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Bacterial Gene Detection Kit

# BactFinder™

# Instruction Manual

**--- Features ---**

This product is a kit for detecting bacterial genes extracted from cell culture media by real-time PCR.

**--- General Precautions ---**

1) Read this instruction manual carefully before use. Use the product in accordance with the operating procedures described in the instruction manual. We do not guarantee the reliability of the product if it is used in a manner other than that described in the operating instructions or for other than the intended purpose of use.
2) All specimens should be treated as potentially infectious and handled with extreme caution while wearing protective equipment (glasses, gloves, masks, etc.).
3) This reagent is for research and should not be used for any other purpose. It is not intended for diagnostic purposes.

**--- Kit Composition ---**

| | | |
|---|---|---|
| (1) DNA Polymerase | ..... | 1 |
| (2) 10x PCR Buffer | ..... | 1 |
| (3) 50 mM $MgCl_2$ | ..... | 1 |
| (4) 10 mM each dNTPs | ..... | 1 |
| (5) 10x PP Mix*1 | ..... | 1 |
| (6) Positive Control (100 copies/μL) | ..... | 1 |
| (7) Internal Control*2 | ..... | 1 |
| (8) DNA Free Water | ..... | 1 |
| (9) 1.5mL tube for reagent preparation | ..... | 12 |
| (10) PCR Reaction 8-Strip, Cap | ..... | 12 each |

1: 10x PP Mix contains fluorescent probes, so take care to shield it from light.

2: Internal Control is used as a reference. If a sample contains a large amount of non-target nucleic acids or inhibitors, it may not be detected.

**---Purpose of Use ---**

Detection of bacterial genes extracted from cell culture media.

**--- Operating Precautions ---**

1) If probes or primers in the reaction reagent are degraded by nuclease contamination, accurate detection will not be possible. As well as from the experimental equipment, nuclease contamination can come from the sweat and saliva of the user, so take care during operation.
2) To prevent sample contamination, it is recommended that sample preparation and nucleic acid extraction is physically isolated from reaction reagent preparation. If this is difficult, UV irradiation and cleaning of the workspace should be performed before moving on to the next step.
3) After using reagents, store with the lid of the reagent tube tightly closed. Since they contain fluorescent reagents, take care to shield them from light.
4) The reagent tubes should be used with care and not dropped from a high place or subjected to strong impact as they may break.
5) The Positive Control should be well mixed and spun down before use.
6) When dispensing reagents, always use new disposable tips to prevent contamination between samples.
7) Operate the real-time PCR device according to its instruction manual.
8) This kit uses the real-time PCR method, and amplification and detection are performed simultaneously, so there is no need to use the amplified product after the reaction is finished for electrophoresis or other purposes. Do not remove the amplified product from the PCR tube, etc., as this may cause contamination.

**--- Administration and Dosage ---**

**[Equipment]**

Prepare the following equipment as necessary.

Mixer, micropipette, microtubes (sterilized, low adsorption, DNase/ RNase Free), filter tips (sterilized, low adsorption, DNase/RNase Free), centrifuge, heat block, real-time PCR device capable of detecting FAM and HEX

**[Operating Instructions]**

**1. Nucleic acid extraction**

Nucleic acids should be extracted from specimens using a commercially available nucleic acid extraction kit. It is recommended to evaluate the extraction efficiency in advance.

Add an appropriate amount of Internal Control per sample as a reference and perform nucleic acid extraction.

**2. Reagent Preparation**

1) Thaw DNA Free Water, 10x PCR Buffer, 10 mM each dNTPs, 50 mM $MgCl_2$, and 10x PP Mix, mix thoroughly with a mixer and spin down. Keep DNA Polymerase on ice after spinning down.
2) On ice, prepare a master mix of PCR reaction solution.
   Prepare the required amount of master mix considering dispensing losses, etc. (See “Composition of PCR reaction solution” below).
3) Dispense 15 μL of the master mix prepared in step 2) into PCR tubes or plates.
4) Add 10 μL of DNA Free Water as the Negative Run Control and 10 μL of Positive Control as the Negative Run Control. Measure the Negative Control and Positive Control for each test.
5) Add 10 μL of the nucleic acid extract of each sample.
6) Mix the reaction solution in the PCR tube or plate by pipetting or mixer and spin down.

Composition of PCR reaction solution

| Reagent | Amount (for 1 reaction) |
|---|---|
| DNA Free Water | 8.5 μL |
| 10x PCR Buffer | 2.5 μL |
| 10x PP Mix | 2.5 μL |
| 10 mM each dNTPs | 0.5 μL |
| 50 mM $MgCl_2$ | 0.75 μL |
| DNA Polymerase | 0.25 μL |
| Total | 15 μL |

**3. Real-time PCR detection**

An example protocol for CFX96™ Real-Time PCR Detection System（Bio-Rad）Connect is shown below.

1) Set the fluorescence detection wavelength of the real-time PCR device to FAM - HEX.
2) The settings of the real-time PCR program should be set based on the following protocol. For the setting method, follow the instruction manual of the real-time PCR device being used.

RT-PCR program

| Temperature | Time | Cycles | Fluorescence detection |
|---|---|---|---|
| 95°C | 10 sec. | 1 | No |
| 95°C | 10 sec. | 45 | Yes (FAM - HEX) |
| 66°C | 1 min. | | |

Do not perform fluorescence detection in the first cycle of the PCR step due to the possibility of background signal detection.

If there is a Passive Reference setting, select "None" and start measurement.

**--- Determination of Results ---**

The results are determined using the analysis software of the real-time PCR device; if a Cq value is calculated, it is considered positive, and if not, it is considered negative.

CFX96™ Real-Time PCR Detection System（Bio-Rad）Connect recommends analysis using the regression method and baseline subtracted curve fit mode. After analysis, check the amplification curve and confirm that the Cq values are calculated correctly. If the regression method does not analyze correctly due to the purity of the nucleic acid extract, poor mixing of reagents, foaming, etc., use the single threshold method or other methods for analysis. Follow the instructions in the manual provided with the software.

**--- Notes on the Determination ---**

1) All bacterial items and Positive Control are detected by FAM. All references (Internal Control) are detected by HEX. If the real-time PCR system being used is not compatible with HEX, try using a fluorochrome with a fluorescence wavelength close to the maximum fluorescence wavelength of HEX.
2) Reference (Internal Control) may not be detected if large amounts of other nucleic acids are present.
3) Depending on the real-time PCR device and analysis method, Cq values may be calculated from back-noise signals in very rare cases. After the test, be sure to check the amplification curve and confirm that the obtained Cq value is derived from the nucleic acid amplification.

**--- Performance ---**

**1. Sensitivity and accuracy**

When the Negative Control is tested as a sample no fluorescence amplification is detected; when the Positive Control is tested as a sample, a positive result is obtained within 35 cycles.

**2. Product performance data**

For detailed performance data, contact the manufacturer's sales representative or customer support representative.

**--- Precautions During Use or Handling ---**

**1. Handling (hazard prevention)**

1) Specimens should be treated as potentially infectious.
2) If the reagent gets into the eyes or mouth, take first aid measures such as rinsing thoroughly with water, and if necessary, consult a physician or other healthcare professional.
3) DNA Polymerase contains more than 40% glycerin. Do not use near fire.

**2. Use**

1) Store at -30°C to -15°C according to storage instructions.
2) Do not use reagents after their expiration date.
3) Do not mix reagents with different serial numbers.
4) Do not use for any other purpose.

**3. Disposal**

1) To avoid contamination, dispose of used tubes and plates in a double sealed plastic bag without opening the lid as medical waste and according to waste regulations. To prevent PCR amplification products from scattering, do not autoclave.
2) Dispose of used reagents, equipment, etc. as medical, industrial, or infectious waste in accordance with the Act on Waste Management and Public Cleaning and the Water Pollution Prevention Act.

**--- Storage and Shelf-life ---**

**[Storage]**

Store at -30 to -15°C.

**[Shelf-life]**

6 months from date of manufacture

The expiration date is on the label on the outer packaging and container.

**--- Packaging ---**

BactFinder™ : for 50 tests ..... Code 69263

**--- Related Products ---**

FungiFinder™ : for 50 tests ..... Code 69264

**--- Contact ---**

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