Vibrio parahaemolyticus toxR/tdh dual Real-time PCR Diagnostic kit

Product No. MX-2201

Intended Use

This kit is used for the in vitro detection of *toxR, tdh* two-fold *Vibrio parahaemolyticus* nucleic acid, the experimental results are only for basic research to provide a reference, not as a basis for clinical diagnosis.

Specification 24 Reactions

Principle and Interpretation

This kit adopts two-fold fluorescence PCR technology, which is suitable for the in vitro detection of *Vibrio parahaemolyticus* specific gene *toxR* and virulence gene *tdh* nucleic acid. Each reaction system contains two pairs of specific primers and probes for *Vibrio parahaemolyticus* species identification and virulence gene detection. Based on the CT value of the fluorescent PCR amplification product, it is determined whether the strain is *Vibrio parahaemolyticus* or not and whether it is pathogenic or not based on the virulence gene.

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:

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 200uL 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

Stage	Cycles	Temperature	Time	Step	Fluorescent Signal# Acquisition
Pre-mutability	1	95℃	3min	pre-mutability	No
Real-time PCR	40	95℃	30sec	mutability	No
		60℃	30-60 sec*	metallurgy	Yes

*: When using different types of instruments for time setting, please follow the requirements of the instrument instruction manual for experimental operation, generally set at 30 sec.

#: The Vibrio parahaemolyticus-specific gene toxR is fluorescently labeled as FAM, the virulence gene tdh is fluorescently labeled as VIC, and the quenching motifs are all TAMRA.

Result Analysis

Blank control: no FAM fluorescence signal was detected and no typical amplification curve appeared.

Negative control: no FAM fluorescence signal was detected and no typical amplification curve was observed.

Positive control (with causative gene): a FAM fluorescent signal was detected, a typical amplification curve was present, and the Ct value was <30.0.

All of the above need to be satisfied at the same time, otherwise this experiment is invalid.

Sample test results:

Ct values ⩾35 were determined to be negative;

Samples were judged to be positive with a Ct value <30 and a typical amplification curve;

If the Ct value is 30≤Ct<35 and a typical amplification curve is present, the DNA is reextracted for testing. If the result is still 30 ≤ Ct value < 35, the sample is judged as positive; Ct value ≥ 35, the result is judged as negative.

Limitations of Method

The target sequences detected by this kit are the conserved regions of specific genes in *Vibrio parahaemolyticus*, 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.