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Escherichia coli Universal Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK545

Shipping and Storage

1. Store in dark at -20℃, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20℃ without affecting the expiration date. Avoid repeated freezing and thawing, freezing and thawing 6 times will not affect the detection effect.

Component

Component	Specification/Volume μL/Quantity		Main components
	25T	50T	
qPCR premix (containing enzymes)	400μL	800μL	Tris, KCl, MgCl2, dNTPs, Taq enzymes, etc
Primer probe for Escherichia coli universal type	100μL	200μL	Primer probe
Positive control Escherichia coli universal type	500μL	500μL	Plasmids containing target detection gene fragments
Negative control	500μL	500μL	normal saline

Description

This reagent kit is designed based on the principle of fluorescence PCR technology, with specific primers and Taqman probes designed for the universal type of Escherichia coli. It is detected by a fluorescence PCR detector to achieve the detection of universal nucleic acid of Escherichia coli.

Application

This kit is used for qualitative detection of Escherichia coli universal nucleic acid and for auxiliary diagnosis and epidemiological monitoring of Escherichia coli universal infection.

Applicable instruments

Suitable for ABI 7500 Bio-Rad CFX96, Roche Lightcycler480I, Lightcycler480II, cobas Z480, Real time fluorescence quantitative PCR instruments such as Hongshi SLAN-96S and SLAN-96P.

Specimen collection

1. Sample types: feces, vomit, and other samples.
2. Storage conditions: The collected specimens should be sent for testing in a timely manner. Those tested within 24 hours should be stored at 4℃, and those tested beyond 24 hours should be stored at -70℃, avoiding repeated freezing and thawing.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Melt the components of the reagent kit at room temperature, shake thoroughly and mix well, then centrifuge immediately. Calculate the number of reagents used N (N=number of samples+1<positive control>+1<negative control>), configure the reaction system according to the table below, add each component to the same appropriate volume centrifuge tube, mix thoroughly, and centrifuge immediately to prepare the reaction system mixture. Transfer it to the PCR reaction tube/plate at a rate of 20μL/well and transfer it to the sample processing area.

Component	Volume (μL)
qPCR premix (containing enzymes)	16μL
Primer probe for Escherichia coli universal type	4μL
Total volume (reaction system mixture)	20μL

2. Sample Processing (Sample Processing Area)

2.1. Nucleic acid extraction:

Select the appropriate nucleic acid extraction kit to extract nucleic acid, and follow the instructions of the corresponding kit for specific operations.

2.2. Sampling:

Add 5μL of processed nucleic acid, negative control, and positive control to the PCR reaction tube/plate that has been mixed with the reaction system mixture, resulting in a final volume of 25μL. Cover the tube tightly or seal it with a membrane, and perform transient centrifugation followed by detection on a fluorescence PCR amplification instrument.

3. Amplification testing (nucleic acid amplification area)

step	Cycles	Temperature	Time
Pre denaturation	1 cycle	95℃	5min
Sex change	40cycles	95℃	10sec
Annealing/extension/fluorescence detection*		55℃	40sec

Note: Fluorescence detection at 55℃ in step 2, using FAM as the detection channel.

The ABI series fluorescence PCR instrument does not select ROX calibration, and the quenching group is selected as None.

4. Result analysis

According to the analysis of the image, adjust the start and end values (it is recommended to start from 3-15 and end from 5-20, and adjust the amplification curve of the negative control to be flat or below the threshold line). Click on the analysis button and view the results on the report interface.

Quality control standards

1. Negative control: Ct value>38 or not detected.
2. Positive control: The amplification curve is S-shaped and the Ct value is ≤ 35.
3. The above requirements must be met simultaneously for the same experiment, otherwise this experiment will be considered invalid.
4. Each detection target requires a positive and negative control, and the baseline threshold is adjusted for different targets based on their corresponding negative results.

Result interpretation

1. FAM channel detects the universal type of Escherichia coli.
2. Negative: Ct value>38 or not detected.
3. Positive: The amplification curve is S-shaped and the Ct value is ≤ 35.
4. Suspicious: The amplification curve shows an S-shaped pattern, and the Ct value is between 35 and 38, requiring retesting; If the retest results are consistent, the judgment result is positive. If the CT value is greater than 38 or not detected, the judgment result is negative.

Limitations of detection methods

1. Improper sample collection, transportation, and storage, as well as improper transportation, storage, and configuration of reagents, can all affect experimental results and even lead to false negative results.
2. If there is laboratory contamination, reagent contamination, or sample cross contamination, false positive results may occur.

Performance indicators of reagent kit

1. Minimum detection limit: 500 copies/mL.
2. Specificity: No cross reactivity against other pathogens that may cross the detection target.

Note

1. Each stage of PCR operation should be strictly partitioned to avoid cross contamination.
2. The components of the reagent kit should be thoroughly melted and mixed before use, and centrifuged for a few seconds before use.
3. Each component shall not be interchanged with other products or corresponding ingredients of different batch numbers.
4. If the specimen to be tested is not tested in a timely manner, it should be stored at -20℃ or -70℃.
5. The processing of samples should strictly follow biosafety regulations.
6. PCR operators should have experience and receive professional training.
7. This kit is only used for scientific research purposes and is not intended for clinical diagnosis.