# BactoReal® Kit *Escherichia coli*

## Manual

**For use with the**

- **ABI PRISM® 7500 (Fast)**
- **Mx3005P®**
- **LightCycler® 480**


**For veterinary use only**

REF **DVEB01911, DVEB01913** Σ **100**

REF **DVEB01951, DVEB01953** Σ **50**

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## Index

## 1. Product description

BactoReal® Kit *Escherichia coli* is a real-time PCR assay for detection of *E. coli* DNA. This test was developed and validated for the ABI PRISM® 7500 (Fast) instrument (Life Technologies), LightCycler® 480 (Roche) and Mx3005P® (Agilent), but is also suitable for other real-time PCR instruments. This test allows the rapid and sensitive detection of DNA of *Escherichia coli* purified from sample material (e.g. with the QIAamp DNA Mini Kit).

BactoReal® Kit *Escherichia coli* detects the dxs gene of *E. coli* and *Shigella*. A probe-specific amplification-curve at 530 nm (FAM channel) indicates the amplification of *E. coli* DNA.
An internal positive control system for detection in VIC/HEX channel, (554 nm, order no. DVEB01911 or DVEB01951) or Cy5 channel (667 nm; order no. DVEB01913 or DVEB01953) excludes false-negative interpretation of results due to inhibition of real-time PCR (see 8. Interpretation of PCR-data).
When using PCR-platforms not validated by ingenetix, an evaluation of the multiplex-PCR is recommended. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

BactoReal®, MycoReal, ParoReal and ViroReal® Kits are optimized to run under the same thermal cycling conditions. RNA and DNA material can be analysed in one run.

## 2. Pathogen information

*Escherichia coli* is a gram negative bacterium that is a member of the normal gut flora of humans and animals. Most *E. coli* strains are harmless, but some serotypes carry virulence factors which enable them to be pathogenic in target hosts. These pathogenic *E. coli* are implicated in a number of diseases including diarrhoea, septicaemia and urinary tract infections in humans and animals. The dxs gene (1-deoxyxylulose-5-phosphate synthase gene) can be found in all *E. coli* strains.

**References:**
Lois LM, Campos N, Putra SR, Danielsen K, Rohmer M, Boronat A. 1998. Cloning and characterization of a gene from *Escherichia coli* encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamin, and pyridoxol biosynthesis. Proc Natl Acad Sci U S A. 95:2105-10.

## 3. Principle of real-time PCR

A specific DNA sequence of the pathogen genome is amplified and the generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows for a sequence-specific detection of PCR amplificates.

## 4. General Precautions

The user should always pay attention to the following:
- Always include a negative control per PCR-run (water instead of sample).
- Optional: for valid interpretation of results, a negative control should be included during DNA-extraction (for example extraction of water instead of sample material), in order to exclude false-positive results due to contamination with *Escherichia coli* DNA during extraction.
- Be careful when handling the positive control.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated workspace.
- Periodically decontaminate benches and devices.
- Use sterile pipette tips with filters.
- Thaw all components thoroughly at room temperature before starting an assay. When thawed, mix the components and centrifuge briefly.
- For MSDS, see www.ingenetix.com.

## 5. Contents of the Kit

### 5.1. BactoReal® Kit *Escherichia coli* order no. DVEB01911 or DVEB01951

| Labelling | Content | Amount | | Storage |
|---|---|---|---|---|
| | | DVEB01911 | DVEB01951 | |
| *Escherichia coli* Assay Mix (green cap) | Primer and probe (FAM) for detection of *E. coli* | 2 x 50 µl | 1 x 50 µl | -20°C |
| CR-1 Assay Mix (yellow cap) | Primer, probe (VIC/HEX) and target for detection of IPC | 2 x 50 µl | 1 x 50 µl | -20°C |
| *Escherichia coli* Positive Control (red cap) | Control-DNA (approx. 10,000 target copies/µl) | 1 x 25 µl | 1 x 25 µl | -20°C |
| DNA Reaction Mix (white cap)# | Reaction Mix | 2 x 500 µl | 1 x 500 µl | **-20°C until first use, then at +4°C** |
| Water (blue cap) | Water | 1 x 1000 µl | 1 x 1000 µl | -20°C to +4°C |

#DNA Reaction Mix contains uracil-N glycosylase (UNG)

### 5.2. BactoReal® Kit *Escherichia coli* order no. DVEB01913 or DVEB01953

| Labelling | Content | Amount | | Storage |
|---|---|---|---|---|
| | | DVEB01913 | DVEB01953 | |
| *Escherichia coli* Assay Mix (green cap) | Primer and probe (FAM) for detection of *E. coli* | 2 x 50 µl | 1 x 50 µl | -20°C |
| CR-3 Assay Mix (yellow cap) | Primer, probe (Cy5) and target for detection of IPC | 2 x 50 µl | 1 x 50 µl | -20°C |
| *Escherichia coli* Positive Control (red cap) | Control-DNA (approx. 10,000 target copies/µl) | 1 x 25 µl | 1 x 25 µl | -20°C |
| DNA Reaction Mix (white cap)# | Reaction Mix | 2 x 500 µl | 1 x 500 µl | **-20°C until first use, then at +4°C** |
| Water (blue cap) | Water | 1 x 1000 µl | 1 x 1000 µl | -20°C to +4°C |

#DNA Reaction Mix contains uracil-N glycosylase (UNG

The components of BactoReal® Kit *Escherichia coli* are stable until the expiry date stated on the label. Repeated thawing and freezing should be avoided. Please protect kit components from light.

## 6. Additionally required materials and devices

- Reagents and devices for DNA-extraction
- PCR-grade water
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and VIC/HEX or Cy5 channel
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material

## 7. Preparation of real-time PCR

Please make sure that at least one negative control (water, blue cap), as well as one positive control (red cap) and one extraction negative control (optional, recommended) are included per PCR run.
Ingenetix highly recommends performing PCR analyses in duplicates, which increases the probability of detection of the pathogen and facilitates interpretation of results.

### 7.1. Pipetting scheme

| | | Per sample |
|---|---|---|
| **Preparation of Master Mix** (mix well) | Water* | 3.0 µl |
| | DNA Reaction Mix (2x) | 10.0 µl |
| | *Escherichia coli* Assay Mix | 1.0 µl |
| | CR Assay Mix | 1.0 µl |
| | **Total volume Master Mix** | **15.0 µl** |
| **Preparation of PCR** | Master Mix | 15.0 µl |
| | Sample* | 5.0 µl |
| | **Total volume** | **20.0 µl** |

*1-8 µl of the sample can be used. When using an amount other than 5 µl of the sample, the amount of $H_2O$ has to be changed accordingly.

**Positive Control:** As positive control please use 1 µl of the *Escherichia coli* Positive Control + 4 µl $H_2O$.
Optional: a 1:10 dilution of the positive control can be used and defined as second standard value (approx. 1000 target copies/µl).

### 7.2. Programming of the temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

**Select dyes:** FAM-TAMRA for detection of *E. coli*
Cy5-NONE (CR-3 Assay Mix) **or** VIC-TAMRA (CR-1 Assay Mix) for detection of IPC
**Select reference dye (passive reference)**: ROX
**Sample Volume:** 20 µl
**Temperature Profile:**

| **Program 1** | **Program 2** | **Program 3** |
|---|---|---|
| Cycles: 1<br>Analysis: None | Cycles: 1<br>Analysis: None | Cycles: 45<br>Analysis: Quantification<br>Acquisition at 60° |
| 50°C<br>2 min* | 95°C<br>20 sec | 95°C<br>5 sec<br>60°C<br>1 min |

For ABI PRISM® 7500:
Ramp speed: Without "fast cycling" parameter

For LightCycler® 480 instrument:
Detection format: 2 Color Hydrolysis Probe (dyes see above)

***Note:** If viral RNA should be also detected in the same PCR run, program 1 has to be prolonged to 15 min at 50°C. This temperature profile can be used for all BactoReal®, MycoReal, ParoReal and ViroReal® kits for the detection of DNA or RNA.

## 8. Interpretation of PCR-data

Examples for interpretation of positive reactions are shown in the amplification plots below.

**For a valid interpretation, the following criteria must be fulfilled:**

| | Ct/Cp (FAM channel) *Escherichia coli* target | Ct/Cp IPC target | Interpretation |
|---|---|---|---|
| Negative control | Negative / >34* | 36.0 ± 2 | Valid |
| Positive control (undiluted, 1 µl/PCR) | 27.0-30.0 | 36.0 ± 2 | Valid |
| Or: positive control (1:10 diluted, 1 µl/PCR) | 30.0-33.0 | 36.0 ± 2 | Valid |
| Extraction negative control (optional) | Negative / >34* | 36.0 ± 2 | Valid |
| Negative sample | Negative | 36.0 ± 2 | Valid |
| Positive sample | Positive** | Positive/Negative | Valid |

* Ct/Cp values >34 result from amplification of low concentrations of *E. coli* DNA present in the reaction mix.
***E. coli* can be found ubiquitously in the environment. Therefore, contamination with *E. coli* DNA may lead to false-positive results. Contamination might happen during sample taking, DNA extraction and preparation of the PCR-reaction or might be due to contaminated reagents. An *E. coli*-specific amplification curve of the sample has to be interpreted in context of the Ct/Cp-values of the negative control: The Ct/Cp value of the sample has to be at least 3 Ct/Cp values lower than that of the negative extraction control as well as from contamination with *E. coli* DNA.

**For analysis of PCR data please proceed as follows:**
For analysis of PCR results gained with BactoReal® Kit *Escherichia coli* please select fluorescence display options FAM channel for the *Escherichia coli* target and VIC/HEX channel (order no. DVEB01911, DVEB01951) or Cy5 channel (order no. DVEB01913, DVEB01953) for the internal positive control target. Samples with a positive Cp or Ct-value are considered positive. Please also check the presence of amplification-curves manually.

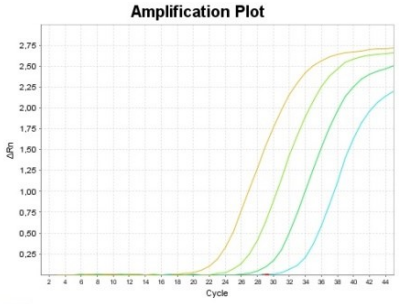
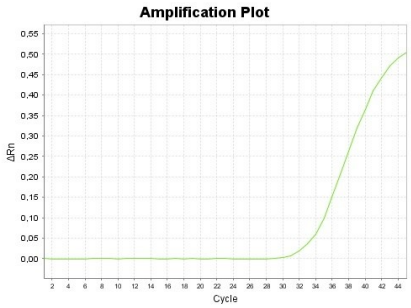
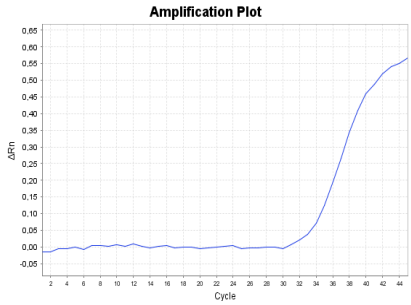
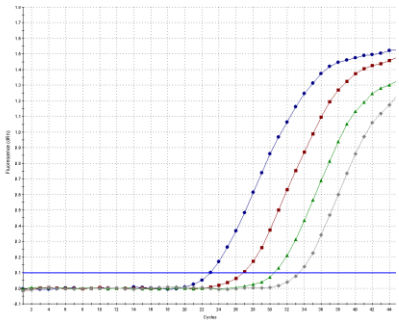
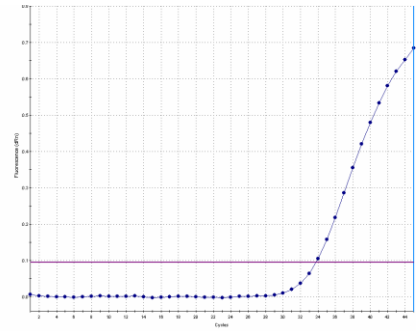
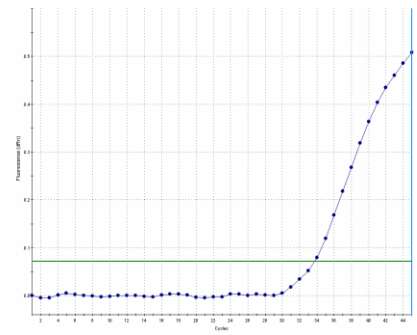
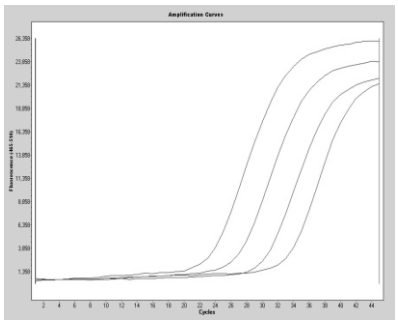
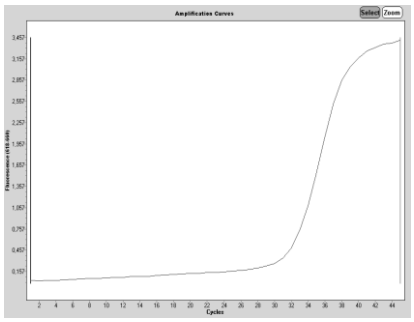
**Once the analysis is completed, the following results are possible:**

**1. Signal in FAM channel:**
→ DNA of *E. coli* was amplified. The sample has to be interpreted as positive (see also criteria for valid interpretation above).
*E. coli* DNA can lead to a reduced or absent fluorescence signal of the internal positive control assay (competition of PCR).

**2. No signal in FAM channel:**
→ No *E. coli* DNA is detectable in the sample. The sample has to be interpreted as negative.
An inhibition of PCR cannot be excluded.

**2a. No signal in FAM channel but signal of the internal positive control:**
→ No *E. coli* DNA is detectable in the sample. The sample has to be interpreted as negative.
The positive signal of the internal positive control assay excludes a putative PCR inhibition.

**2b. No signals in FAM channel and no signal with internal positive control:**
→ No interpretation statement can be made.
Information about possible sources of error and their solution can be found in 9. Troubleshooting.

| Detection of ***Escherichia coli*** | Detection of internal positive control **CR-3** | Detection of internal positive control **CR-1** |
|---|---|---|
|  |  |  |
| **ABI Prism® 7500:** FAM channel, 530 nm<br>1:10 serial dilution of *E. coli* DNA | **ABI Prism® 7500:** Cy5 channel, 667 nm<br>Internal positive control | **ABI Prism® 7500:** VIC channel, 554 nm<br>Internal positive control |
|  |  |  |
| **Mx3005P®:** FAM channel<br>1:10 serial dilution of *E. coli* DNA | **Mx3005P®:** CY5 channel<br>Internal positive control | **Mx3005P®:** HEX channel<br>Internal positive control |
|  |  | |
| **LightCycler® 480:** FAM channel<br>1:10 serial dilution of *E. coli* DNA | **LightCycler® 480:** Cy5 channel<br>Internal positive control | |

## 9. Troubleshooting

**1. No *E. coli* specific signal with positive control:**

- Incorrect programming of the temperature profile of the real-time PCR instrument.
  → Compare the temperature profile with the protocol (see 7. Preparation of real-time PCR).
- Incorrect configuration of the PCR reaction.
  → Check your work steps (see 7. Preparation of real-time PCR) and repeat the PCR, if necessary.

**2. No signal with the internal positive control and no *E. coli* specific signal with the sample:**

- The PCR reaction was inhibited. No interpretation can be made.
  → Make sure that you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions.
  → If no operating mistakes during extractions can be retraced, it is recommended to repeat the PCR with lower amounts of DNA-eluate (1/5 or 1/10 of sample volume + the adequate amount of $H_2O$).
- Incorrect PCR conditions.
  → Check the PCR conditions and repeat the PCR, if necessary.

**3. *Escherichia coli* specific signal (Ct/Cp values <34) with the negative control (see 8. Interpretation of PCR-data):**

- A contamination occurred during preparation of the PCR.
  → Repeat the PCR with new reagents in replicates.
  → Strictly pipette the positive controls at last.
  → Make sure that work space and instruments are decontaminated at regular intervals.

**4. *Escherichia coli* specific signal (Ct/Cp values <34) with the negative control of DNA-extraction (see 8. Interpretation of PCR-data):**

- A contamination occurred during extraction.
  → Repeat the extraction and PCR using new reagents.
  → Make sure that work space and instruments are decontaminated at regular intervals.

## 10. Specifications

BactoReal® Kit *Escherichia coli* was evaluated with the ABI PRISM® 7500 (Fast) instrument (Life Technologies), with the LightCycler® 480 (Roche) and the Mx3005P® (Agilent). For further validation data please contact ingenetix.

### 10.1. Detection limit

The DNA reaction mix contains residual *E. coli* DNA, which gives rise to a positive amplification at Ct >34. Therefore, the detection limit is 1000 copies/reaction.

### 10.2. Analytical specificity

The specificity is ensured by the selection of highly specific primers and probes. The primers and probes were checked for possible homologies to currently published sequences by sequence comparison analyses. This also validated the detection of so far known *E. coli* strains. BactoReal® Kit *Escherichia coli* shows cross reaction with *Shigella*.

## 11. Annex – symbols

| | | | |
|---|---|---|---|
| LOT | Batch code | | Use by |
| REF | Catalogue number | | Manufactured by |
| Σ | Contains sufficient for <n> tests | | Store at |