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Order No. 34-7025/-7100/-7250

AquaScreen® Escherichia coli

Detection Kit for qPCR

Instructions for Use

Reagents for 25/100/250 reactions

Manufacturer:

Minerva Biolabs GmbH, Koepenicker Strasse 325, 12555 Berlin, Germany

FOR USE IN RESEARCH AND QUALITY CONTROL

CELL CULTURE CONTAMINATION & QUALITY CONTROL
CLINICAL | WATER | VETERINARY DIAGNOSTICS
MYCOPLASMA | BACTERIA | VIRUSES



Symbole / Symbols



Chargen-Nr. / Lot No.



Artikel-Nr. / Order No.



Verfallsdatum / Expiry date



Lagerung bei / Store at



**Enthält Reagenzien für 25, 50, 100 oder 250 Bestimmungen
Contains reagents for 25, 50, 100 or 250 tests**



Hersteller / Manufacturer

INDICATION

AquaScreen® *Escherichia coli* PCR Detection Kit for the quantitative detection of *Escherichia coli* (*E. coli*) in water samples, prepared with the AquaScreen® FastExtract kit.

EXPLANATION OF THE TEST

Escherichia coli is a type of faecal coliform bacteria commonly found in the intestines of animals and humans. The presence of *Escherichia coli* in water is a strong indication of recent sewage or animal waste contamination which may contain disease-causing organisms like *Giardia* spp. and *Cryptosporidium*. According to the WHO guideline values for bacteriological quality of drinking water, treated water entering the distribution system and treated water in the distribution system, *Escherichia coli* shall not be detectable in any 100-ml sample.

AquaScreen® *Escherichia coli* is an in vitro assay for the quantitative detection of *Escherichia coli* in water samples. Compared to culture methods that can detect *Escherichia coli* first after a few days, the PCR-based detection of this pathogens is completed within 6 hours. PCR also allows for a higher specificity without annoying background growth simplifying result reading drastically. The quantitative result of PCR is linear up to 10^6 particles/sample with no need for preparing dilutions of the sample material.

TEST PRINCIPLE

The amplified *uidR* gene is highly conserved within the species *Escherichia coli*. It allows detection of all *E. coli* serogroups including enteroinvasive *E. coli* (EIEC) and *Shigella* spp. which evolved from the same ancestor. Cross reactivities to other water born microorganisms are not known. The detection limit is 10 genome equivalents per PCR. Because of the wide linear range reliable results without a time-consuming conditioning of the sample material are possible.

The kit contains the nucleotide dUTP instead of dTTP. The heat-labile Uracil-DNA Glycosylase (UNG) is suitable to prevent carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplified reactions and the pretreatment of all successive PCR mixtures with the heat-labile UNG. UNG is not included. The *E. coli* Mix also contains all required reagents like primer, probes and polymerase.

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 are stored until use at +2 to +8 °C. The lot specific Certificate of Analysis can be downloaded from our website (www.minervabiolabs.com).

Kit Component Label Information	Quantity			Cap Color
	25 reactions Order No. 34-7025	100 reactions Order No. 34-7100	250 reactions Order No. 34-7250	
E coli Mix	1 x freeze-dried	4 x freeze-dried	10 x freeze-dried	red
Rehydration Buffer	1 x 1.8 ml	1 x 1.8 ml	3 x 1.8 ml	blue
Positive Control DNA	1 x freeze-dried	1 x freeze-dried	1 x freeze-dried	green
Internal Control DNA	1 x freeze-dried	1 x freeze-dried	2 x freeze-dried	yellow
PCR grade Water	1 x 2.0 ml	1 x 2.0 ml	1 x 2.0 ml	white

NEEDED, BUT NOT INCLUDED IN THE KIT

The kit contains all reagents for analysis. General industrial supplies and reagents, usually available PCR laboratories are not included:

- qPCR device with filter sets for the detection of the fluorescence dyes FAM™ and ROX™
- PCR reaction tubes for the specific qPCR device
- 1.5 ml reaction tubes, DNA- and RNA-free
- Micro centrifuge for 1.5 ml PCR reaction tubes
- Pipettes with corresponding filter tips to prepare and dispense the reaction mix (10, 100 und 1000 µl)
- Optional: For calibration we recommend our *Escherichia coli* DNA Calibration Reagent (Cat.-No. 52-0083).

SPECIMEN

The preparation of the sample material is explained in the AquaScreen® FastExtract manual.

The extracts can be stored at a temperature of at least -18 °C for a period of one year. Repeated freezing and defrosting, or storage in the refrigerator for more than 12 hours should be avoided. The sample should not contain more than 100 µg/ml DNA.

PRECAUTIONS

For in vitro diagnostic use. This kit should be used only by trained persons. All samples should be considered potentially infectious and handled at the local or national regulations. This kit does not contain hazardous substances and may be disposed of according to local regulations.

TEST PROCEDURE

The test should be carried out with negative and positive controls and patient samples in duplicate. For quantification, a dilution series of an appropriate standard (Cat. No. 52-0083) is required. All reagents and samples must be equilibrated to 2 to 8 °C prior use.

1. Rehydration of the Reagents

After reconstitution, the reagents should be stored at 2 to 8 °C for up to 4 days or below -18 °C till the expiration date stated on the box label. Repeated freezing and thawing should be avoided and reconstituted controls (internal amplification control and the positive control) stored in aliquots.

1.	E coli Mix Internal Control Positive Control	red cap yellow cap green cap	Spin all freeze-dried components for 5 sec at maximum speed of the mini centrifuge
2.	E coli Mix	red cap	Add 365 µl rehydration buffer (blue cap)
3.	Internal Control	yellow cap	Add 300 µl PCR grade Water (white cap)
4.	Positive Control	green cap	Add 300 µl PCR grade Water (white cap)
5.	E coli Mix Internal Control Positive Control	red cap yellow cap green cap	Incubate 5 min at room temperature
6.	E coli Mix Internal Control Positive Control	red cap yellow cap green cap	Vortex DNA briefly and spin for 5 sec

2. Preparation of the Reaction Mix

Prepare the required amount of master mix at room temperature in a 1.5 ml reaction tube for all control and test reactions.

1.		for 1 reaction	for 25 reactions
	E coli Mix	14.0 µl	350.0 µl
	Internal Control DNA	1.0 µl	25.0 µl

2. Homogenize the reaction mix by carefully snapping the tube

3. Add 15 µl to each PCR tube, discard remaining material.

3. Loading the test tubes

-
- Negative Control:
1. Add 10 μl elution buffer from DNA extraction (ref. chapter *Sample Material*) or PCR Grade Water (white cap).
-
2. Sample: Add 10 μl of each sample.
-
3. Positive Control: Add 10 μl Positive Control (green cap).
-
4. Spin all PCR tubes briefly, load the qPCR cycler and start the program.
-

Preparation of the reaction mix and sample loading should not take more than 45 minutes to avoid a reduction in the fluorescent signal. The pipetting sequence should be respected and the tubes closed after each sample load.

4. Starting the reaction

-
1. Load the cycler, check each PCR tube and the cycler lid for tight fit.
-
- Program the qPCR cycler or check stored temperature profiles.
2. See Appendix for temperature profiles of selected qPCR cyclers. Programs for additional cyclers might be available on request.
-
3. Start the program and data reading.
-

5. Result reading

-
1. Save the data at the end of the run.
-
2. Read the channels for the fluorescence dyes FAMTM and ROXTM and show the 2nd deviation of the data.
-
3. Read the calculation of the Ct values for the negative controls, the positive controls and the samples.
-
4. Evaluate in accordance with the laboratory and instrument-specific reference ranges.
-

Reference range for selected qPCR devices:

qPCR device	Manufacturer	Evaluating the threshold cycle		
		positive	borderline	negative
LightCycler [®] 1.2	Roche Diagnostics	< 40	\geq 40	no Ct
ABI Prism [®] 7500	Applied Biosystems	< 40	\geq 40	no Ct
Rotor-Gene [®] 6000	Corbett Research	< 40	\geq 40	no Ct
Mx3005P [®]	Agilent Technologies	< 40	\geq 40	no Ct

INTERPRETATION OF RESULTS

The presence of *Escherichia coli* in the sample is indicated by an increasing fluorescence signal in the FAM™ channel during PCR. The concentration of the contaminant can be calculated by a software comparing the crossing cycle number of the sample with a standard curve created in the same run.

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the ROX™ channel, provided the Internal Control was added to the master mix. *Escherichia coli* DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of Internal Control in the PCR mix, the signal strength in this channel is reduced with increasing *Escherichia coli* DNA loads in the sample.

If a 10 µl test volume is used, then the total quantity of genomic equivalents present in the water sample can be calculated by multiplying the qPCR result by 10 (elution volume of extraction 100 µl).

Example: A 500 ml water sample is used for the analysis. On the basis of the real-time PCR, a result of 60 DNA copies is determined using a test volume of 10 µl. 60 multiplied by 10 totals 600. Result: in 500 ml sample water, there were 600 intact *Escherichia coli* particles. That results in 120 *Escherichia coli* particles per 100 ml of water.

Detection of <i>Escherichia coli</i> FAM™ channel	Internal control ROX™ channel	Interpretation
positive	irrelevant	<i>Escherichia coli</i> positive
negative	negative	PCR inhibition
negative	positive	<i>Escherichia coli</i> negative

NOTES ON THE TEST PROCEDURE

1. This leaflet must be widely understood for a successful use of the 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 test method can affect the results.
3. Inhibition may be caused by the sample matrix, but also by sample elution buffer of DNA extraction kits not recommended or validated. Negative controls should always be completed with the use sample elution buffer.
4. For each test setup, at least one negative control may be included also including sample preparation. Positive controls facilitate the evaluation of the test. Typical Ct values for the internal control and positive control are shown on the Certificate of Analysis and can be used for orienting quality control.
5. The use of control samples is advised to secure the day-to-day validity of results. The controls should be carried out in the same manner as the samples. It is recommended to run laboratory specific control samples with a high, medial and low (e.g. 3x LOD₉₅) level or establish commercial controls, for example, Minerva Biolabs Quantification Standard *Escherichia coli* (Order No. 52-0083). Participation in external quality control programs is recommended.

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

LightCycler is a registered trademark of a member of the Roche Group.

ABI Prism and FAM are registered trademarks of Applied Biosystems Corporation.

Rotor-Gene is a registered trademark of Qiagen GmbH.

Mx3005P is a registered trademark of Agilent Technologies, Inc..

Programming and data recording of different qPCR devices

1. LightCycler® 1.2, 1.5, 2.0 and 480

Program 1: Pre-incubation

Cycles 1
Analysis Mode None

Temperature Targets Segment 1

Target Temperature [°C] 95
Incubation time [min] 2:00
Temperature Transition Rate [°C/s] 20.0
Secondary Target Temperature [°C] 0
Step Size [°C] 0.0
Step Delay [Cycles] 0
Acquisition Mode None

LightCycler® 2.0:

Please check the correct settings for the „seek temperature“, this should be adjusted on at least 90°C.

Program 2: Amplification

Cycles 45
Analysis Mode Quantification

Temperature Targets	Segment 1	Segment 2	Segment 3	Segment 4
Target Temperature [°C]	95	55	60	72
Incubation time [s]	0	5	7	5
Temperature Transition Rate [°C/s]	20.0	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0	0
Step Size [°C]	0.0	0.0	0.0	0.0
Step Delay [Cycles]	0	0	0	0
Acquisition Mode	None	None	Single	None

Program 3: Cooling

Cycles 1
Analysis Mode None

Temperature Targets Segment 1

Target Temperature [°C] 40
Incubation time [s] 30
Temperature Transition Rate [°C/s] 20.0
Secondary Target Temperature [°C] 0
Step Size [°C] 0.0
Step Delay [Cycles] 0
Acquisition Mode None

Result Reading:

- Select the fluorescence channels F1 and F2 or Channel 1 and 3 at the LC 2.0
- Click on *Quantification* to generate the amplification plots and the specific *ct*-values
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered as negative.

target	<i>E. coli</i>	Internal control
channel for LC 1.2, 1.5	F1 (530)	F2 (640)
channel for LC 2.0, 480	channel 1 (530)	channel 3 (610)

2. RotorGene® 6000 (5-plex)

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Please check the correct settings for the filter combination:

Target	<i>E. coli</i>	Internal Control
Channel	Green	Orange
Wave length	470-510 nm	585-610 nm

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95°C for 5 sec
Annealing	55°C for 10 sec —> acquiring to Cycling A (green and orange)
Elongation	72°C for 7 sec
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and orange) according to the following table and start data analysis by double click.
- The following windows will appear:
 - Quantitation Analysis - Cycling A* (green or orange)
 - Quant. Results - Cycling A* (green or orange)
 - Standard Curve - Cycling A* (green or orange)
- In window *Quantitation Analysis*, select first *linear scale* and than *slope correct*
Threshold setup (not applicable if a standard curve was carried with the samples and auto threshold was selected)
 - In window *CT Calculation* set the threshold value to 0-1
 - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The *ct*-values can be taken from the window *Quant. Results*.
- Samples showing no *ct*-value can be considered as negative.

3. ABI Prism® 7500

Detector Settings:

E. coli Target Probe: Reporter - FAM™, Quencher - none
Internal Control Probe: Reporter - ROX™, Quencher - none

**The ROX™ Reference needs to be disabled. Activate both detectors for each well.
Measurement of fluorescence during extension.**

Program Step 1: Pre-incubation

Setting Hold
Temperature 95 °C
Incubation time 3:00 min

Program Step 2: Amplification

Cycles 45
Setting Cycle
Denaturing 95 °C für 30 sek
Annealing 55 °C für 30 sek
Extension 60 °C für 45 sek

Result Reading:

- Enter the following basic setting at the right task bar:

Data: Delta RN vs. Cycle
Detector: FAM™ and ROX™
Line Color: Well Color

Target	<i>E. coli</i>	Internal Control
Channel	FAM™	ROX™

- Open a new window with for the *Graph settings* by clicking the right mouse button

Select the following setting and confirm with *ok*:

Real Time Settings: Linear
Y-Axis Post Run Settings: Linear and Auto Scale
X-Axis Post Run Settings: Auto Scale
Display Options: 2

- Initiate the calculation of the ct-values and the graph generation by clicking on *Analyze* within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no ct-value can be considered as negative.

4. Mx3005P®

- Mark the wells in use in the setup menu, tab “Plate Setup”
- Select FAM™ und ROX™ for “Collect Fluorescence Data”
- Check that the Reference Dye function is set to “none” according to the ground settings of the device
- Specify the type of sample (negative control, positive control, calibrators, sample) by using the tab “well type”
- Program the temperature profile by using the tab “Thermal Profile Design”:
 - Segment 1 (Pre-Melt): 2 min, 95 °C
 - Segment 2:
 - 30 sec, 95 °C
 - 30 sec, 55 °C - data collection end
 - 45 sec, 60 °C - data collection end
 - 45 cycles
- Start the program in the menu “Run” by clicking on “Start” in the window “Run Status”

Interpretation of the raw data:

- Select the “Analysis” menu, click the tab “Analysis Selection/Setup” and mark all positions for analysis
- Activate the following options in window “algorithm enhancement”:
 - Amplification-based threshold
 - Adaptive baseline
 - Moving average
- Set the threshold to automatic in tab “Results” and “Amplification Plots”
- Read the *Ct values* in “Text Report”

Related Products

Aqua Screen® Fast Extract Kit

32-1010/1050/1200	DNA Extraction Kit for water samples	10/50/250 extractions
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AquaScreen® Detection Kits for qPCR

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

PCR Calibration Reagents, 100 µl each, 1x10⁶ genomes/µl

52-0101	<i>Legionella pneumophila</i> DNA Calibration Reagent
52-0071	<i>Pseudomonas aeruginosa</i> DNA Calibration Reagent
52-0083	<i>Escherichia coli</i> DNA Calibration Reagent

DNA Remover™

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4 x 500 ml

DNA Remover™ Wipes

15-2001	DNA Decontamination Reagent, in spender box	120 wipes
15-2002	DNA Decontamination Reagent, refill sachets	5 x 120 wipes



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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



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