

PCR-STE^C Detection Kit

(Catalog# K1452-96; 96 Rxns; Storage at -20°C)

I. 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.

PCR-STE^C Detection Kit is an ideal tool for a specific, rapid, and reliable amplification and detection of the virulence-associated genes *stx1*, *stx2* and *eae* by the real-time PCR method (Multiplex PCR). The kit includes all reagents required in a comfortable ready-to-use Multiplex PCR MasterMix. The optimized MasterMix contains a Buffer, dNTPs, Hot-start DNA Polymerase, DNA-free water, MgCl₂ and 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 MasterMix. 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.

Additionally, the kit includes both Positive Control (stabilized solution of E.coli O157 genomic DNA) and Negative Controls (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.

II. Applications:

- An ideal tool for specific, rapid, and reliable amplification and detection of the virulence-associated genes *stx1*, *stx2* and *eae* by real-time PCR method (Multiplex PCR).

III. Sample Type:

- Food samples

IV. Kit Contents:

Components	K1452-96	Part Number
Multiplex PCR Master Mix	2 Vials	K1452-96-1
PCR Positive Control	1 Vial	K1452-96-2
PCR Negative Control	1 Vial	K1452-96-3
DNA Lysis Buffer	2 Vials	K1452-96-4

V. User Supplied Reagents and Equipment:

- Thermal Cycler such as Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers
- PCR tubes

VI. Storage Conditions and Reagent Preparation:

All the reagents are shipped in dry ice and stored at -20°C upon receipt. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months.

VII. Assay Protocol:

- Use an appropriate procedure for extraction of nucleic acid from Gram-negative bacteria. Optionally you can use our DNA Lysis Buffer suitable for DNA extraction.
- Pipet 15 µl PCR mix into each PCR tube.
- 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).
- Mix carefully but thoroughly by pipetting up and down. Do not vortex.
- Place the PCR tube in the real time thermal cycler. Cycle the samples as described above

PCR Cycling Conditions:

Step	Time	Temperature
Initial PCR activation step	10 min	95°C
40 Cycles	15 sec	95°C
Denaturation, Annealing and Extension	1 min	60°C*
Melt Analysis	Refer to instrumental instructions	

*Data collection at 60°C for channels green (FAM), yellow (HEX), orange (ROX) and red (CY5)

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

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

VIII. Related Products:

Product Name	Cat. No.	Size
PCR-Salmonella Detection Kit	K1447	96 Rxns
PCR-Salmonella-Listeria Detection Kit	K1448	96 Rxns
PCR-Listeria monocytogenes Detection Kit	K1449	96 Rxns
PCR-Legionella spp Detection Kit	K1450	96 Rxns
PCR-Legionella spp Plus Detection Kit	K1451	96 Rxns
PCR-Campylobacter Detection Kit	K1453	96 Rxns
Coronavirus Rapid RT-qPCR Detection Kit	K1461	100 Rxns

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