AquaScreen® Pseudomonas aeruginosa

qPCR Detection Kit

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The AquaScreen® Pseudomonas aeruginosa qPCR Detection kit is specifically designed for the quantitative detection of *Pseudomonas aeruginosa* in water samples prepared with the AquaScreen® FastExtract kit.

Pseudomonas aeruginosa is a microorganism commonly found in faeces, soil, water, and sewage. Also, it proliferates in aqueous or moist environments such as sinks, water baths, hot water systems, showers, and spa pools but also on organic materials like fruits and vegetables. *Pseudomonas aeruginosa* is a typical nosocomial pathogen causing health-care associated infections with potentially serious complications (e.g. wound infections).

As for all water pathogens, successful prevention is achieved through simple, routine screening aiming at rapid and specific pathogen detection. Traditional culture-based methods unfortunately require time-consuming procedures (e.g. for bacteria growth or colonies analysis).

TEST PRINCIPLE

The AquaScreen® Pseudomonas aeruginosa Kit uses qPCR for quantitative detection of *Pseudomonas aeruginosa*. In contrast to more time-consuming culture-based methods, AquaScreen® assays need less than six hours including sample preparation and qPCR to reliably detect *Pseudomonas aeruginosa*. Additionally, compared to the culture method, this qPCR assay is superior in terms of sensitivity as well as specificity. In fact, conventional culture fails to detect viable but non-culturable particles and therefore minimal *Pseudomonas aeruginosa* amounts and, on the other hand, can still lead to misidentification of closely related non pathogenic species.

The AquaScreen® qPCR assay is insensitive to contamination with other bacterial genera. Furthermore, linear quantification is obtained up to 1 x 10^6 particles per sample, therefore requiring no sample material dilution and demonstrating unprecendented assay robustness.

The PCR system targets the ecfX gene encoding an extra-cytoplasmic sigma factor specific for *Pseudomonas aeruginosa*. Compared to target regions such as 16S rDNA the species-specific gene ecfX gene allows reliable discrimination of *P. aeruginosa* from other species of the genus. Cross-reactivity to other waterborne microorganisms is not known.
The kit contains all necessary qPCR components including hot-start Taq polymerase, primers, probes, and dNTPs. False negative results caused by PCR inhibition and/or DNA extraction issues can be reliably identified by using the provided Internal Control DNA. The amplification of the Internal Control DNA is detected at 610 nm (ROX™ channel), whereas the *Pseudomonas aeruginosa*-specific amplification is detected at 520 nm (FAM™ channel).
The P. aeruginosa Mix contains dUTP instead of dTTP to facilitate the degradation of amplicon carry-over by use of uracil-DNA glycosylase (UNG). Applying this strategy reduces the probability of false positives. Please note that UNG is not included in the AquaScreen® qPCR kit.

REAGENTS

Each kit contains reagents for 25, 100 or 250 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 to +8 °C until use. The rehydrated components must be stored at ≤ -18 °C.

Kit Component	Quantity			Cap Color
	25 reactions Cat. No. 34-6025	100 reactions Cat. No. 34-6100	250 reactions Cat. No. 34-6250	
P. aeruginosa Mix	1 vial lyophilized	4 vials lyophilized	10 vials lyophilized	red
Rehydration Buffer	1 vial 1.8 ml	1 vial 1.8 ml	3 vials 1.8 ml	blue
Positive Control DNA	1 vial lyophilized	1 vial lyophilized	1 vial lyophilized	green
Internal Control DNA	1 vial lyophilized	1 vial lyophilized	2 vials lyophilized	yellow
PCR Grade Water	1 vial 2 ml	1 vial 2 ml	1 vial 2 ml	white

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 AquaScreen® qPCR kit contains all necessary reagents for the qPCR. Additional consumables and equipment is supplied by the user:

- qPCR device with filter sets for detecting the fluorescent dyes FAM™ and ROX™
- PCR reaction tubes for the specific qPCR device
- 1.5 ml reaction tubes, DNA- and RNA-free
- Microcentrifuge for 1.5 ml PCR reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- For DNA standard curves, we recommend our *Pseudomonas aeruginosa* PCR Quantification Standard (Cat. No. 52-0071).
- For DNA extraction from water samples, we recommend AquaScreen® FastExtract kit (Cat. No. 32-1010/-1050).

SPECIMEN

For sample preparation, please see the Instructions for Use of AquaScreen® FastExtract. Extracted samples must be stored at ≤ -18 °C for max. one year. Repeated freezing and thawing should be avoided as it is detrimental to the integrity of DNA molecules.

PRECAUTIONS

The AquaScreen® qPCR kit 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 a suitable lab coat and disposable gloves.
This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

ADDITIONAL NOTES

⇒ These instructions must be understood to successfully use the AquaScreen® qPCR Detection kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents of the kit must not be used beyond their shelf life.

⇒ Follow the exact protocol. Any deviation may affect the test method and results.

⇒ PCR inhibition is likely to be caused by the sample matrix, or, in case of extracted DNA, by the elution buffer. We recommend our AquaScreen® FastExtract kit for sample preparation. Any other DNA extraction kit needs to be qualified for this use.

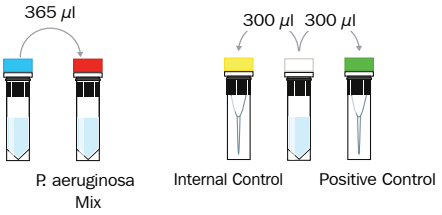
⇒ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative controls are essential in case of troubleshooting.

⇒ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA level (e.g. 3x LOD 95). Please note that Minerva Biolabs also offers to participate in external quality control programs.

PROCEDURE – OVERVIEW

1. Reagents preparation

P. aeruginosa Mix
Positive Control
Internal Control

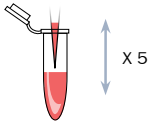


for 5 min RT
briefly
for 5 sec

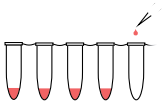
2. Reaction mix preparation

1 reaction:
14 µl P. aeruginosa Mix
1 µl Internal Control

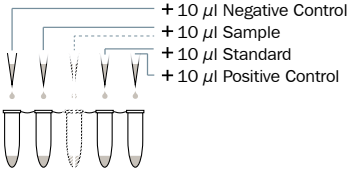
Mix by pipetting up-and-down



aliquot 15 µl reaction mix

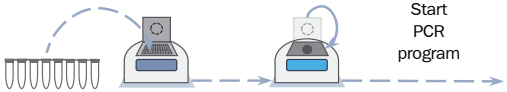


3. Addition of samples and controls

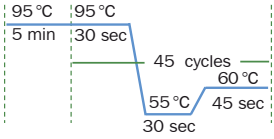


Full speed
5 sec

4. PCR amplification



Start
PCR
program



P. aeruginosa Mix
Rehydration Buffer
Positive Control
Internal Control
PCR Grade Water

incubate
vortex
centrifuge
add

This procedure overview is not a substitute for the detailed manual.

MB_SI_AquaScreen_P_aeruginosa_02_EN

PROCEDURE – STEP BY STEP

1. Reagents preparation

After reconstitution, the reagents can be stored at +2 to +8 °C for up to one day. Long-term storage must be at ≤ -18 °C. Repeated freeze/thaw of rehydrated components must be avoided as it might affect the assay sensitivity. We recommend storing these components in aliquots.

1.	P. aeruginosa Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Spin down all lyophilized components at max. speed for 5 sec
2.	P. aeruginosa Mix	Red cap	Add 365 µl Rehydration Buffer (blue cap)
3.	Internal Control DNA	Yellow cap	Add 300 µl PCR Grade Water (white cap)
4.	Positive Control DNA	Green cap	Add 300 µl PCR Grade Water (white cap)
5.	P. aeruginosa Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Incubate at room temperature for 5 min
6.	P. aeruginosa Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Vortex briefly and spin down for 5 sec

2. Reaction mix preparation

The following steps (2. Reaction mix preparation, 3. Addition of samples and controls, and 4. PCR amplification) should be performed within 45 minutes to avoid a significant reduction of the fluorescent signal.

We recommend following strictly this protocol and pipetting sequence:

1.	Calculate the required volume of master mix for all the control and test reactions and prepare it as indicated below, at room temperature, and in a 1.5 ml reaction tube.		
		for 1 reaction	for 25 reactions
	P. aeruginosa Mix	14 µl	350 µl
	Internal Control	1 µl	25 µl
2.	Mix by pipetting up and down (5 times).		
3.	Add 15 µl to each PCR tube and close the lid.		

3. Addition of samples and controls

⇒ Please note that a standard curve is required for quantification. We recommend our PCR Quantification Standards as templates for the generation of standard curves (e.g. *Pseudomonas aeruginosa*, Cat. No. 52-0071).

⇒ Run positive and negative control samples (e.g. no template control, NTC) in duplicate in each PCR.

1.	Negative Control: Add 10 µl of elution buffer used for DNA extraction or PCR Grade Water (white cap).
2.	Test sample/standard curve sample: Add 10 µl of each sample.
3.	Positive Control: Add 10 µl of Positive Control (green cap).
4.	Close the PCR tubes tightly and spin briefly.

4. PCR amplification

1.	Place the PCR tubes in the qPCR cycler and close the lid.
2.	Program the cycler as indicated below: 1 cycle — 95 °C for 5 min 45 cycles — 95 °C for 30 sec (Denaturation) 55 °C for 30 sec (Annealing) 60 °C for 45 sec (Elongation and data collection) **Fluorescent dyes: FAM™ and ROX™**
3.	Start the program.

This assay has been successfully performed on the following qPCR devices:

qPCR device	Manufacturer
CFX96 Touch™	Bio-Rad
LightCycler® 1.2	Roche Diagnostics
ABI Prism® 7500	Applied Biosystems
Rotor-Gene® 6000	Corbett Research
Mx3005P™	Agilent Technologies

DATA INTERPRETATION

The presence of *P. aeruginosa* is indicated by an increasing fluorescence signal in the FAM™ channel.
We recommend evaluating the progression of the amplification curve for any sample, including the controls. The quantification is based on threshold cycle (Ct) values and a DNA standard curve run in parallel with the samples. The exact procedure to obtain the Ct values, perform baseline calculation/normalization, and threshold setting depends on the particular qPCR device and software in use. Please refer to the documentation of your specific device for further details.

A positive PCR result is indicated by a Ct < 40. PCR reactions with Ct ≥ 40 are considered negative.
In addition, a successful PCR reaction (no inhibition) displays an increasing fluorescent signal in either the FAM™ and/or the ROX™ channel (given that the Internal Control was added to the master mix prior to PCR). The *P. aeruginosa* target DNA might compete with the Internal Control in the PCR. High levels of *P. aeruginosa* DNA in the sample would therefore lower the Internal Control signal in the ROX™ channel.

The following table will help with the interpretation of the PCR results:

Detection of *Pseudomonas aeruginosa* FAM™ channel	**Internal control ROX™ channel**	**Interpretation**
positive	irrelevant	*P. aeruginosa* positive
negative	negative	PCR inhibition
negative	positive	*P. aeruginosa* negative

Running quantification standards allows quantification of the *Pseudomonas* load in the sample. *P. aeruginosa* particles per sample can be then calculated as illustrated by the following example:

DNA copies / qPCR reaction (as determined per interpolation on the standard curve)	60
Sample volume used for DNA extraction (ml)	500
Volume used for elution (µl)	100
Sample volume used for qPCR (µl)	10 (1/10 of the eluate)
DNA copies / sample	60 × 10 (volume correction factor)

In the example above, for the analyzed 500 ml water sample, the equivalent in DNA of 600 *P. aeruginosa* particles were detected. This amount may include viable and cultivable *P. aeruginosa* and viable but non-cultivable *P. aeruginosa* (VBNC-state) as well as dead yet still intact *P. aeruginosa*.

ASSAY CHARACTERISTICS

1. Sensitivity and linear range

The assay detects from 1 genome equivalent (GE) per reaction of *P. aeruginosa* DNA. Robust and linear detection of the bacteria is achieved in the range 50 - 1 x 10^6 GE / reaction.

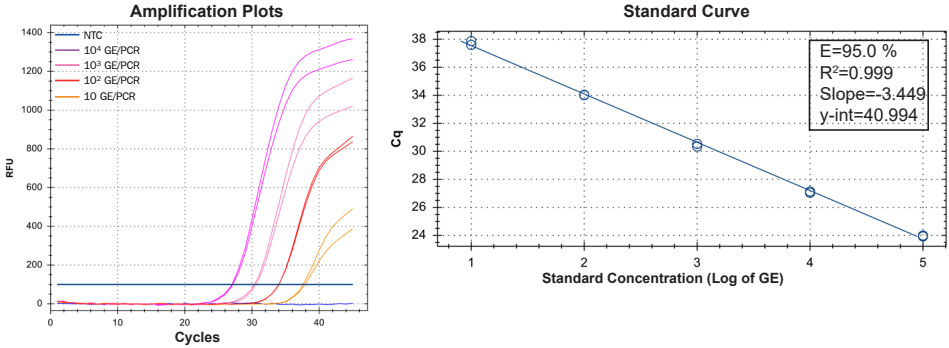


Fig. 1. Performance of the AquaScreen® qPCR. On the left, amplification curves obtained with the kit using serial dilutions of *P. aeruginosa* (PCR Quantification Standard, in duplicates) and a CFX96 Touch™ qPCR System (Bio-Rad). The legend shows the used concentrations in genome equivalents (GE) per PCR reaction. The graph on the right represents the standard curve obtained using the average Ct values for the amplification plots on the left at the specified standard concentrations. In the inset the parameters calculated for this standard curve by linear regression.

2. Specificity

The assay specifically detects *Pseudomonas aeruginosa*.
The following bacteria are not detected by the assay, indicating lack of cross-reactivity with these species:

Bacillus cereus	*Legionella pneumophila*
Bacillus subtilis	*Micrococcus luteus*
Clostridium acetobutylicum	*Proteus mirabilis*
Enterococcus feacalis	*Salmonella enterica*
Enterobacter aerogenis	*Staphylococcus aureus*
Lactobacillus acidophilus	

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

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registered trademark and FAM and ROX are trademarks of Applera Corporation or its subsidiaries in the US and certain other countries. CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. Mx3005P is a trademark of Agilent Technologies. Rotor-Gene is a registered trademark of Qiagen GmbH. Aqua-Screen is registered trademark of Minerva Biolabs GmbH.

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

Sample Preparation

32-1010/-1050	AquaScreen® FastExtract (Microbial DNA from water samples)	10/50 extractions
56-1010/1050/1200	Venor®GeM Sample Preparation Kit (Mycoplasma DNA)	10/50/200 extractions

Contamination Control Kits for conventional PCR

11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 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

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

PCR Clean™

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

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

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/3×50 ml

WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200× concentrate	15×10 ml/3×50 ml/500 ml

RELATED PRODUCTS

AquaScreen® FastExtract

DNA-based system for quantitative detection of water pathogens.
AquaScreen® combines water filtration, lysis of the collected microorganisms, DNA extraction and elution of the DNA in minimal volumes ready for PCR analysis.

Features	
Description	Rapid DNA extraction from water samples
Recommended Use / Scope	AquaScreen® FastExtract can be used with your established suction device (47 mm frit) for the extraction of legionella and other microbial contaminations. AquaScreen® FastExtract is optimized for high flow and throughput and provides high quality DNA for subsequent PCR analysis.
Kit Components	Membrane filters Incubation dishes Incubation, collection and sample storage tubes Lysis, wash and elution buffers
Package Sizes	Cat.-No. 32-1010 10 extractions Cat.-No. 32-1050 50 extractions
Required lab devices & reagents	Vacuum pump Micro centrifuge Filtration system, 47 mm frit Pipetting equipment and filtered tips Incubator (37 °C for petri dishes, 56 °C for reaction tubes) Ethanol (96-100 %)
Shelf Life and Storage	Components are maintainable at room temperature for at least 6 months.
Compliance	AFNOR XP T90-471 and ISO/TS 12869:2012 in combination with AquaScreen® qPCR kits

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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