minerva biolabs®

AquaScreen® FastExtract (Fx)

DNA Extraction Kit for Water Samples

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

LOT	**Lot No.**
REF	**Cat. No.**
	Expiry date
	Storage temperature
Σ	**Number of reactions**
	Manufacturer

INDICATION

The AquaScreen® product line consists of reliable test systems for quantitative detection of various waterborne pathogens.

The AquaScreen® FastExtract kit is specifically designed for the extraction microbial DNA from water samples. The kit complies with the design requirements of ISO/TS 12869. The extracted DNA can be used directly with our AquaScreen® qPCR kits for sensitive and reliable detection of legionella and other bacteria in water samples.

The current version of AquaScreen® FastExtract kit (**Product Version 2**) includes components with further optimized performance and a dedicated protocol to achieve higher robustness with a wider range of samples.

TEST PRINCIPLE

The AquaScreen® FastExtract works robustly with various volumes of water samples, with 0.4 μm filter membranes to capture the microorganisms before DNA isolation. Bacterial cells are then lysed by a combination of detergents and chaotropic salts directly on the filter membrane.

The subsequent DNA extraction procedure is very simple and consists of four general steps:
(1) Cell lysis,
(2) Selective binding of DNA to spin columns,
(3) Removal of residual contaminants and inhibitors, and
(4) Elution of purified DNA
The DNA is eluted with minimal volumes and is ready-to-use for PCR. The entire procedure takes less than an hour.

CONTENT

Each kit contains reagents and consumables for 10 or 50 extractions. The expiry date of the unopened package is given on the label. The kit components are stored at room temperature to preserve product stability and ensure maximal performance. Ensure that the specified volumes of ethanol (absolute) are added to Wash Buffer 1 and Wash Buffer 2 before starting. Dissolve any visible precipitate in the Lysis Buffer by moderate warming.

Kit Component	Quantity	
	10 extractions Cat. No. 32-1010	**50 extractions Cat. No. 32-1050**
Membrane Filter	10	50
Incubation Dishes	10	50
Incubation Tubes	10	50
Spin Columns	10	50
Collection Tubes	10	50
Sample Storage Tubes	10	50
Lysis Buffer	25 ml	110 ml
Binding Buffer	10 ml	25 ml
Wash Buffer 1	3 ml (add 3 ml ethanol (abs.) before first use)	15 ml (add 15 ml ethanol (abs.) before first use)
Wash Buffer 2	4 ml (add 16 ml ethanol (abs.) before first use)	12 ml (add 48 ml ethanol (abs.) before first use)
Elution Buffer	1 × 2 ml	2 × 2 ml

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The kit contains reagents and consumables for extracting microbial DNA from water samples. Additional consumables and equipment are supplied by the user:

- Equipment for water filtration with 47 or 55 mm filters (manifold, pump, frit, tubing, etc.; Please contact our technical customer service for further assistance on this matter)
- Ethanol > 96 % abs.
- Reaction tubes (1.5 ml or 2 ml)
- Microcentrifuge and heat block for 1.5 ml (or 2 ml) reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 µl)
- Incubator (37 °C) for petri dishes

PRECAUTIONS

The AquaScreen® FastExtract is for research use only. The kit should be used by trained laboratory staff only.

All samples should be considered as potentially infectious and handled with all due care and attention.

Always wear suitable lab coat, disposable gloves, and protective goggles. The sample preparation waste containing Wash Buffer 1 and Wash Buffer 2 may form highly reactive compounds if in contact with bleach. DO NOT add bleaching agents or acidic solutions to the sample-preparation waste. If any liquid is spilt, clean with suitable laboratory detergent and water.

The kit substances may be discarded according to local regulations.

The Binding Buffer contains propan-2-ol: flammable and irritant. The Wash Buffer 1 contains guanidinium thiocyanate: harmful and irritant.

The hazard (H) statements according to the European Directive 1907/2006/EC (REACH) are listed below.

Component/GHS symbols	Hazards	
Binding Buffer 	H225 H319 H336	Highly flammable liquid and vapour. Causes serious eye irritation. May cause drowsiness or dizziness.
Wash Buffer 1 	H302+H332 H314 H318 H412	Harmful if swallowed or if inhaled. Causes severe skin burns and eye damage. Causes serious eye damage. Harmful to aquatic life with long lasting effects.

See safety data sheets for detailed information.

ADDITIONAL NOTES

⇒ These Instructions for Use must be understood to successfully use the AquaScreen® FastExtract (FX) kit. Any deviation from the given protocol may affect the results.

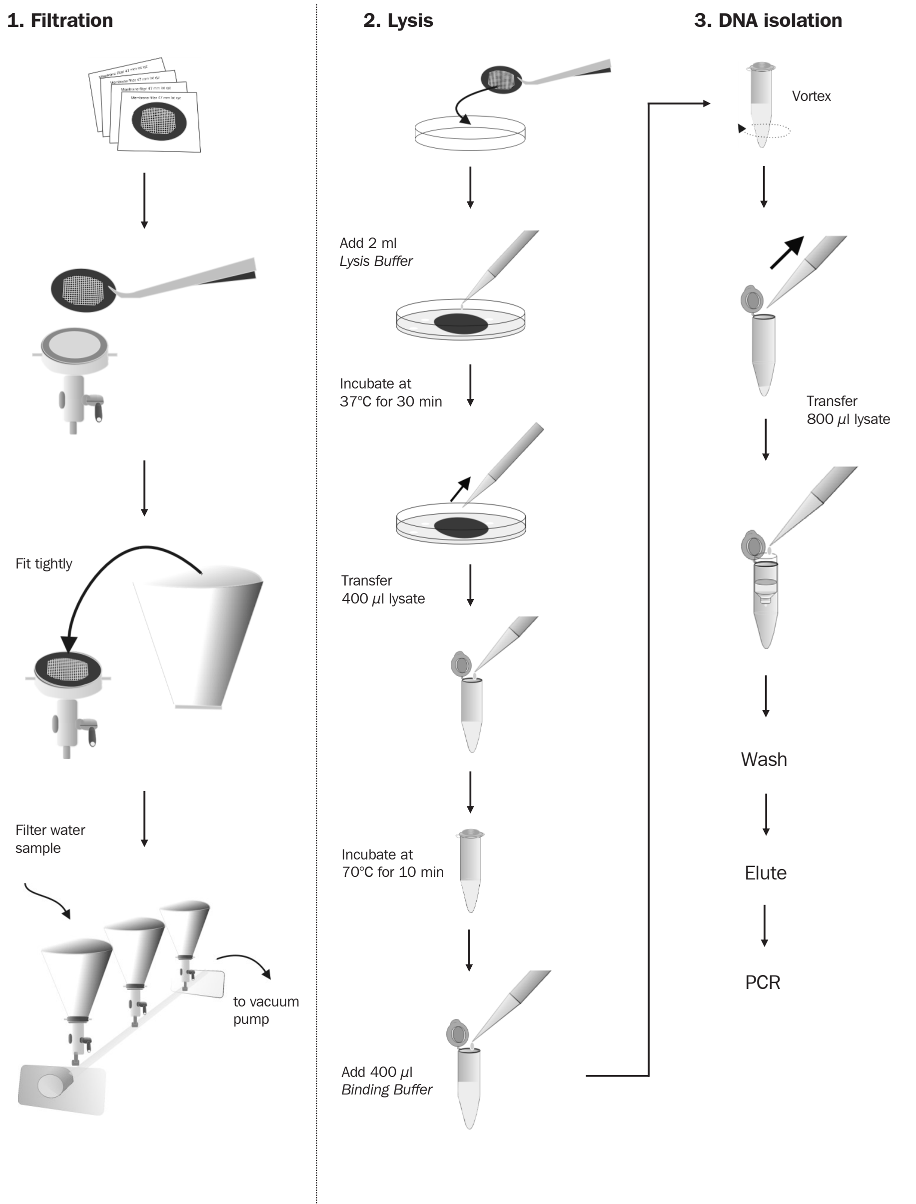
⇒ The reagents and components should not be mixed with reagents and components from different lots but used as an integral unit. The reagents must not be used beyond shelf life.

⇒ We recommend including control samples on a regular basis to monitor the reliability of your results. It is also advantageous in case of troubleshooting.

⇒ Do not use other alcohols than ethanol, as it will lead to inconsistent yields.

⇒ Pre-heating of Elution Buffer improves the yield significantly.

PROCEDURE – OVERVIEW



PROCEDURE – STEP BY STEP

Preparation of sample material

Drinking water, bathing or pool water and wastewater released from suspended particles can be used as sample material. The sample conditioning affects the reliability of the test findings and must be in accordance with the guidelines of water sampling procedures (ISO Norm 5667-1 to 5667-10). Water samples should be kept at +2 to +8 °C and tested within two days after collection. Preservation with chemical means may lead to cell lysis and is therefore not recommended, as intact microorganisms are required to be efficiently captured on the filter. Water samples contaminated with suspended particles or fixed volatile contents have to be purified by filtration with a folded paper filter. The samples <u>must not</u> be centrifuged for purification.
We recommend a sample volume of 1000 ml. However, the kit works with a minimum volume of 100 ml. Extracellular free DNA present in the sample will not be captured by the filter and is therefore not detectable. The test does not differentiate between "viable and cultivable" and "viable but not cultivable" (VBNC) microorganisms.

1. Filtration

1.1	Carefully remove a Membrane Filter from the package and moisten the filter briefly using the particular water sample to be tested.
1.2.	Insert the Membrane Filter in the filtration device and filter the required volume of water sample.

2. Lysis

⇒ Set the heat block to 70 °C and the incubator to 37 °C

2.1	Transfer the Membrane Filter <u>upside down</u> to an Incubation Dish.
2.2.	Pipet 2 ml of Lysis Buffer onto the Membrane Filter.
2.3.	Immerse the filter in the Lysis Buffer by carefully rocking the dish and incubate at 37 °C for 30 min.
2.4.	Rinse the filter with the Lysis Buffer and rock the dish for 10 sec, in order to obtain a homogeneous lysate.
2.5.	Transfer 400 µl of the lysate into the Incubation Tube and incubate at 70 °C for 10 min.
2.5a.	<u>Optional:</u> Use the remaining lysate to set up further sample subsets (400 µl each) for parallel incubation. This will increase the total DNA yield, upon subsequent pooling (see 3.2a.). Do not forget to adjust the calculations (see „4. Calculations"), accordingly.
2.6.	Spin down the sample and add 400 µl Binding Buffer. Vortex immediately and thoroughly in order to prevent any precipitation of nucleic acids. Do not centrifuge the sample and proceed immediately with step 3.1..

3. DNA isolation

⇒ Before first use, reconstitute Wash Buffer 1 and Wash Buffer 2 with absolute ethanol as stated on the bottle label.

⇒ Set the heat block to 70 °C and pre-heat the Elution Buffer.

3.1	Transfer the mix from step 2.6 (~800 μl) to a Spin Column placed on a Collection Tube, without moistening the rim of the spin column.
3.2.	Centrifuge the Spin Column at ≥ 10,000 × g for 1 min. Discard the flow-through and reassemble the same Spin Column and Collection Tube.
3.2a.	Optional: If further aliquots of the same sample were prepared (see Lysis step 2.5a), transfer additional ~ 800 μl to the same Spin Column and repeat step 3.2. Adjust calculations (see „4. Calculations“), accordingly.
3.3.	Add 500 μl of Wash Buffer 1. Centrifuge at ≥ 10,000 × g for 1 min. Discard the flow-through and reassemble Spin Column and Collection Tube.
3.4.	Add 500 μl of Wash Buffer 2. Centrifuge at ≥ 10,000 × g for 1 min. Discard the flow-through and Collection Tube. Place the Spin Column on a new Collection Tube.
3.5.	Centrifuge at full speed for 3 min to remove residual Wash Buffer 2.
3.6.	Discard the Collection Tube and place the Spin Column into a Sample Storage Tube.
3.7.	Pipet 60 μl of pre-heated Elution Buffer (70 °C) in the Spin Column directly onto the silica membrane. The membrane's surface should be covered with Elution Buffer.
3.8.	Incubate at room temperature for 2 min, then centrifuge at 8,000 × g for 2 min to elute.
3.9.	The eluate contains purified microbial DNA and can be used directly for PCR or stored at +2 to +8 °C for a week. Long-term storage should be below -18 °C.

4. Calculations

The number of microbial particles in your sample is determined by means of quantitative real-time PCR. Calculate the number of genomes per PCR by using a DNA standard curve. We recommend our PCR Quantification Standards for your species of interest, as templates to obtain the DNA standard curve as well as our AquaScreen® qPCR Detection kits (see last page or go to our website), for reliable detection of waterborne pathogens.
The following equation should be used to calculate the number of particles in your sample:

$$\text{Genomes per PCR} \times \text{scaling factor} \times \text{correction factor} \times \frac{1000}{\text{Sample volume (ml)}} = \text{particles per liter}$$

The **scaling factor** is 5 and accounts for the subdivision of volumes (400 μl out of 2 ml lysate accounting for 1/5 of the total available sample volume), according extraction protocol. Please note that you must adjust the scaling factor if more than 400 μl of the sample lysate (max 2 ml, see 3.2a.) were used for DNA isolation.
<u>Note:</u> The scaling factor is 2.5 if 2x 400 μl lysate was used for extraction. If 3x 400 μl of lysate were used for extraction, the scaling factor will be 1.67.
The **correction factor** accounts for the volume of DNA used in qPCR. Therefore, if 10 μl of the 60 μl eluate are used, the correction factor will be 6.
Here is an example to illustrate the calculation:
⇒ The sample volume is 500 ml
⇒ 400 μl of the lysate are used for extraction > Scaling factor is 5
⇒ Elution volume is 60 μl; 10 μl are used for qPCR > Correction factor is 6
⇒ Number of genomes per PCR is 27
⇒ The equation is therefore

$$27 \text{ genomes per PCR} \times 5 \times 6 \times \frac{1000}{500 \text{ (ml)}} = 1620 \text{ particles per liter}$$

Thus, there are 1620 intact particles per liter in the original water sample.

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 from the use, the results of use, or the inability to use this product.

Trademarks
AquaScreen, Venor, Onar, Mynox, and ZellShield are registered trademarks and PCR Clean, WaterShield, Mycoplasma Off, and SwabUp are trademarks of Minerva Biolabs GmbH, Germany.

Related Products

AquaScreen® Detection kits for qPCR

33-2025/-2100/-2250	AquaScreen® Legionella species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® Legionella pneumophila	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® Pseudomonas aeruginosa	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® Escherichia coli	25/100/250 reactions

Contamination Control Kits for conventional PCR

11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/100/250 reactions
12-1025/-1100/-1250	Onar® Bacteria Detection Kit	25/100/250 reactions

Contamination Control Kits for qPCR

11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit (Mycoplasma DNA)	10/50/200 extractions

Mycoplasma Elimination

10-0200/0500/1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 10^8 genomes / vial

52-0116	*Acholeplasma laidlawii*
52-0129	*Mycoplasma arginini*
52-0117	*Mycoplasma fermentans*
52-0115	*Mycoplasma gallisepticum*
52-0130	*Mycoplasma hyorhinis*
52-0112	*Mycoplasma orale*
52-0119	*Mycoplasma pneumoniae*
52-0103	*Mycoplasma salivarium*
52-0124	*Mycoplasma synoviae*
52-0164	*Spiroplasma citri*
52-5571	*Bordetella pertussis*
52-0083	*Escherichia coli*
52-0101	*Legionella pneumophila*
52-0071	*Pseudomonas aeruginosa*

See MB homepage for further available species

Genomic DNA Extracts, 10±2 ng/vial

51-1370	*Legionella dumoffii*
51-1533	*Legionella jordanis*
51-0101	*Legionella pneumophila*
51-1514	*Legionella pneumophila subs. fraseri*
51-1515	*Legionella pneumophila subs. pascullei*
51-0071	*Pseudomonas aeruginosa*
51-0083	*Escherichia coli*

See Minerva homepage for further available species

PCR Clean™

15-2025/15-2200/15-2500	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4×500 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes

SwabUp™ Lab Monitoring Kits

181-0010/-0050	Sample collection and DNA extraction	10/50 samples

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant Spray, spray bottle, refill canister	1 l/5 l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes in refill pack	5×50 wipes

Minerva Biolabs GmbH
Schkopauer Ring 13
D-12681 Berlin, Germany

www.minerva-biolabs.com
Ordering: order@minerva-biolabs.com
Support: support@minerva-biolabs.com

USA & Canada

Minerva Biolabs Inc.
1 Jill Ct., Building 16, Unit 10
Hillsborough, NJ 08844
USA

www.minervabiolabs.us
Ordering: order@minervabiolabs.us
Support: help@minervabiolabs.us

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