Bacteria DNA Detection Kit (qPCR) V01

Product Name
Bacteria DNA Detection Kit (qPCR)

Package Specifications
50 Reactions

Expected Use
The kit is used for qualitative detection of Bacteria DNA contamination in cells, cell and gene therapies, and other biological products.

Product Description
The kit utilizes PCR-fluorescent probe technology to design specific primer and probes targeting the conserved region of Bacteria 16S rRNA coding sequences, enabling targeted amplification of Bacteriai DNA. It features simple operation, rapid detection, high specificity, and high sensitivity.

Internal control (IC) can be added during the sample extraction phase to evaluate extraction efficiency, or incorporated during the PCR amplification reaction phase to monitor potential inhibition of the amplification process and prevent false-negative results.

This kit contains UDG enzyme to prevent contamination of amplification products.

This kit is designed for use with Zhuhai Hengqin Baorui Nucleic Acid Extraction or Purification Kit, with a detection limit of 10 CFU/ reaction.

Components
The kit contains the following components:

Components	Specification (50 Reactions)
Bac qPCR MIX	1.0 mL × 1 tube
Bac positive control	1.5 mL × 2 tubes
Bac negative control	1.5 mL × 2 tubes
Bac internal control *	1.0 mL × 1 tube
instructions	1 copy

explain ：

*When performing both bacteria and fungi DNA detection, add only one internal control (Fun or Bac) to the sample.

Components in different batch numbers of reagent kits are not interchangeable.

Reagents required for experimental procedures but not included in the kit: nucleic acid extraction or purification kit.

Storage Conditions and Shelf Life
1.The kit shall be stored at-18℃ or below in a light-protected environment, with a shelf life of 24 months.

2.Avoid repeated freeze-thaw cycles for the kit; the number of freeze-thaw cycles should not exceed 7.

3.Product batch number and expiration date: Refer to the product outer packaging.

Applicable Devices
Including but not limited to the following models: SLAN-96P and SLAN-96S fully automated medical PCR analysis systems; ABI7500 and ABI QuantStudio™ 5 real-time fluorescent quantitative PCR systems; Roche LightCycler 480 fluorescent quantitative PCR system; and Perlor CFX96 quantitative PCR system.

Related Equipment
Clean benches or biosafety cabinets, handheld centrifuges, vortex mixers, real-time PCR instruments, and pipettes of various specifications.

Protocol
1.Pre-experimental preparation

The procedure should be performed under aseptic conditions, preferably in a laminar flow hood or biosafety cabinet. After ultraviolet irradiation for 30 minutes, the work surface, pipettes, and centrifuge tube racks should be sprayed with 75% alcohol and dried.

2.Preparation of Reagents

Remove Bac qPCR MIX, Bac internal control, Bac negative control, and Bac positive control from the kit, thaw at room

temperature, and mix thoroughly by vigorous shaking before instant centrifugation for subsequent use.

Based on the number of samples to be tested, calculate the required number of reaction wells (N), typically with 2 replicates. N = (Number of samples to be tested + 1 template-free control (NTC) + 1 negative control sample (NCS) + 1 positive control sample (PC)) × 2.

According to the calculated detection quantity, Bac qPCR MIX was aliquoted into 96-well PCR plates or PCR eight-channel tubes at a volume of 20 μL per well.

3.Nucleic Acid Extraction and Purification

Perform nucleic acid extraction and purification on negative controls and test samples using nucleic acid extraction or purification kits, following the operational procedures specified in the kit. The internal control addition volume is 10 μL, and the sample volume is 200 μL.

4.Amplification and Detection

4.1 After aliquoting Bac qPCR MIX to reaction tubes at 20 μL per tube, prepare the reaction mix by adding negative control, positive control, and the prepared target nucleic acid according to the following table:

Components	Single well volume (μL)
Bac qPCR MIX	20
Template DNA	10
Total Volume	30

*If the internal control function in the kit is not used, no internal control needs to be added during extraction, the CY5 channel should not be set in the program configuration, and CY5 results need not be considered during result analysis.

If no internal control is added during sample extraction but is introduced during amplification, 1 μL of internal control should be added per reaction when preparing the MIX. When aliquoting the qPCR MIX, 21 μL per reaction should be dispensed, resulting in a total PCR reaction volume of 31 μL per reaction.

4.2 The layout of perforated plates can refer to the table below

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC					S1	S1					PC
B	NTC					S2	S2					PC
C						S3	S3					
D						S4	S4					
E						S5	S5					
F						S6	S6					
G	NCS					S7	S7					
H	NCS					S8	S8					

This example demonstrates the detections of 1 template-free control NTC, 1 negative control sample NCS, 1 positive control PC, and 8 test samples, with each test performed in duplicate wells.

*For actual testing, adjust the layout according to sample quantity by referring to this example.

4.3 Transfer the prepared reaction mixture to the reaction tube, cover the tube, mix thoroughly, and perform instantaneous centrifugation before transferring it to the fluorescence PCR instrument.

4.4 Place the reaction tube into the sample slot of the fluorescence PCR instrument and run the following program on the fluorescence PCR instrument:

Step	Condition	Cycles
UDG treatment	50℃: 2 minutes	1
Pre-denaturation	95℃: 3 minutes	1
PCR amplification	95℃: 10 seconds, 62℃ (fluorescence collection): 30 seconds	40
cooling	25℃*: 10 seconds	1

Select FAM and CY5 as the fluorescence channels, where FAM is the target gene and CY5 is the internal control gene. For ABI series instruments, choose ROX as the reference fluorescence.

If the instrument does not support 25℃, set to 37℃.

5.Interpretation of Results

5.1 Threshold Line Setting

The threshold line is adjusted based on instrument noise conditions, with the threshold line set beyond the background signal fluctuation position. For manual adjustment, it should be precisely positioned above the highest point of the negative control amplification curve. Threshold line setting should be based on laboratory validation data, with the requirement to meet the detection limit as the benchmark.

5.2 Experimental Establishment Conditions

Quality control sample	FAM	CY5
NTC	Double well Ct ≥ 37 or no significant peak in the amplification curve	/
NCS	Double well Ct ≥ 37 or no significant peak in the amplification curve	Double well Ct value <35 with effective "S" -shaped amplification
PC	Double well Ct value <35 with effective "S" -shaped amplification	/

Quality control standards should be based on laboratory validation data, with the detection limit requirement as the benchmark.

5.3 Interpretation of Results

FAM	CY5	Result Evaluation
Among the two duplicate wells, more than one well showed Ct values <37 and effective "S" -shaped amplification.	Double well Ct value <35 with effective "S" -shaped amplification	Positive
	Double well Ct ≥ 35 or no significant peak in the amplification curve	Positive, with inhibition
Double well Ct ≥ 37 or no significant peak in the amplification curve	Double well Ct value <35 with effective "S" -shaped amplification	Negative
	Double well Ct ≥ 35 or no significant peak in the amplification curve	The result cannot be determined, with inhibition observed

If the CY5 channel is suppressed, retesting is required or the inhibitory factors should be identified and eliminated.

Notes

1. The kit should be stored at-18℃ or below.
2. Before conducting the experiment, please read the kit instructions carefully and follow the operational procedures strictly.Precise control of time and reagent volume during operation will yield optimal results.
3. Ensure that consumables used for nucleic acid extraction are clean and DNase/RNase.free The extraction process should be completed as quickly as possible, followed by proceeding to the next experimental step or cryopreservation.
4. Do not use expired components or mix components from different batches.
5. Freezing storage reagents should be completely thawed at room temperature before use, followed by instantaneous centrifugation to ensure complete sedimentation of the liquid at the tube bottom. Avoid repeated freeze-thaw cycles to prevent degradation of reagent performance.
6. Use new disposable polyethylene gloves to seal the reaction tube lid to avoid contact with the reaction tube using bare hands or used gloves. During testing, employ disposable powder-free latex gloves without fluorescent substances.
7. The laboratory shall strictly implement zoned management in accordance with relevant regulations, conducting genetic testing in the sequence of solution preparation area → template extraction area → amplification area → analysis area. Strict requirements must be enforced regarding personnel, equipment, reagents, and airflow direction within each zone.
8. Samples and positive controls should be promptly sealed after use to avoid contamination between components and aerosols, which may lead to false positives.
9. Amplification products must not be opened, and experimental waste should be collected promptly and disposed of harmlessly away from the PCR laboratory.
10. If the target in the sample is strongly positive, competitive inhibition within the system may affect the detection of the internal standard.

Disclaimer

This product is for research use only, and the liability of our company for this product is limited to the value of the product itself.

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