ab287888 – PCR-STEC Detection Kit

For the rapid and reliable amplification and detection of the virulence-associated genes stx1, stx2 and eae by real-time PCR method

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287888

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Multiplex PCR Master Mix	2 Vial	–20°C
DNA Lysis Buffer	2 Vials	–20°C
PCR Positive Control	1 Vial	–20°C
PCR Negative Control	1 Vial	–20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- PCR tubes
- Real Time Thermal cycler

Assay Protocol

1. Use an appropriate procedure for extraction of nucleic acid from Gram-negative bacteria. Optionally you can use the provided 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.
    a. For the negative control, add 5 µl H2O, PCR-grade (PCR Negative Control).
    b. 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

PCR Cycling Conditions:

Step	Time	Temperature (°C)
Initial PCR Activation Step	10 Minutes	95
40 Cycles	15 Seconds	95
	1 Minute	60
MeltAnalysis	Refer to Instrumental Instructions	

ΔNote: *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		
+	+	+	+/-	Positive
-	-	-	+	Negative
-	-	-	-	Inhibition△

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)