Real-time PCR Diagnostic Kit for Rapid Identification of E. coli O157:H7/NM

Product No. MX-1701

Intended Use

Enterohemorrhagic Escherichia coli (EHEC) is a group of *Escherichia coli* bacteria that can cause hemorrhagic diarrhea and enteritis in humans. Serotype O157:H7 was used as the representative strain.This product is the Real-time PCR Diagnostic Kit for Rapid Identification of *E. coli* O157:H7/NM, which can specifically amplify the specific DNA nucleic acid fragments of *E. coli* O157:H7/NM in the sample, and determine whether the sample contains *E. coli* O157:H7/NM by Ct value.

Specification 24 Reactions

Principle and Interpretation

This kit uses real-time fluorescent PCR technology to perform specific amplification of *E. coli* O157:H7/NM by specific primers of *E. coli* O157:H7/NM. The fluorescence-labeled probes were used to label and track the amplification products, the reaction process was monitored online in real time, and whether the products were amplified or not could be analyzed in combination with corresponding software.

Kit Contents

No.	Components	Amount
1	PCR Master mix (Lyophilization)	8 Tubes/Row x 3
2	PCR Buffer	1 mL/Tube
3	Lysis Buffer	5 mL/ Bottle

Self-provided materials

Sterilized 1.5mL centrifuge tube; sterilized 0.2mL PCR tube and tip; crushed ice or ice box; micropipette; centrifuge; vortex oscillator; metal bath; sterilized ultrapure water.

Directions

1. Sample Preparation:

Method: Reference to this manual:

a) Samples from culture: Take 1mL of the bacteria culture into a 1.5mL tube, centrifuge at 3000 ×g for 10min or 10000×g for 2min, discard the supernatant, add 200μL lysis buffer, mix well by vortex, heat at 99℃ in metal bath or boil in a water bath for 10 minutes, cooling down in ice bath, centrifuge at 12000×g for 2min, and take supernatant to a new clean tube as DNA template.

b) Samples from colony: Take one colony using the inoculating loop, suspend in 200μL lysis solution, mixed by vortex, heat at 99℃ in metal bath or boil in a water bath for 10 minutes, cooling down in ice bath, centrifuge at 12000×g for 2 min, and take supernatant to a new clean tube as DNA template.

2. Prepare the PCR Reaction Mix

① Take out the PCR Master mix (Lyophilization), add 23μL PCR Buffer to the tube, mix well by pipette or vortex.

② Add 2μL DNA template from Step 1(Sample Preparation) into PCR tubes, with a total reaction volume of 25μL.

③ The corresponding positive control, negative control and sterile deionized water were added to the positive control, negative control and blank control systems respectively.

3. Reaction Condition

Stage	Cycles	Temperature	Time	Step	Fluorescent Signal# Acquisition
Pre-mutability	1	95℃	3min	pre-mutability	No
Real-time PCR	35	95℃	30sec	mutability	No
		60℃	30sec	metallurgy	Yes

#: The *E. coli* O157:H7/NM-specfic gene has a fluorescent motif of FAM and VIC.

Result Analysis

Sample Tube To Be Tested		Negative Control		Result Interpretation
FAM(518nm)	VIC(553nm)	FAM(518nm)	VIC(553nm)	
Amplification	Amplification /No Amplification	No Amplification	Amplification	Positive（Fig.1、2）
No Amplification	Amplification	No Amplification	Amplification	Negative（Fig.3）
Amplification/ No Amplification	Amplification /No Amplification	Amplification	Amplification	Contamination
No Amplification	No Amplification	No Amplification	No Amplification	Inhibition

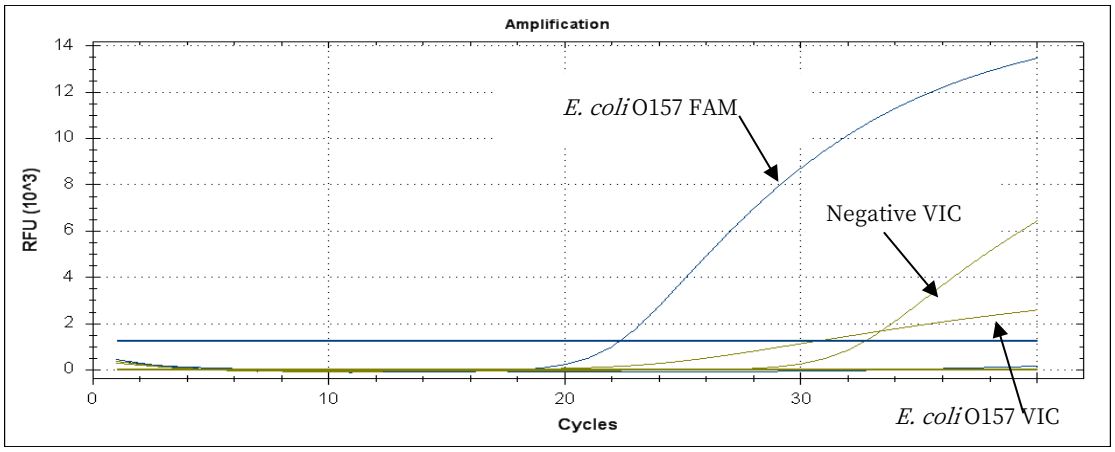

FIG.1

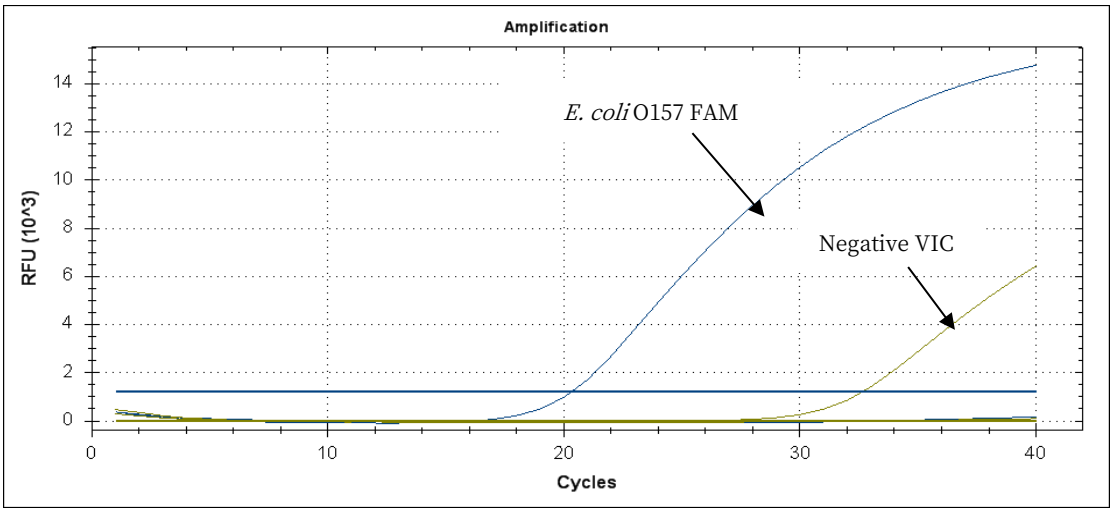

FIG.2

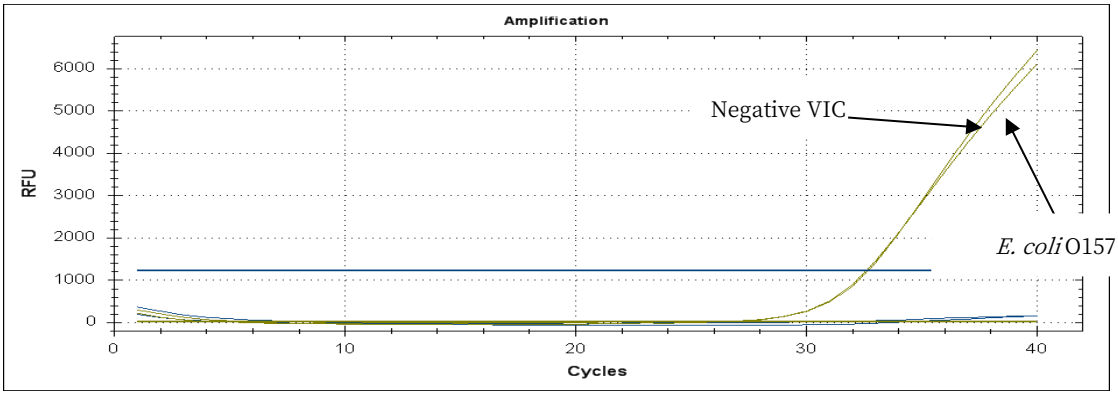

FIG.3

Limitations of Method

The target sequences detected by this kit are the conserved regions of specific genes in *E. coli* O157:H7/NM, which are highly conserved and stable. If the bacteria have genetic mutations at the target sequence, false negative results may occur. In addition, the procedure of sample collection, preparation, transportation and storage will also affect the test results.

Storage Conditions

Storage at 2-8 ℃, or store at -20 ℃ when not used for a long time.

Shelf Life

24 month.

Announcements

1. Please read the instruction of this kit carefully before the experiment and strictly follow the operation steps.
2. All components in this kit should be fully melted and mixed before use, and the high speed centrifugation for a short time is necessary.
3. This kit must be stored away from light, and the centrifuge tube and Tips with the DNase and RNase free should be autoclave before used. The whole operation process and the PCR laboratory should comply with the requirements of regulations such as "Administrative Measures for Clinical Gene Amplification Testing Laboratories in Medical Institutions" and "Working Guidelines for Clinical Gene Amplification Testing Laboratories in Medical Institutions" issued by the NHFPC. The waste and amplification products produced during the test should be properly treated to prevent cross-contamination.

For laboratory use in industry or R&D purpose. Not for drug, household or other uses.