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Enteropathogenic Escherichia coli Detection Kit (Real-Time PCR Method)

Product Number: DTL0671

Shipping and Storage

1. Store below 30℃. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used the all components should be stored at (-20±5)℃. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. It is not recommended to repeat the freeze-thaw cycle more than 7 times.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
EPEC PCR Master Mix	Lyophilized powder ×1 Bottle
Exogenous Internal Control	Lyophilized powder ×1 Tube
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

Note: Do not mix reagents from different batches.

The reaction system is lyophilized powder contains all components required for fluorescence PCR, including Taq enzyme, primers, probes, dNTPs, and Mg^{2+}.

Description

This kit utilizes TaqMan probe-based real-time fluorescence PCR technology, employing a pair of primers specific to pathogenic Escherichia coli and a specific probe. Fluorescence PCR technology is used to amplify and detect the DNA of pathogenic Escherichia coli in vitro, for use in the clinical etiological diagnosis of suspected infectious samples.

Application

This kit is suitable for detecting pathogenic E. coli in water samples, feces, and suspected contaminated water and food samples, and is used for the auxiliary diagnosis of pathogenic E. coli infections.

Applicable instruments

Real-time fluorescence PCR instrument with FAM channel and VIC channel.

Specimen collection

1. Applicable sample type: Wound exudate, Suspected contaminated food or water.
2. Sample collection: Feces samples: Collect 0.5~1mL of stool sample, add 100-200μL of fecal sample (if using solid fecal samples, collect samples the size of a soybean) to a 1.5mL EP tube, add PBS buffer to bring the volume to 1mL (solid fecal samples need to be thoroughly ground), vortex 3 times for 10 seconds each time. Then let stand for 10 minutes, then centrifuge at 8,000 rpm for 5 minutes, and collect the supernatant for further nucleic acid extraction or short-term storage at -20°C; Suspected contaminated food sample: The suspected contaminated food is cut by surgery and mixed evenly, take 0.05g and grind it in a grinder.Add 1.5mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5mL sterilized centrifuge tube, centrifuge at 8000rpm for 2 minutes,collect 100μL of supernatant in 1.5mL sterilized centrifuge tube;

Suspected contaminated water: Collect directly 100μL suspected contaminated water for use.

3. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2℃ ~ 8℃. If long-term preservation is needed, it should be stored below -70℃, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the EPEC PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent, and then subpackage it into PCR reaction tubes according to 19μL/ tube.

Take out the Exogenous Internal Control and open the tube cap,add 100μL of Redissolved Diluent,then cover the tube,mix evenly for 15 seconds with a vortex shaker, centrifuge for 3 seconds with a palm centrifuge, and then subpackage it into PCR reaction tubes according to 1μL/ tube.

The total test reaction system mixed well between the EPEC PCR Master Mix and Exogenous Internal Control is 20μL/tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid(NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA is not used immediately, it should be stored below -20℃. For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note: The Negative Control and the Positive Control does not require nucleic acid extraction.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube, and close the tube cover.
Negative Control/ Positive Control	Add 5μL of negative control and positive control to the reaction tube, and cover the tube tightly.

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be mix evenly for 5 seconds with a vortex shaker,centrifuged for 5 seconds on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the negative control, positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect EPEC DNA, select VIC channel to detect the Internal Control. Set the Reaction Volume per Well to 25μL.

(Note: For ABI series instruments, select 'None' under 'Quencher' , and select 'None' as the dye to use as the passive reference.)

Recommended reaction program setting:

Step	Cycles	Temperature	Time	Collect fluorescence signal
1	1 cycle	95℃	2min	No
2	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis:

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3-15, the End value can be set at 5-20, the

amplification curve of the negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6.Quality control', and then go to the Plate window to record the Ct value.

6. **Quality control**

Negative control: FAM detection channel has no obvious amplification curve.

Positive control: FAM detection channel has an obvious amplification curve, and the Ct value ≤32.00.

Exogenous Internal Control: VIC detection channel has an obvious amplification curve, and the Ct value ≤32.00.

The above requirements must be met at the same time in the same experiment, otherwise this experiment is invalid and needs to be repeated.

Explanation of Test Result

1. Positive:Ct value ⩽40.00 and the curve has a clear index growth curve.
2. Negative:The FAM channel of sample test results have no Ct value and no specific amplification curve,and the VIC channel is ⩽32.00.
3. Suspicious samples: If the sample test result is 40.00<Ct value⩽45.00, it is recommended to repeat the test. If the test channel is still 40.00<Ct value⩽45.00 and the curve has an obvious growth curve, it is judged as positive, otherwise it is negative.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. If cross-contamination is not controlled during the sample extraction process, false positive results will occur.
3. Contamination from the Positive Control or amplified products (amplicons) can lead to false positive results.
4. Genetic mutations or rearrangements in the target sequence may lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Degradation of reagent performance due to improper transportation, storage, or inaccurate preparation may lead to false negative results or inaccurate quantification.
7. The results of this test are for reference only. Clinical diagnosis should be based on a combination of clinical symptoms, other laboratory findings, and epidemiological data.

Performance Parameters

1. Minimum detection limit: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: The coefficient of variation (CV, %) of the Ct value of the pathogen detection channel is ⩽5.00%.
3. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.

Note

1. Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochlorite or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".