testing of human samples without extensive validation.

This kit should be used only by trained persons.

All samples should be considered potentially infectious and handled according to local or national regulations.

This kit substances may be disposed of according to local regulations.

Always wear a suitable lab coat, disposable gloves, and protective goggles. The sample-preparation waste contains Wash Buffer 1 and 2, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water.

The risk phrases applying to Wash Buffer 1 and Wash Buffer 2 are:

R 22 Harmful if swallowed.

R 36/38 Irritating to eyes and skin.

R 52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

The risk and safety phrases applying are:

S 13 Keep away from food, drink and animal feed.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36 Wear suitable protective clothing.

S 46 If swallowed, seek medical advice immediately and show container or label.

PROCEDURE

Preparation of Sample Material

Drinking water, bathing or pool water and waste water released from suspended particles can be used as sample materials. The sample conditioning affects the reliability of the test findings and must be in accordance with the guidelines of the ISO Norm 11731. For additional guidelines pertaining to the water sampling procedure, please refer to ISO Norm 5667-1 to 5667-10 or contact your national environmental agency. The water samples should be kept at room temperature, and tested within two days of collection. Preservation with chemical means is not possible as intact microorganisms are needed for the filtration procedure. Water samples contaminated with suspended particles or fixed volatile contents have to be purified by filtration with a folded paper filter. The samples may not be centrifuged for purification. For the testing procedure, at least 100 ml is minimally required, however a sample volume of 1000 ml is recommended. Free DNA of already lysed microorganisms in the sample passes through the membrane filter and is not detectable in the test system. The test does not differentiate between “viable and cultivable” and “viable but not cultivable” (VBNC) legionella.

Filtration of the Water Sample

1.	After carefully removing a membrane filter from the packaging moisten the filter for a few seconds using the sample material.
2.	Insert the membrane filter into the filtration system and filtrate the amount of water required.

Lysis

Note: Dissolve possible precipitate in the Lysis Buffer by heating it up to 70 °C if necessary.

1.	Transfer the membrane upside down into the incubation disks.
2.	Pipette **2 ml** of prewarmed **Lysis Buffer**.
3.	Submerge the membrane in the Lysis Buffer by carefully rocking the dish and incubate at 37°C for 30 min.
4.	Rinse the membrane and homogenize the lysis buffer by rocking the dish for 10 seconds.
5.	Pipette 500 µl of the lysate into the Incubation Tube and incubate for 15 min at 56 °C.
6.	Add **250 µl of ethanol** and vortex 5 sec.

DNA Isolation

Reconstitute Wash Buffer 1 and Wash Buffer 2 with absolute ethanol as stated on the bottle label.
Pre-heat Elution Buffer to 70 °C.

1.	Take one Spin Column per sample from the kit and place it into a Collection Tube. Mark the sample identification on the lit of the spin column and fill **700 µl of the sample lysate** into the Spin Column without moistening the rim of the Spin Column.
2.	Centrifuge the system for 1 min at 10,600 x g (approx. 10,000 rpm with a micro centrifuge). Discard the flow through from the Collection Tube and reassemble the Spin Column and the Collection Tube.
	Optional: For highest sensitivity this process can be repeated with the remaining lysate until all liquid is retrieved from the membrane. Please note that in the calculations the increased sample volume needs to be considered accordingly.
3.	Add **500 µl of Wash Buffer 1**. Centrifuge the system for 1 min at 10,600 x g (approx. 10,000 rpm with a desk centrifuge), discard the flow through and re-assemble the Spin Column.
4.	Fill the spin column with **500 µl of Wash Buffer 2**. Centrifuge the system for 1 min at 10,600 rpm (10,000 x g), take the Spin Column out of the Collection Tube and discard the Collection Tube with the containing Wash Buffer 2. Reassemble the Spin Column using a fresh Collection Tube.
5.	Centrifuge for 1 min at full speed (approx. 13,200 rpm) in order to remove the remaining Wash Buffer 2.
6.	Discard the collection tube containing the Wash Buffer 2 and place the spin column into a Sample Storage Tube.
7.	Pipette **60 µl of pre-warmed elution buffer** (70 °C) into the Spin Column directly onto the center of the silica membrane. The complete membrane should be covered with Elution Buffer. Secure the Sample Storage Tube and incubate for 5 min at room temperature.
8.	Following the incubation, centrifuge the system for 2 min at 10,600 rpm (10,000 x g).
9.	Remove the Spin Column and use the eluate directly for the PCR procedure. The extract is stable for approximately 1 week at +2 °C to +8 °C and can be kept at -18 °C for long term storage.

Calculations

With the use of quantitative real-time PCR and a standard curve generated with the titrated DNA standard (Cat. No. 52-0101), the quantity of legionella particles in the extract and subsequently in the sample volume can be determined. If a 10 µl test volume is used, then the total quantity of genomic equivalents present in the water sample can be calculated:

$$\text{genomes/PCR} \times 25{,}71 \times \frac{1000}{\text{sample volume in [ml]}} = \text{legionella particles per liter}$$

Example: A 500 ml water sample is used for the analysis and extracted in 60 µl. On the basis of the real-time PCR, a result of 30 DNA copies is determined using a test volume of 10 µl.

-> 30 genomes/PCR x 25,71 x 2 = 1542 legionella particles per liter

500 ml sample water contained 771 intact legionella particles (viable cultivable, viable but not cultivable (VBNC), and dead but still intact legionella). That results in 154 legionella particles per 100 ml of water.

NOTES ON THE PROCEDURE

1. This leaflet must be widely understood for a successful use of AquaScreen® *FastExtract* (FX) kit. The reagents supplied should not be mixed with reagents from different lots and used as an integral unit. The reagents of the kit should not be used beyond its shelf life.
2. Any deviation from the extraction method can affect the results.
3. For each setup, the use of control samples is advised to secure the day-to-day validity of results. at The spike with an Internal Amplification Control facilitate the evaluation of the extraction.
4. Do not use other alcohols than ethanol, because other alcohols may cause inconsistent yields.
5. Pre-heating of Elution Buffer increases the yield drastically.
6. The extract is stable for approximately 1 week at +2 °C to +8 °C and can be kept at -18 °C for long term storage.

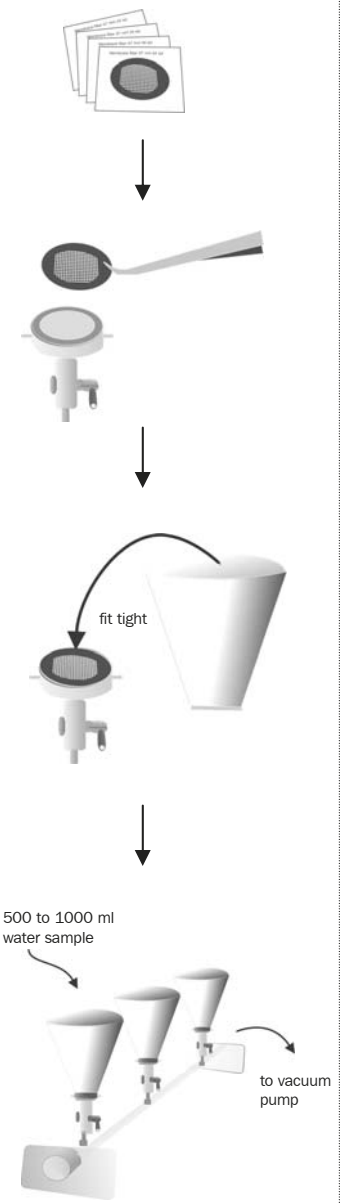
APPENDIX

Limited Product Warranty

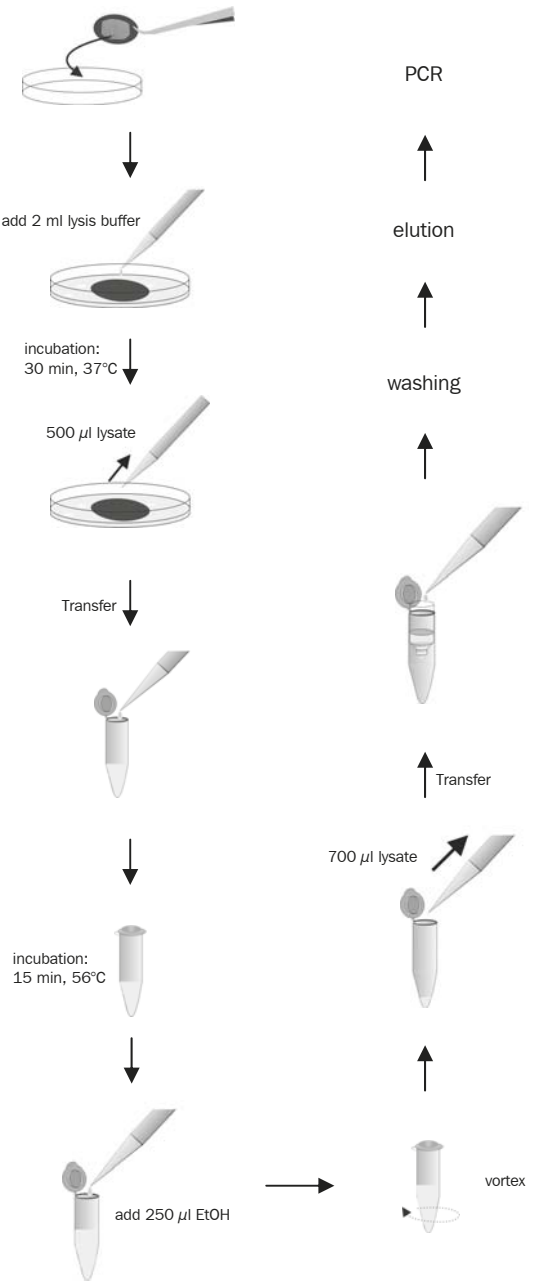
This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Process:

1. Filtration:



2. Lysis



32-1_052012 (en)
last update 05-2012

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Trademarks

AquaScreen® is a registered trademark of Minerva Biolabs.

Related Products

MB *Taq* DNA Polymerase		
53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase	50/100/200/250 units
Quantification Standards, 100 µl each, 1x10^6 genomes/µl		
52-0101	*Legionella pneumophila* DNA Standard	
DNA Remover™		
15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4 x 500 ml
AquaScreen® *Fast Extract* Kit		
32-1010/1050/1200	Extraction Kit	10/50/250 extractions
AquaScreen® Detection Kits		
34-2025/0100/2250	AquaScreen® *Legionella species*	25/100/250 tests
33-2025/0100/2250	AquaScreen® *Legionella pneumophila*	25/100/250 tests
34-6025/0100/2250	AquaScreen® *TotalBacteria*	25/100/250 tests

Manufacturer

Minerva Biolabs GmbH
Koepenicker Str. 325
D-12555 Berlin
Germany

Ordering

Tel. +49 (0)30 2000 437-0
Fax +49 (0)30 2000 437-9
order@minerva-biolabs.com

Product Information

www.minerva-biolabs.com
info@minerva-biolabs.com

Technical Service

Tel. +49 (0)30 2000 437-0
support@minerva-biolabs.com

Made in Germany

Minerva Biolabs GmbH develops and manufactures products in accordance with EN ISO 9001:2008 and EN ISO 13485:2003 quality system requirement.
Reg.No. SY 60026567 0001 & SX 60025009 0001

minerva biolabs®

...as precise as diagnostics should be™