Instructions for use
InviScreen® O157:H7 Detection Kit

INVITEK diagnostics

ALS Life Sciences Portugal, S.A.
Zona Industrial de Tondela, ZIM II, Lote 6,
3460-070 Adiça - Tondela, Portugal

Phone: +351 232 817 817
info@invitek.com
invitek.com

REF 6015003201 | Σ 50 tests | -25 °C -15 °C | RUO

1. Intended use

The InviScreen® O157:H7 Detection Kit is designed for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157:H7 serotype in food samples. This kit is intended to be used by trained laboratory professionals in accordance with ISO 13136. The PCR kit is a reliable and efficient tool that enables the rapid and accurate identification of the most common STEC strain to cause illness in people, the O157:H7 serogroup, in various food matrices, including but not limited to meats, dairy products, vegetables, and processed foods. This kit should be used in conjunction with the InviScreen® STEC Detection Kit that detects specific genetic markers associated with Shiga toxin genes.

2. Product description

The InviScreen® O157:H7 Detection Kit follows the ISO 13136 standard method. The method uses the Real time PCR technique to detect the presence of the *rfbE* and *fliC* genes which code the virulence marker and its H-antigen-associated gene, respectively, and are specific of the O175:H7 serotype. The method provided in this kit is suitable for application with various samples types, including products intended for human consumption, animal feed, environmental samples in food production and handling areas, as well as environmental samples in primary production settings. The amplification detection within this kit is based on Taqman technology, whereby fluorescence is detected upon the degradation of the hydrolysis probe during the PCR reaction.

3. Kit contents

REF.	COMPONENT	FUNCTION	CAP COLOR	QUANTITY
D30.01	Primer/Probe Mix[1]	Targeted detection	●	1 tube, 500 µL
D30.02	qPCR Master Mix[1]	Amplification	●	1 tube, 500 µL
D30.03	Negative Control	Negative Control	●	1 tube, 50 µL
D30.04	Positive Control	Positive Control	●	1 tube, 50 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 [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] It is recommended to carefully homogenize samples that are contain more than one ingredient in its composition and are not originally in a powdered or granulated form.
[3] The assay was validated on a Bio-Rad CFX96.
[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 a reaction mix according to the table below:

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

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 DNA 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 °C	10 min	1
2	Denaturation	95 °C	15 sec	
3	Hybridization/extension plate reading *	59 °C	30 sec	40
4	Extension	72 °C	20 sec	

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

c. Results Interpretation

Target DNA amplification is monitored in FAM (O157) and HEX (H7) channels. After setting the threshold baseline, the analysis outcome should be interpreted according to the scenarios referred bellow.

A result is considered positive when Ct ≤ 40.

RESULT	O157 (FAM)	H7 (HEX)
Positive	+	+
Negative	-	+/-
Inconclusive [6,7]	-/?	-/?

[6] 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.
[7] 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

Positive results are expressed as “Presumptive detection of STEC of O157:H7 serogroup in the test portion of x g or x ml”

Negative results are expressed as “E. coli O157:H7 not detected 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
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® O157:H7 Detection Kit was designed to specifically detect *E. coli* O157:H7 serogroup. 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					
	stx1	stx2	eae	O157 (rfbE)	H7 (fliC)	Agreement
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:H7 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 *rfbE* and *fliC* genes detected in both samples.