Multiplex PCR Diagnostic Kit for Rapid Identification of Diarrheogenic Escherichia coli

Product No. MX-2101

Intended Use

This kit is used for in vitro detection of the nucleic acids of EAEC, EPEC, STEC/EHEC, ETEC and EIEC, and the experimental results only provide reference for basic research, not as a basis for clinical diagnosis..

Specification 24 Reactions

Principle and Interpretation

This kit is suitable for the detection of Diarrheagenic *Escherichia coli* (EPEC, EHEC, ETEC, EIEC, EAEC) nucleic acids in vitro. Each reaction system contained twelve pairs of specific primers for species identification and virulence gene detection of *Escherichia coli*. The virulence gene species contained in the strain were determined according to the band size of PCR amplification products and their pathogenic types were determined.

Kit Contents

No.	Components	Amount
1	PCR Master mix (Powder)	24 test /Tube
2	RNase-Free ddH_2O	1 mL/Tube
3	Lysis Buffer	5 mL/ Bottle
4	0.2 mL 8-Tube PCR Strips	3

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:

①Reference to GB 4789.6 2016-6.5.2.

②Reference 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 (Powder), add 575μL RNase-Free ddH_2O to the tube, mix well by pipette or vortex.

Note: If more PCR mix remain, recommanded aliquot to the 0.2mL PCR tube for storage for long time use.

② Take 23μL PCR mix to 0.2mL PCR tube.

③ Add 2μL DNA template from Step 1(Sample Preparation) into PCR tubes, with a total reaction volume of 25μL.

④ 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

The PCR program: Pre-denaturation for 5min at 95°C, 40 amplification cycles of 30s at 95°C, 30s 63°C and 1.5 min at 72°C, with a 10 min extended elongation step at 72°C.

Put the PCR reaction tube into the PCR instrument, verify that the PCR reaction conditions and start the reaction procedure.

Result Analysis

1. Electrophoresis analysis of PCR amplification products: If agarose gel electrophoresis is used for separation and identification of the amplification products, the recommended concentration is 2% agarose gel, and the length of the gel is not less than 10cm; The recommended voltage is the product of the distance between positive and negative electrodes (cm) of the electrophoresis tank and 5(V/cm), and the electrophoresis time is generally 30-45min.

2. The results showed that >97% of *Escherichia coli* (Including diarrheal and non-diarrheal large intestine) had *uidA*(1487bp) gene. On the basis of *uidA* gene, if the amplification of virulence gene, it is diarrhea *Escherichia coli*. According to DNA Marker and (or) positive control, the amplification strip size was determined, and virulence gene types were determined, and virulence gene combinations were combined to determine the final pathogenic types, as shown in the following table:

Type	Target strip size and determination		
EAEC	*astA* (102bp) *aggR* (400bp) *pic* (1111bp)	*astA, aggR, pic* （One or more positive）	*uidA*（1487bp） +/-
EPEC	*escV* (544bp) *bfpB* (910bp)	*bfpB* (+/-) , *escV* (+) , *stx1* (-) , *stx2* (-)	
STEC/EHEC	*escV*(544bp) *stx1* (244bp) *stx2* (324bp)	*escV* (+/-) , *stx1* (+) , *stx2* (-) ,*bfpB* (-) ; *escV* (+/-) , *stx1* (-) , *stx2* (+) , *bfpB* (-) ; *escV* (+/-) , *stx1* (+) , *stx2* (+) , *bfpB* (-)	
ETEC	*lt* (655bp) *stp* (157bp) *sth* (171bp)	*lt, stp, sth* （One or more positive）	
EIEC	*invE*(766bp)	*invE* (+)	

Note: *astA* and *pic* genes can be transferred between bacteria, and when *astA* and other virulence genes are positive at the same time, *astA* and *pic* genes are not used as the basis for type determination. If *uidA*, *invE* and *pic* are positive at the same time, the strain is determined to be EIEC. If *uidA*, lt and *astA* test are positive at the same time, the strain is determined to be ETEC.

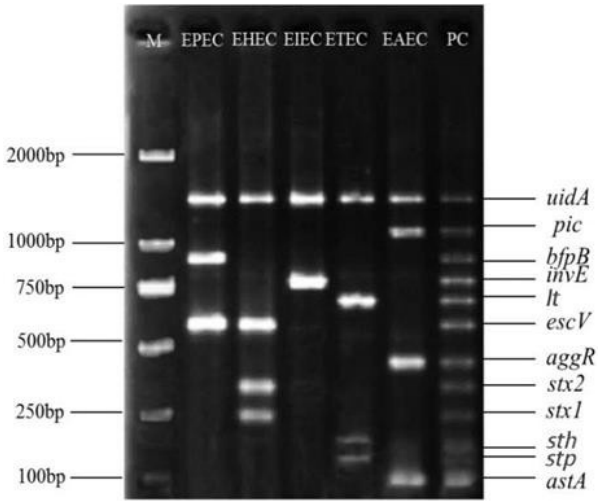

FIG.1 Electrophoretic picture of five diarrheal *Escherichia coli* species and positive control

M: DL 2000 DNA Ladder; PC: 12 positive controls.

In this figure, five types of diarrogenic *Escherichia coli* carry typical virulence genes

Limitations of Method

The target sequences detected by this kit are the conserved regions of specific genes in Diarrheogenic *Escherichia coli*, 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.