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# PCR-STEC 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-STEC 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 $H_2O$). 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:

1. 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.
2. Pipet 15 µl PCR mix into each PCR tube.
3. For the samples of interest, add 5 µl of the extracted DNA sample.
   For the negative control, add 5 µl $H_2O$, PCR-grade (PCR Negative Control).
   For the positive control, add 5 µl E. coli O157 Control Template (PCR positive Control).
4. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
5. 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:***

1. 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.
2. The quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value.
3. 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.
4. 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 |

***FOR RESEARCH USE ONLY! Not to be used on humans.***