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Catalog No: BF-82929675

[Enterococcus avium Probe Realtime PCR Kit]

User Manual

Disclaimer: Products are intended for research use only

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Product Description

Enterococcus avium Probe Realtime PCR Kit is a PCR kit intended for research use in nucleic acid analysis under controlled laboratory conditions using probe-based real-time PCR. Purified DNA is directly amplified using sequence-specific primers and probes. During amplification, fluorescence signals are generated through probe cleavage, allowing real-time monitoring of amplification under controlled laboratory conditions. The resulting signals may be used for comparative analysis of target nucleic acids.

Enterococcus avium Probe Realtime PCR Kit has the following features:

1. One-step PCR workflow to reduce handling steps and minimize contamination risk.
2. Demonstrates high sensitivity under optimized laboratory conditions.
3. High specificity based on primer-probe design.
4. Includes a non-infectious plasmid-based positive control for assay verification.
5. Compatible with commonly used DNA purification methods.
6. Suitable for research applications including assay development and method evaluation.
7. Broad dynamic range under laboratory conditions.
8. For research use only. Not intended for diagnostic or therapeutic use.

Input Sample Requirements

DNA samples should be prepared using standard nucleic acid purification methods suitable for downstream PCR analysis. Commercially available column-based or magnetic bead-based DNA extraction methods are recommended.

Kit Components

Component Name	Description	Size	Vial	Lid Color
2× Probe qPCR Master Mix	Contains optimized buffer and enzyme mix	500 µL	0.5 mL Vial	Blue
Primer-Probe Powder	Lyophilized target-specific primer and probe set	50 T	1.5 mL Vial	Brown
Positive Control (1E7 copies/µL)	Non-infectious plasmid-based control	0.5 mL	0.5 mL Vial	Yellow
Nuclease-free water / Dilution Buffer	For reaction setup	1 mL	1.5 mL Vial	Green
Manual	Instruction booklet	1 Set	N/A	N/A

Storage and Handling

Reagents are shipped on ice and should be stored at −20°C upon receipt. The kit is stable for at least 12 months when stored under recommended conditions.

Avoid repeated freeze–thaw cycles. All components should be thawed completely at room temperature prior to use, kept on ice during use, and returned to −20°C promptly after use.

Before reaction setup, gently mix thawed components by inversion or brief vortexing and collect contents by quick centrifugation if necessary.

Materials and Equipment Required but not Supplied in the Kit

Unless otherwise indicated, all materials are available through major laboratory suppliers.

Class	Items
General laboratory equipment and consumables	Benchtop microcentrifuge, micropipettes, vortex mixer, disposable powder-free gloves, sterile filter pipette tips
Kit-specific laboratory equipment and consumables	Manual or automated nucleic acid purification systems, real-time PCR instruments and compatible consumables, PCR reaction setup workstation, appropriate control materials for assay verification

Procedural Guidelines

Use purified DNA as starting material.

Perform all procedures at room temperature (20–25°C), unless otherwise specified.

Use sterile, disposable Nuclease-free pipette tips and microcentrifuge tubes.

Wear appropriate personal protective equipment (PPE), including disposable gloves, when handling reagents and DNA.

Procedures

Section 1: Preparation of Control Dilutions

Prepare serial dilutions of the Positive Control to generate a range of concentrations (e.g., 1E1–1E6 copies/µL) for assay evaluation.

Note: Due to the high concentration of the Positive Control, dilution steps should be performed in a designated area to avoid contamination. The Positive Control is a non-infectious plasmid construct.

1. Label six microcentrifuge tubes (1–6).

2. Add 45 µL of Dilution Buffer to each tube.

3. Add 5 µL of Positive Control (1E7 copies/µL) into tube 6 and mix thoroughly.

4. Perform serial 10-fold dilutions by transferring 5 µL sequentially from tube to tube.

5. Mix each dilution thoroughly and keep on ice until use.

These dilutions may be used for assay performance evaluation.

Section 2: Preparation of Reagents and Controls

6. Primer-Probe Reconstitution

The Enterococcus avium Probe Realtime PCR Kit Primer-Probe component is provided in lyophilized form.

Add 165 µL of Nuclease-free water to reconstitute the lyophilized Primer-Probe Powder.

Mix gently by vortexing for 10 seconds and briefly centrifuge to collect contents.

Keep the reconstituted solution on ice before use.

Store any remaining solution at −20°C and avoid repeated freeze–thaw cycles.

7. Reaction Setup Planning

Determine the number of reactions based on sample number and experimental design.

Include appropriate controls such as:

- Positive Control (for assay verification)

- Negative Control (nuclease-free water)

Adjust total reaction numbers accordingly.

Section 3: qPCR Reaction Setup (20 µL per reaction)

Prepare the reaction mixture on ice as follows:

Component	Volume
2× Probe qPCR Master Mix	10 µL
Primer-Probe Mix	3 µL
Template DNA or control	7 µL
Total	20 µL

Mix gently and centrifuge briefly.

Perform real-time PCR according to instrument guidelines.

Section 4: PCR Cycling Conditions

1. Perform real-time PCR using the recommended cycling conditions below. Optimization may be required depending on instrument platform and experimental conditions.

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	45
Annealing/Extension	60°C	30 sec	45
Extension & Signal Acquisition (FAM channel)	72°C	1 min	45

Section 5: Data Analysis (For Research Use Only)

1. General Data Review

After completion of the qPCR run, review amplification plots and ensure that the threshold is set within the exponential phase of amplification and above background signal. For instrument-specific analysis procedures, refer to the corresponding user manual.

2. Control Performance Evaluation

Verify that control reactions perform as expected:

- Positive Control: amplification signal should be observed
- Negative Control (no-template control): no amplification signal

If control performance does not meet expectations, results should not be interpreted and the experiment should be repeated.

3. Quantitative Analysis (Optional)

If quantitative analysis is performed, serial dilutions of the Positive Control may be used to generate a standard curve under laboratory conditions. The relationship between fluorescence signal and target concentration may be used for comparative analysis.

4. Qualitative Assessment (Optional)

Amplification results may be evaluated based on Ct values:

- Ct values may be used for comparative analysis of target signal levels under experimental conditions.
- Absence of amplification or late Ct values may indicate low or undetectable levels

Results should be interpreted within the context of experimental design and research objectives.

5. Important Notice

This kit is intended for research use only. Results are for analytical and investigational purposes and are not intended for diagnostic applications.

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