Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 02
Revision date: 05/06/2023

1. Identification

Product name **PRImeDETECT™ STEC Detection Kit**
(Shigatoxigenic *Escherichia coli*: STEC)

Cat. No **FP0036** (96 reactions)

2. Description

Shiga toxin-producing *Escherichia coli* (STEC) are important enteric pathogens worldwide, causing diarrhea with or without blood visibly present and hemolytic uremic syndrome. Pathogenic **STEC** are characterized by the production of Shiga-toxin **(stx)** and are often shown to produce attaching and effacing lesions on intestinal mucosa. This latter property is encoded by genes, including **eae**, grouped together in a pathogenicity island referred to as the 'locus of enterocyte effacement'.

PRImeDETECT™ STEC Detection Kit is based on amplification and detection of the virulence-associated genes **stx1, stx2** and **eae** by the real-time PCR method (multiplex PCR).
All reagents required for qPCR are provided ready to use as Multiplex PCR Master Mix (1 vial). The Multiplex PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and MgCl2 to perform the number of reactions indicated in the kit. The Multiplex PCR Master Mix also includes an internal amplification control (IAC) whose detection indicates the absence of PCR inhibitors. Primers and probes for the amplification of IAC as well as for the amplification of the target gene are included in the Master Mix. The probe for the detection of target gene is labelled with the FAM (stx1), HEX (stx2) and ROX (eae) fluorochrome, whereas the probe for the detection of IAC is labelled with the CY5 fluorochrome.
In addition, the kit includes positive control DNA (stabilized solution of *E. coli* O157 genomic DNA) and negative control (Nuclease-free, PCR-grade H2O). The positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a negative control reaction should be included every time the kit is used

3. Composition

✓ Multiplex PCR Master Mix (1 vial)

✓ PCR Positive Control (1 vial)

✓ PCR Negative Control (1 vial)

4. Features

- ✓ **Amplification limit:** 5 DNA copies per reaction
- ✓ **Quantification Dynamic range:** 4 logs
- ✓ **Simultaneously** detect and differentiate the major STEC-associated virulence marker genes: stx1, stx2 and eae in the same PCR amplification reaction.
- ✓ **Inclusivity:** specifically reacts with all strains of *E. coli* with the indicated target genes (stx1, stx2 and eae).
- ✓ **Compliance: ISO/TS 13136:2012**
- ✓ **Exclusivity:** No cross-reactions with other strains of Non- Shiga toxin-producing *E. coli* (STEC).
- ✓ **Detection:** probe labelled with fluorescent dyes– FAM-BHQ1 ***(stx1)***, HEX-BHQ1***(stx2)***, ROX-BHQ2 ***(eae)*** and CY5-BHQ1**(IAC).**
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

5. Storage specifications

PRImeDETECT™ STEC Detection Kit is shipped at ambient temperature. On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month.

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6. PCR reaction

1. Pipet 15 µl PCR mix into each PCR vessel.
2. For the samples of interest, add 5 µl of the extracted DNA sample.
   For the negative control, add 5 µl H2O, PCR-grade (PCR Negative Control).
   For the positive control, add 5 µl *E. coli* O157 Control Template (PCR positive Control).
3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
4. Place the PCR vessel into the real time thermal cycler. Cycle the samples as described above

7. PCR cycling conditions

Step	Time	Temperature
1 Cycle: Initial PCR activation step	10 min	95 ˚C
40 Cycles: Denaturation, Annealing and Extension	15 sec 1 min	95 ˚C 60 ˚C*

*Data collection at 60˚C for channels green (FAM), yellow (HEX), orange (ROX) and red (Cy5).

8. Analysis of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data.

Quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value. The positive control is intended to verify correct assay performance and should not be used as the sole reference for quantitative analysis.

A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold value for detector. It is recommended to analyze each fluorescence channel separately. A reaction will be considered negative whenever no amplification curve is produced or fluorescence does not cross the threshold and there is an amplification curve for IAC at the expected Ct.

Interpretation of results:

Detector HEX	Detector ROX	Detector FAM	IAC Detector Cy5	Interpretation
(+)	(+)	(+)	(+) **or (-)**	Positive
(-)	**(-)**	**(-)**	(+)	Negative
(-)	**(-)**	**(-)**	**(-)**	Inhibition*

**The sample might contain PCR inhibitors. In this case the test needs to be repeated with diluted sample.*

9. Further information

Product Use Limitations This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Safety Information When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.