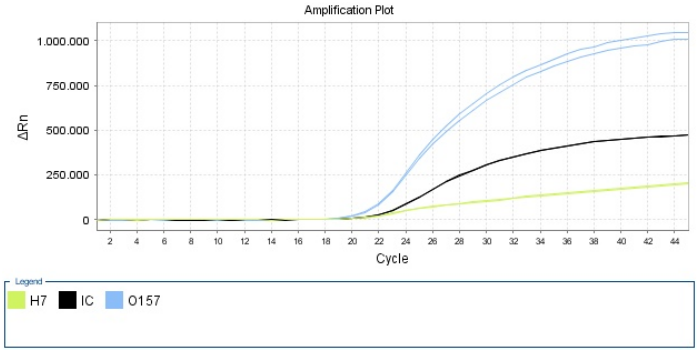
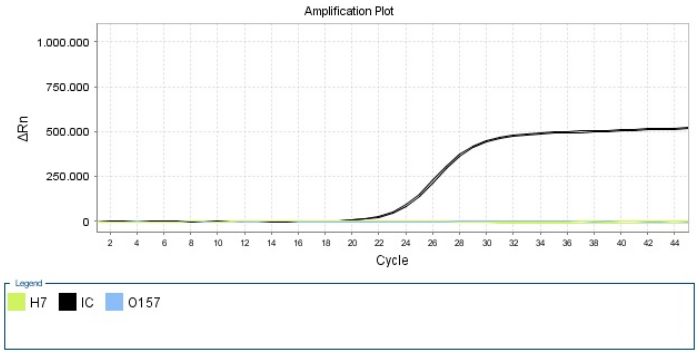
Positive Control –



Negative Control –



11. SPECIFICITY/INCLUSIVITY

a) 100 % Exclusivity, determined using 13 strains of closely related organisms or occurring in the same habitat, including non-pathogenic *E. coli* and other serotypes of *E. coli* (Table 1).
b) 100 % Inclusivity, determined in 9 strains of *E. coli* O157:H7 and *E. coli* O157 (Table 1).

12. PERFORMANCE CHARACTERISTICS

A detection limit of 1 to 10 Cells per 25g of food sample can be achieved after enrichment. The SUPREME REAL TIME DETECTION KIT *E. coli* O157:H7 has a reaction sensitivity of 1 pg of target DNA.

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BPMR is certified ISO 9001:2015

BIOPREMIER

SUPREME
REAL TIME DETECTION KIT

Escherichia coli O157:H7/O157 (duplex)

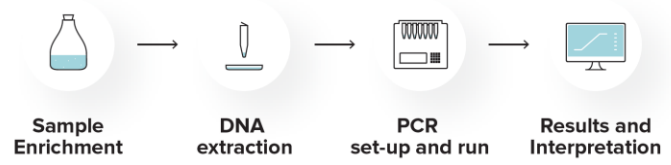
Ref: BIOPSFS-0059

1. PATHOGEN DESCRIPTION

Escherichia coli (E. coli) is a bacterium that is commonly found in the gut of humans and warm-blooded animals and most of the strains are harmless. Some strains however, such as enterohemorrhagic *E. coli* (EHEC) O157:H7 can cause serious foodborne outbreaks that cause diarrhoea, fever, and vomiting in humans.
E. coli O157:H7 is recognized by its somatic (cell wall) antigen (O157) and its flagella antigen (H7). In addition, *E. coli* O157:H7 is known to produce Shiga-like toxins, which cause severe symptoms.
The reservoir of this pathogen appears to be mainly cattle, in addition to others such as ruminants, mammals, and birds.
E. coli O157:H7 is transmitted to humans primarily through the consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Fecal contamination of water and other foods and cross-contamination during food preparation (with beef and other meat products, contaminated surfaces, and kitchen utensils) will also lead to infection.

2. INTENDED USE

SUPREME REAL TIME DETECTION KIT *E. coli* O157:H7 / O157 (duplex) is a kit for the detection of pathogenic *E. coli* O157 and allows the simultaneous detection of the serotype O157:H7 DNA in a real-time PCR based on the use of TaqMan technology, providing a simple, reliable and rapid result for the detection of *E. coli* O157:H7 / O157 infection. The kit includes an Internal Control (IC). The IC is used for the evaluation of PCR inhibitors in the sample, or for the evaluation of problems that occurred during PCR preparation/amplification. The kit includes Master mix for the target and IC, primers and TaqMan® probes, labelled with non-fluorescent quenchers. The signal for the detection of the targets is detected in the FAM (*E. coli* O157) and HEX / VIC (serotype H7) channels, and the one for the IC in the ROX channel. The kit contains Uracil-DNA Glycosylase (UDG), preventing DNA contamination with PCR products. Included in the kit is also a positive control, allowing an evaluation of the primers and probes used for the detection of the targets and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.
The kit was validated in the instruments ABI PRISM® 7500 Fast. The kit is compatible with all thermocyclers working in **FAM, JOE / VIC/ HEX** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Food use only.
The procedure includes the following main steps:



3. TRADEMARKS AND DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

4. LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

5. QUALITY CONTROL

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

6. WARNINGS AND PRECAUTIONS

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.

7. CONTENTS AND STORAGE CONDITIONS

The kit should be stored between -20°C and 5°C and protected from exposure to light. Repeated thawing and freezing (>5) should be avoided, as this may reduce the sensitivity. Considerate to freeze in aliquots to maintain the performance of the assay.
The kit contains reagents for 100 reactions.

CONTENTS	UNITS	COMPOSITION
Master Mix (blue cap)	2 tube (2 x 683µl)	Buffer, dNTPs, Primers and Probes
Enzymes (yellow cap)	1 tube (1 x 220µl)	DNA polymerase, UDG and storage buffer
Negative Control (clear cap)	1 tube (1 x 130µl)	Nuclease-free water
Positive Control (red cap)	1 tube (1 x 130µl)	Target DNA

8. MATERIALS 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 / DNA extraction kit (example: BIOPEXT-0400/ BIOPEXT-0609)

9. PROCEDURE

9.1. ENRICHMENT / DNA EXTRACTION

Recommended a pre-enrichment according to ISO/TS 13136 or *E. coli* isolated colonies.

9.2. DNA EXTRACTION

1. Collect 1 mL of enriched sample and centrifuge at 10,000 - 12,000 g for 5 min.
2. Discard all the supernatants.
3. Wash the pellet: add 1 mL of 0.9% NaCl solution or PBS.
4. Centrifuge at 10,000-12,000 g for 5 min and discard all the supernatant.
5. Use kit or suitable protocol for DNA extraction. Such as BIOPREMIER DNA Rapid Extraction Buffer (ref: BIOPEXT-0400) or BIOPREMIER DNA Extraction Kit from Food (ref: BIOPEXT-0609) (not included).

9.3. PCR PREPARATION

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly (do not vortex the tube Enzymes) and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	Nº OF SAMPLES	
qPCR reaction	**1**	**10 (10 + 1)**
Master Mix	13 µl	143 µl
Enzymes	2 µl	22 µl
Total Volume	**15 µl**	**165 µl**

Note: Prepare the PCR reaction for each sample, or in alternative, prepare a Master Mix for the total number of reactions plus 10% (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 15 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 5 µL of Negative Control tube (Clear Cap); Pipette 5 µL of DNA sample per well; and for the positive control, pipette 5 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	TEMPERATURE	CYCLES	TIME	ACQUISITION
Incubation	37 ºC	1	15 min	No
UDG Inactivation	95 ºC	1	5 min	No
DNA Amplification	94 ºC	45	15 s	No
	60 ºC		30 s	Collect Data
	72 ºC		15 s	No

Sample Volume: 20 µl
Passive reference dye: None
Detection format: Hydrolysis Probe

TARGET	CHANNELS
Detection of *E.coli* O157	FAM | Excitation at 465 nm, Emission at 510 nm
Detection of H7	VIC | Excitation at 540/533 nm, Emission at 580 nm
Detection of Internal Control (IC)	ROX | Excitation at 533 nm, Emission at 610 nm

10. 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. Sample results should be assessed 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:

	E. coli O157 DETECTION FAM	H7 DETECTION VIC	IC DETECTION ROX
Negative Control	Negative	Negative	Positive
Positive Control	Positive	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:

E. coli O157 DETECTION FAM	H7 DETECTION VIC	IC DETECTION ROX	INTERPRETATION
Positive	Positive	Positive/Negative**	Positive for *E. coli* O157:H7
Positive	Negative	Positive/Negative**	Positive for *E. coli* O157
Negative	Negative	Positive	Negative
Negative	Positive	Positive/Negative**	Negative (other flagellated H7 bacteria)
Negative	Negative	Negative	Invalid Result*

*When both *E. coli* and IC detection are Negative, means the presence of PCR inhibitors in the sample. Dilute the sample or perform another DNA extraction.
** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.

Table 1: Exclusivity and Inclusivity of the SUPREME Real Time Detection Kit *E. coli* O157:H7 / O157 (duplex)

ID	Strain	Source	SUPREME Kit Result		Result comparison
			O157	H7	
MB 113	Escherichia coli serotype O6, biotype 1		NEG	NEG	Matched
MB 132	Escherichia coli O157:H7		POS	POS	Matched
MB 189	*E. coli*		NEG	NEG	Matched
MB 190	*E. coli*		NEG	NEG	Matched
MB 239	*E. coli*		NEG	NEG	Matched
MB 240	Escherichia coli O157:H7		POS	POS	Matched
MB 260	Escherichia coli O157, stx-		POS	NEG	Matched
MB 264	Escherichia coli BLSE+		NEG	NEG	Matched
MB 265	Escherichia coli O157		POS	NEG	Matched
MB 358	Escherichia coli O157:H7, eae +		POS	POS	Matched
MB 607	*E. coli*		NEG	NEG	Matched
MB 619	Escherichia coli O145		NEG	NEG	Matched
MB1027_A	Escherichia coli O26		NEG	NEG	Matched
MB1027_B	Escherichia coli O11		NEG	NEG	Matched
MB1028_A	Escherichia coli O103		NEG	NEG	Matched
MB1028_B	Escherichia coli O145		NEG	NEG	Matched
SO 673	Escherichia coli O157:H7	EQC *- PHE	POS	POS	Matched
SO 674	Escherichia coli O157:H7	EQC *- PHE	POS	POS	Matched
M223D11A	Escherichia coli O157:H7	EQC* - FAPAS	POS	POS	Matched
M223D11B	E. coli / P. putida/ C. freundii	EQC *- FAPAS	NEG	NEG	Matched
SO 697	Escherichia coli O157:H7	EQC* - PHE	POS	POS	Matched

*EQC – External Quality Control