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Bifidobacterium spp. (BD) Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK276

Shipping and Storage

1. Store away from light at -20°C; shelf life is 12 months.
2. Low-temperature transportation shall not exceed 5 days; after opening, store away from light at -20°C with no impact on shelf life. Avoid repeated freeze-thaw cycles; up to 10 freeze-thaw cycles will not affect detection performance.

Component

Component	50T
Bifidobacterium Primer Mix	100μL
Probe qPCR Amplification Mix	900μL
LactobacillusPositive control	100μL
Lactobacillus Negative control	100μL

Description

Specific primers and probes are designed targeting the highly conserved genomic regions of Bifidobacterium for this kit. When the reaction system contains Bifidobacterium genomic template, the PCR reaction proceeds and releases fluorescent signals. A real-time fluorescent quantitative PCR instrument is used to monitor and output signals of corresponding channels in real time during PCR, so as to realize qualitative analysis of detection results.

Application

This kit is applicable to nucleic acid detection of Bifidobacterium. Bifidobacterium is one of the important components of intestinal flora in humans and animals. Some Bifidobacterium strains can be used as probiotics in food, medicine and feed. It is used for auxiliary diagnosis of Bifidobacterium and shall not be used for definitive diagnosis.

Applicable instruments

ABI series, Bio-Rad series, Roche LC480, QIAGEN Rotor-Gene series, Tianlong Gentier-96E/96R series, Yaru MA6000, Hongshi SLAN-96S/96P and other multi-channel real-time fluorescent quantitative PCR instruments.

Specimen collection

1. Sample Selection: Bifidobacterium is the dominant bacterium in the intestinal tract of breastfed infants, accounting for 99% of the total microbial community in feces of breastfed infants. It is also the dominant normal resident bacterium in adult intestines. It can exist in the small intestine, but its quantity is lower than that in the large intestine and feces. Common samples include food samples, dairy products, feces, etc. Appropriate samples shall be selected for detection according to specific clinical conditions during collection.
2. Aseptic Operation: Aseptic operation shall be adopted during sample collection to avoid contamination by external bacteria.
3. Storage: Samples can be stored at 2~8°C for no more than 72 hours; long-term storage is available below -70°C, but repeated freeze-thaw cycles shall be avoided (maximum 5 freeze-thaw cycles).

Protocol

1. **Sample processing (sample processing area)**

Commercially available kits are recommended for DNA/RNA extraction; strictly follow the instruction manual of the

corresponding kit. Contamination shall be avoided during sample nucleic acid extraction.

2. Reagent preparation (reagent preparation area)

Calculate the required number of PCR reaction tubes as N based on the total number of samples to be tested (N = number of samples + 1 negative control tube + 1 positive control tube; prepare one extra reaction for every 10 samples). The reaction system for each test is formulated as shown in the table below:

Reagent	Volume
Bifidobacterium Primer Mix	2μL
Probe qPCR Amplification Mix	18μL
Total Volume	20μL

3. Sample addition (sample processing area)

Transfer 5 μL each of the DNA/RNA extracted in Step 1, positive control and negative control into corresponding reaction tubes respectively, cap tightly, mix thoroughly and perform brief centrifugation.

4. PCR amplification (nucleic acid amplification zone)

4.1. Place the reaction tubes to be tested into the reaction slot of the real-time fluorescent quantitative PCR instrument;

4.2. Set channels and sample information; set the reaction system to 25μL; Fluorescence channel settings: Reporter Dye: FAM, Quencher Dye: NONE; DO NOT select ROX reference fluorescence;

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	95℃	2min	No
2	40 cycles	95℃	10sec	No
		55℃	30sec	Yes

Note: For ABI series fluorescent PCR instruments, ROX correction shall not be selected during setup, and Quencher shall be set to None.

5. Result analysis and judgment

5.1. Kit validity judgment

Positive Control: Typical S-shaped amplification curve or Ct value ≤ 35.

Negative Control: Ct value > 40 or no Ct value, the curve is a straight line or slight slant without exponential growth phase..

5.2. Specimen result judgment

Positive: S-shaped amplification curve with Ct value ≤ 35.0 in detection channel and obvious exponential growth curve;

Suspicious: S-shaped amplification curve with Ct value ranging from 35.0 to 40 in detection channel. Retest is recommended. If Ct value remains between 35.0 and 40 with obvious growth curve after retest, judge as positive; otherwise negative.

Negative: Specimen Ct value > 40 or no Ct value detected.

Limitations of detection methods

1. False-negative results may occur when the concentration of target nucleic acid in the test sample is lower than the minimum detection limit of this kit.
2. Improper operation during sample collection, transportation, storage and processing may cause DNA/RNA degradation, leading to false-negative results.
3. Cross-contamination during sample collection, transportation, storage and processing may lead to false-positive results.

Performance

1. Minimum detection limit of the product: 200 Copies/mL;
2. Linear detection range: 200 ~ 1×10^{10} copies/mL;
3. Precision: Intra-assay and inter-assay coefficient of variation (CV) of precision reference samples ≤ 5%;
4. High specificity: No cross-reactivity with other pathogens that infect the same site or cause similar symptoms.

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Note

1. All operations must be strictly carried out in accordance with the instructions;
2. The various components in the reagent kit should be naturally melted, completely mixed, and briefly centrifuged before use;
3. The reaction solution should be stored away from light;
4. Try to avoid the presence of bubbles during the reaction, and cover the tube tightly;
5. Use disposable suction tips, disposable gloves, and specialized work clothes for each area;
6. Sample processing, reagent preparation, and sample addition should be carried out in different areas to avoid cross contamination;
7. After the experiment is completed, treat the workbench and pipette with 10% hypochlorous acid, 75% alcohol, or a UV lamp;
8. All items in the reagent kit should be treated as contaminants and handled in accordance with the "Biosafety Guidelines for Microbial Biomedical Laboratories".