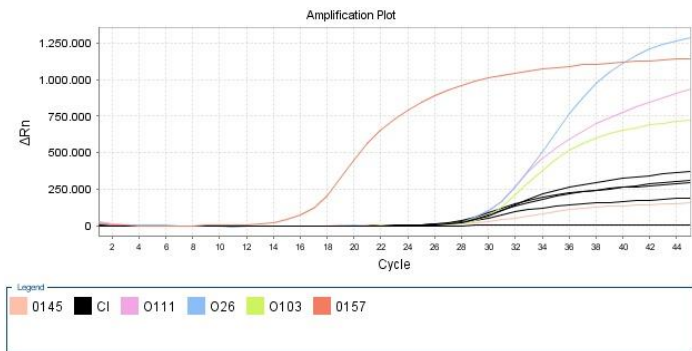
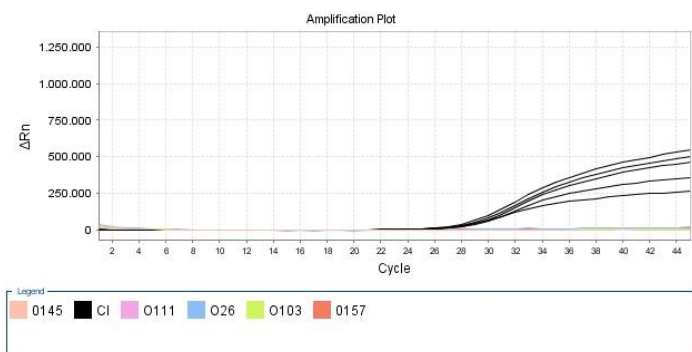


Positive Control –



Negative Control –



12. SPECIFICITY/INCLUSIVITY

- 100 % Exclusivity, determined using 30 strains of closely related organisms or occurring in the same habitat.
- 100 % Inclusivity, determined in 45 *Escherichia coli* strains and 5 positive cultures.



REAL TIME DETECTION KIT

E. coli serogroup determination O26, O103, O111, O145, O157

Ref: BIOPFS-0045

1. PRODUCT DESCRIPTION

Pathogen detection kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific pathogen. The assay is based on 5' nuclease real time PCR reactions to amplify a unique genomic sequence in the target microorganism.

2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence in a reaction containing among other components, a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. The DNA molecule denatures when this solution is heated, separating into two strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands and the DNA polymerase synthesizes a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence (amplicons). When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target amplicons. In Real Time PCR, the signal is measured in each cycle, using in most cases specific fluorescent probes. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus the number of cycles, allowing the determination of the presence or absence of the target organism.

3. PATHOGEN DESCRIPTION

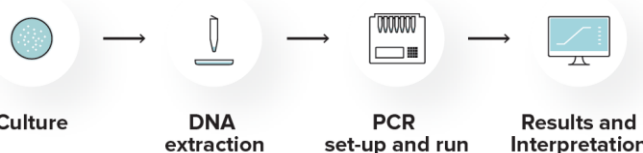
Escherichia coli, which is part of the microbiota of the intestine, can cause enteric/diarrhoeogenic or extra-intestinal infections in humans. Infectious enteric *E. coli* can present different virulence determinants corresponding to different pathotypes. These include the verocytotoxigenic *E. coli* (VTEC), comprising the enterohaemorrhagic *E. coli* (EHEC), and the enteropathogenic *E. coli* (EPEC). VTEC is characterized by the production of verotoxin (Vtx), encoded by *vtx1* or *vtx2* genes (also known as Shiga-like toxins - Stx - corresponding to *stx1* and *stx2* genes). Shiga toxin-producing *E. coli* (VTEC) can cause diarrhea as well as more severe diseases in humans such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). Although VTEC may belong to a large number of serogroups, those that have been firmly associated with the most severe forms of the disease, in particular HUS, belong to O157, O26, O111, O103, and O145 (ISO/TS 13136:2012(E), 2012). Conventional serotyping is a method based in antibody-antigen agglutination reactions. These is a very complex and time-consuming method. Recently, rapid and sensitive methods have been found to be attractive alternatives to the *E. coli* conventional serotyping method. These methods are PCR-based methods, in particular real time PCR-based, that allow the rapid detection of different antigen genes, which in turn allow the determination of *E. coli* serogroups.

4. INTERNAL CONTROL (IC)

Pathogen detection kits include an Internal Control (IC) in the master mix. This control is amplified at the same time as the target DNA sequence, but using a different set of primers and a probe labeled with a different fluorophore. The inclusion of the IC in each reaction allows the evaluation of PCR inhibitors in negative results.

5. APPLICATION

REAL TIME DETECTION KIT *E. coli* serogroup determination is intended to rapidly detect the five serogroups of this microorganism - *E. coli* O157, O26, O111, O103, and O145. The different serogroups reactions can be performed independently. The kit was validated in the instruments ABI PRISM® 7500 Fast and ThermoScientific® PikoReal. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Food use only. The procedure includes the following main steps:



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Website: <http://www.biopremier.com>

BPMP is certified ISO 9001:2015

6. CONTENTS AND STORAGE

The kit contains reagents for 50 assays for each target

CONTENTS	UNITS	COMPOSITION
Master Mix (blue cap)	● 5 tubes (5 x 840 µl)	Buffer, dNTPs, DNA polymerase
Assay Mix – O26 (brown cap)	● 1 tube (1 x 105 µl)	Primers and Probes
Assay Mix – O103 (brown cap)	● 1 tube (1 x 105 µl)	Primers and Probes
Assay Mix – O111 (brown cap)	● 1 tube (1 x 105 µl)	Primers and Probes
Assay Mix – O145 (brown cap)	● 1 tube (1 x 105 µl)	Primers and Probes
Assay Mix – O157 (brown cap)	● 1 tube (1 x 105 µl)	Primers and Probes
Negative Control (clear cap)	○ 1 tube (1 x 175 µl)	Nuclease-free water
Positive Control (red cap)	● 1 tube (1 x 175 µl)	Target DNA

Store all contents at -20°C and protect them from light as excessive exposure to light may affect the fluorescent probes. Minimize freeze-thaw cycles. Reagents stored as recommended may be used until the expiration date indicated in the tube.

7. MATERIAL REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease-free filter tips
- Real-time PCR instrument
- Tubes/Strips/Multiwell plates and accessories specific for each Instrument
- Lysis buffer (example: BIOPEXT-0400).

8. PRECAUTIONS AND RECOMMENDATIONS

Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials, and equipments for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.

The user should always pay attention to the following:

- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

9. QUALITY CONTROL

In accordance with BPMR's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

10. PROCEDURE

10.1 DNA extraction

1. Resuspend a few colonies in 100 µL of lysis solution - BIOPREMIER DNA Rapid Extraction Buffer (ref: BIOPEXT-0400) (not included).
2. Homogenized the sample vortexing the tube.
3. Place the tube in the heat block at 95-100°C for 10-15 minutes.
4. Centrifuge at 10,000-12,000 g for 2 min.
5. Remove the supernatant to a clean tube. Store at -20°C if you want to reuse. Homogenize and centrifuge 10,000-12,000 g for 2 min before reusing.

10.2 PCR preparation

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	N° OF SAMPLES	
	1	10 (10 + 1)
qPCR reaction		
Master Mix	16 µl	176 µl
Assay Mix – O26 or Assay Mix – O103 or Assay Mix – O111 or Assay Mix – O145 or Assay Mix – O157	2 µl	22 µl
Total Volume	18 µl	198 µl

Note: Prepare the PCR reaction for each sample, or in the alternative, prepare a Master Mix for the total number of reactions plus 10% to cover pipetting losses (e.g.

for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 18 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 2 µL of Negative Control tube (Clear Cap); Pipette 2 µL of DNA sample per well; and for the positive control, pipette 2 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. Place the reactions into the Real Time PCR instrument.

B – Program set up

Prepare the Real-Time PCR instrument according to the following temperature/time program:

PHASE	STEP	TEMPERATURE	TIME	ACQUISITION
Holding Stage	Step 1	50°C	2 min.	No
	Step 2	95°C	5 min.	No
Amplification - 40 cycles	Step 1	95°C	30s	No
	Step 2	60°C	30s	Yes
	Step 3	72°C	30s	No

TARGET	CHANNELS
Detection of <i>E. coli</i> 026, 0103, 0111, 0145 or 0157	FAM
Detection of Internal Control (IC)	ROX

11. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.

Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Assessment of sample results should be performed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted. Interpretation of PCR data:

a) Controls

To validate the assay, the controls must have the following results:

	<i>E. coli</i> 026, 0103, 0111, 0145 or 0157 DETECTION FAM	IC DETECTION ROX
Negative Control	Negative	Positive
Positive Control	Positive	Positive

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples

Interpretation of sample results is summarized in the following table:

<i>E. coli</i> 026, 0103, 0111, 0145 or 0157 DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Positive/Negative**	DNA detected, sample Positive for <i>E. coli</i> serogroup
Negative	Positive	No DNA detected, sample Negative for <i>E. coli</i> serogroup
Negative	Negative	Invalid Result*

*When both *E. coli* and IC detection are Negative, this means the presence of PCR inhibitors in the sample. Dilute the DNA sample (10-fold dilution) or perform another DNA extraction and repeat qPCR.

** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.