Instructions for use
InviScreen® STEC Detection Kit

INVITEK diagnostics

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REF 6015001200 | Σ 100 tests | -15 ºC / -25 ºC | RUO

1. Intended use

The InviScreen® STEC Detection Kit is specifically designed for the qualitative detection of Shiga toxin-producing *Escherichia coli* (STEC) strains in food samples. This kit is intended for use by trained laboratory professionals in accordance with the ISO 13136 standard. The PCR kit provides a sensitive and reliable method for the detection of STEC *E. coli* in various food matrices, including but not limited to meats, dairy products, vegetables, and processed foods. Laboratory personnel should follow the instructions provided in this manual carefully, including the recommended sample preparation procedures, PCR protocol, and result interpretation guidelines. It is important to note that this kit does not differentiate between different serotypes or subtypes of STEC *E. coli*, nor does it determine the presence of specific serogroups. STEC serotyping can be performed using the InviScreen® STEC Serotyping Kit.

2. Product description

The InviScreen® STEC Detection Kit follows the ISO 13136 standard method. The method uses the real-time PCR technique to detect the presence of the *stx1* and *stx2* genes (associated with cytotoxicity) and the *eae* gene associated with the capability of the bacteria to adhere to the intestinal epithelium and cause disease. The method is applicable to products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling, and environmental samples in the area of primary production. Amplification detection is based on Taqman technology, whereby fluorescence is detected upon the degradation of the hydrolysis probe during the PCR reaction. This kit includes an Internal Amplification Control (IAC) detection primer/probe to rule out the presence of PCR inhibitors.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D25.01	Primer/Probe Mix – stx1/stx2 [1]	Targeted detection	Brown	1 tube, 1000 µL
D25.02	Primer/Probe Mix – eae/IAC [1]	Targeted detection	Brown	1 tube, 1000 µL
D25.03	qPCR Master Mix [1]	Amplification	Green	2 tubes, 1000 µL
D25.04	Negative Control	Negative Control	White	1 tube, 200 µL
D25.05	Positive Control	Positive Control	Red	1 tube, 200 µL

[1] Reagents are supplied with a 5% of extra volume

4. Storage

Reagents should be stored sealed at -20 ± 5°C and may be used until the expiration date shown on the package label. Expiry date refers to the product under rightful handling and storage conditions. It is not recommended the use of the kit after the expiry date stated on the box. Avoid unnecessary repeated freeze/thawing cycles. Protect reagents from light exposure to prevent degradation.

5. Equipment and materials required (Not provided)

- Food processor [2]
- DNA extraction kit
- Real-Time PCR instrument [3]
- Spectrophotometer/Fluorometer [4]
- Plates and/or tubes for qPCR
- 1.5 mL microcentrifuge tubes
- PCR cabinet [5]
- Micropipettes (10, 200 and 1000 µL) and filter tips
- Vortex and microcentrifuge

[2] To ensure accurate analysis of samples with multiple ingredients that are not in a powdered or granulated form, it is recommended to homogenize them carefully.
[3] The assay was validated on a Bio-Rad CFX96 instrument.
[4] It is recommended to quantify and access the purity of DNA.
[5] To minimize the risk of contamination with foreign DNA, we recommend that the PCR reaction set-up is performed in a controlled environment.

6. Suitable test sample material

All kind of food and feed samples suitable for Real-Time PCR amplification can be used, if the quality parameters for purity, concentration and integrity are fulfilled. Prepare the test sample in accordance with the specific International Standard ISO/TS 13136 considering the requirements for the specific product concerned.

7. Test Procedure

a. PCR Reaction Preparation

Allow all reagents to thaw at room temperature, vortex and spin briefly to avoid drops on the vial cap. For each DNA sample prepare two reaction mixes [6] according to the table below:

REAGENT	VOLUME
qPCR Master Mix	10 µL
Primer/Probe Mix [6]	10 µL
Total Volume	**20 µL**

[6] For each sample, two PCR reactions (one for stx1/stx2 and one for eae) for each of the target genes should be prepared.

1. Homogenize the reaction mixture and pipette 20 µL into individual wells according to the predicted PCR plate set-up.
2. Add 5 µL of RNA template to each well.

At least one positive control reaction and one negative control reaction must be included in the PCR run, replacing the sample in these wells with 5 µL of Positive Control and 5 µL of Negative Control, respectively.

It is recommended to prepare the reaction mixture carefully in a controlled environment, preferably in a nucleic acid-free zone. The addition of the positive control and sample DNA should preferably be carried out in a separate room.

b. Amplification Protocol

The amplification conditions are as follows:

	STEPS	TEMPERATURE	TIME	CYCLES
1	Enzymatic Activation	95 ºC	10 min	1
2	Denaturation	95 ºC	15 sec	40
3	Hybridization/extension plate reading *	59 ºC	30 sec	
4	Extension	72 ºC	20 sec	

* Fluorescence data must be obtained during this step through FAM, HEX and Cy5 channels.

c. Results Interpretation

The results should be interpreted in accordance with the analysis software recommended by the Real-Time PCR instrument manufacturer. Target DNA amplification is monitored in FAM (stx1 and eae) and Cy5 (stx2) channels.

A result is considered positive when Ct ≤ 40.

RESULT	Target DNA (FAM/Cy5)	IAC (HEX)
Positive	+	+/-
Negative	-	+
Inconclusive [7,8]	-/?	-

[7] PCR inhibitions may be due to the presence of excessive DNA and/or PCR inhibitors. It is recommended to dilute the DNA extracted from the sample 1:10 or 1:100 in DNAse/RNAse free water and repeat the real-time PCR reaction. When applicable, the LOD of the method should be adjusted in accordance with the dilution factor.
[8] The appearance and characteristics of the amplification curves should be thoroughly considered. Incomplete amplification curves often denote low amount of DNA template. In this case, the positivity of the result is dubious, and the Real-Time PCR reaction should be repeated using a superior amount of DNA template.

d. Expression of results

Samples negative for stx gene: STEC not detected in the test portion of x g or x mL.

Samples positive for stx gene: Presumptive detection of STEC in the test portion of x g or x mL.

Samples positive for stx and eae genes: Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x mL.

8. Quality Control

The test can only be considered valid under the following control conditions:

CONTROLS	Target DNA	IAC
Positive Control	+	+
Negative Control	-	+

If no amplification is observed for the positive control, the test results are invalid and must be repeated. The positive control template is expected to amplify before Ct 35.
If amplification is observed for the negative control, it indicates that the reagents have become contaminated while setting up the run, invalidating test results

9. Performance Characteristics

Specificity: InviScreen® STEC Detection Kit was designed to specifically detect STEC *E. coli*. The specificity was evaluated by analysing the following reference materials: *E. coli* O26, O103, O111, O145 and O157 provided by the national reference laboratory.

E.coli Strain/serotype	Target Gene			Agreement
	stx1	stx2	eae	
O26	+	+	+	100%
O104:H4	-	-	-	100%
O103	-	+	+	100%
O111	-	-	+	100%
O145	+	-	+	100%
O157:H7	+	+	+	100%
NCTC9001	-	-	-	100%

Detection Limit and sensitivity: The limit of detection (LOD) is often matrix dependent, and the sensitivity of the analysis may be reduced depending on the total DNA extracted from the actual ingredient in test, but also its quality. This way, the LOD needs to be determined through in-house validation.
Isolated colonies of *E. coli* O26, O104/H4, O111, O145 and O157 strains were suspended in saline solutions and mixed with bacterial suspensions of *E. coli* NCTC 9001 and *S. aureus* NCTC 6571. The bacterial suspensions were used for DNA extraction. The extracted DNA was scalar diluted in nuclease-free water until a minimum dilution of 1 *E. coli* cfu/µl. Three independent assays were performed with two genomic DNA dilutions in triplicate. The LOD was determined as 3 genomic units.
Additionally, a vegetable matrix (lettuce) and a dairy matrix (cheese) were artificially contaminated with 3 CFU and 30 CFU of *E. coli* O157. The contaminated samples followed the complete analytical procedure according to the ISO/TS 13136. Results obtained were concordant with method validation data described in ISO/TS 13136 with *stx1*, *stx2* and *eae* genes detected in both samples.