

Data sheet

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

Cat. No: FP0035 (48 reactions)

Cat. No: FP0036 (96 reactions)

Introduction

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 (*sxt1*), 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 and negative control. 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

Include DNAREady lysis buffer to extract the DNA from the sample prior to PCR detection.

Each kit contains:

- ✓ Multiplex PCR Master Mix (1 vial)
- ✓ DNAREady lysis buffer (1 bottle)
- ✓ PCR Positive Control (1 vial)
- ✓ PCR Negative Control (1 vial)

Shipping and Storage

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.

Technical 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-BHQ1a (*sxt1*), HEX-BHQ1(*stx2*), ROX-BHQ2 (*eae*) and CY5-BHQ1(IAC).
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

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PCR cycling conditions

Step	Time	Temperature
Initial denaturation	10 min	95°C
40 Cycles:	15 sec	95°C
	1 min	60°C
Melt analysis	Refer to instrument instructions	

Analysis of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data. The user may also, optionally, analyze the melt profile of each reaction.

The quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value.

PRODUCT USE LIMITATION

Not For Medical Diagnostic Use.

Please refer to www.canvaxbiotech.com for the Material Safety Data Sheet of the product.

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