SHENTEK

Bacteria DNA Detection Kit

Version: A/0
For Research Use Only
Product No.: 1504632
Reagents for 50 Reactions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

MicroSHENTEK® Bacteria DNA Detection Kit is used together with MicroSHENTEK® Fungi & Bacteria DNA Extraction Kit, to qualitatively determine whether there is bacteria contamination in cells, cell products or vaccines, etc. The assay performance is validated according to the Ch.P, EP, JP and USP qPCR method with a detection limit of not more than 35 CFU/reaction.

The detection kit uses real-time PCR technique to detect possible bacterial DNA in the test samples, and is able to cover about 92% of known bacterial species, which matches nearly 60,000 species (or subspecies) of bacterial DNA sequences. The specificity and coverage have been validated in a variety of matrices, as well as on materials of species, production cells, and engineered strains.

For extraction information, please refer to MicroSHENTEK® Fungi & Bacteria DNA Extraction Kit User Guide (Product No. [illegible]).

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, mask and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
Bac Positive Control (PC)	NNA047	50 µL ×1 tube	-20℃
Bac qPCR Reaction Buffer	NNB019	220 µL × 2 tubes	-20℃, protect from light
Bac Primer & Probe MIX	NNC098	50 µL × 2 tubes	
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 2 tubes	-20℃

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applicable instruments, including but not limited to the following

- SHENTEK-96S Real-time PCR System
- LightCycler 480II Real-Time PCR System

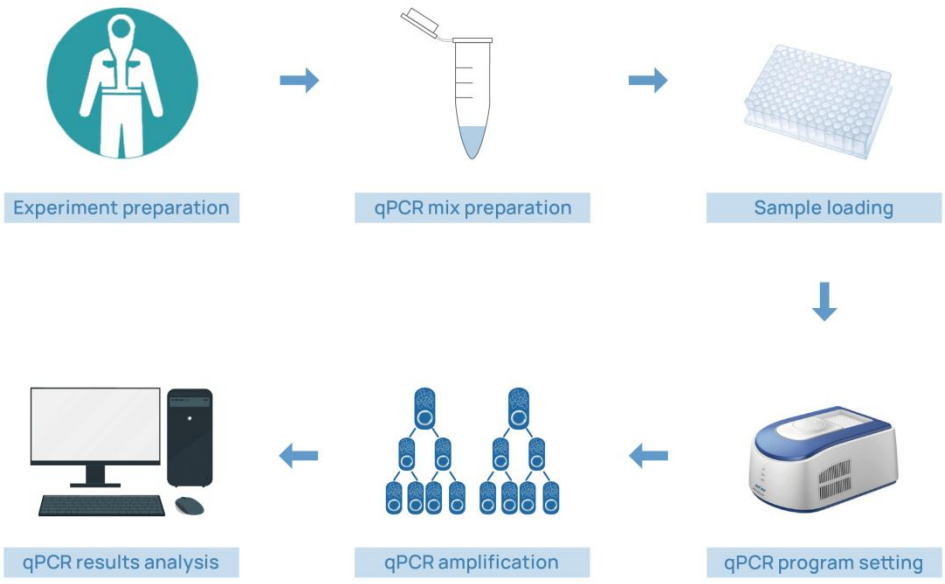
■ Required materials not included in the kit

- Nonstick, Sterile microfuge tubes, 1.5 mL and 2.0 mL
- PCR 8-well strip tubes with caps or 96-well plates with sealing films
- Low retention , Sterile filter tips 1000 µL, 200 µL, 100 µL and 10 µL
- 75% Ethanol
- Medical disposable sharps box
- Nucleic acid scavenger

■ Related equipment

- Single sterile desktop
- Benchtop microcentrifuge
- Microplate and micro tube shaker
- Real-time PCR system
- Pipettes, 1000 µL, 200 µL, 100 µL and 10 µL

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. The work area and environment were properly disinfected to remove residual nucleic acids. Irradiate the tabletop, pipettes and tubes with UV for 60 minutes, and disinfect with 75% ethanol and nucleic acid scavenger.
3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.
4. The laboratory is divided into the Negative area, the Sample area and the Positive area according to the MicroSHENTEK® Fungi & Bacteria DNA Extraction Kit User Guide. Each area is recommended to place a single sterile desktop.

■ qPCR MIX preparation

1. Determine the number of reaction wells according to the number of test samples and control samples with 2 replicates for each sample generally. Number of reaction wells = (1 Positive Co[illegible] (NTC) + 1 Negative Control Sample (NCS) [illegible] test samples)×2
2. Prepare qPCR MIX according to the following table.

Table 2. qPCR MIX preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
Bac qPCR Reaction Buffer	8 µL	264 µL
Bac Primer & Probe MIX	2 µL	66 µL
Total volume	10 µL	330 µL

Note: The preparation of the qPCR MIX should be performed in single sterile desktop in the negative area.

■ qPCR Reaction MIX preparation

1. Vortex each solution and mix well. Then add the solution according to Table 3 to

each well, and the plate layout of 96-well is shown in Table 4.

Table 3. qPCR Reaction MIX preparation in each well

PC (Positive Control)	10 μL qPCR MIX + 20 μL 1E-6 PC*
NTC (No Template Control)	10 μL qPCR MIX + 20 μL DDB
NCS (Negative Control Sample)	10 μL qPCR MIX + 20 μL of purified NCS
PCS (Positive Control Sample)	10 μL qPCR MIX + 20 μL of purified PCS
Test Sample	10 μL qPCR MIX + 20 μL of purified test sample

**PC is prepared by a 10-fold gradient dilution to 1E6-fold before adding to the reaction. To avoid experimental contamination, the dilution must be done in the last step of the experiment.*

Note: If the test samples are acquired from complete extraction and detection [illegible]

Table 4. Example of 96-well plate layout

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

- *This example shows 1 PC, 1 NTC, 1 NCS, 1 PCS, and eight test samples, with duplicate wells for each sample.*
- *In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity.*
- *Follow strictly the operation order: NTC and NCS should be added and sealed successively in the negative area, the test samples should be added and sealed in*

the sample area, and finally PCS and PC (1E-6) should be added and sealed in the positive area.

2. Seal the PCR 8-well strip tubes with caps or the 96-well plate with sealing film. Mix well in microplate or micro test tube shaker, then spin down the reagents for 10 seconds in centrifuge and place it in the qPCR instrument.

■ qPCR program setting

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an example, and set the qPCR program as follows:

1. Click on the Experiment Wizard.
2. Select Step 1 on the well Plate Edit page: Select the reaction wells.
3. Select Step 2: Select the "Micro-Fun&Bac" program in the project.
4. Click "Start" to run the program on the "Experimental Run" page.

If you have alternative qPCR system, set up the instruments as follows:

1. [illegible] method program and select the quantitative PCR assay template.
2. Run a new Probe template, and type th[illegible] FAM in the Reporter Dye drop-down li[illegible] Dye drop-down list, then click OK.
3. Set thermal-cycling conditions:
   a. Set the cycling reaction volume to 30 μL.
   b. Set the temperature and the time as following:

Table 5. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95°C	10:00	1
Denaturation	95°C	00:15	45
Annealing	55°C	00:30	
Extension	72°C*	01:00	

*Instrument will read the fluorescence signal during this step.

Note: If fungi and bacteria are detected by the same qPCR system, add UNG

enzyme at 25℃ for 10 minutes according to the fungal detection program and perform a two-channel assay with FAM and VIC.

■ Result analysis

Take the SHENTEK-96S Real-time PCR System (software version 8.2.2) as an example.

1. Select step 3 in the "Edit" page: define the reaction well, set the sample type for the NTC well as no template control, the PC well as the positive control, the NCS well as the negative control, and the test sample well as unknown .
2. Click "Analysis" on the "Experimental Analysis" page, and the detection values of NTC, NCS, PC, PCS and test samples can be presented in the "Reaction well Information Table".

3. Select Abs Quant / Fit Points analysis type in "Analysis", and click "√" to enter the interface, select Noiseband (Fluoresc) below "Noise Band interface", manually change the Noise Band (Threshold will be consistent with Noise Band), then click "Calculate" at the bottom left to collect the Ct value.

Note: If you use a different instrument or software, refer to the applicable instrument or software documentation. Usually real-time PCR instrument software automatically export the data report.

■ Acceptance Criteria

1. Guidance for the control samples as in the following table.

Table 6. Control sample result analysis

Control samples	FAM
NTC	Ct≥39.00 or absence of specific amplification for duplicate runs
NCS	Ct≥39.00 or absence of specific amplification for duplicate runs
PC	Ct<35.00 and effective "S" -type amplification for duplicate runs
PCS	Ct<39.00 and effective "S" -type amplification for duplicate runs

The control sample results shall be based on method validation data, and considered to satisfy LOD (Limit of Detection) requirement.

2. Guidance for test samples as in the following table.

FAM	Quality control sample	Results judgment
Ct≤39.00 (at least one well) and effective "S" -type amplification for duplicate runs	Meet the requirement	Positive
Ct≥39.00 or absence of specific amplification for duplicate runs	Meet the requirement	Negative
	Ct of PCS ≥39.00	Unable to judge, recommend to retest

- *If the Ct value of negative quality control is <39.00 but the Ct value was higher than 50-100 CFU strains for 2 cycles or more, the negative quality control could meet the requirements.*
- *When the negative and positive controls meet the requirements, if the Ct value of the test samples are <39.00 but higher than 50-100 CFU strains for 2 cycles or more, it could also be* determined *as not detected.*
- *In an event that the sample is special, or some abnormalities occur, or results are difficult to be determined, please contact us for technical support.*

■ Reference

Ch.P<1101> Sterility Tests

Ch.P-General principles-preparation and quality control of animal cell matrix for production and verification of biological products

EP<2.6.21> Nucleic Acid Amplification Techniques.

EP<2.6.7> Mycoplasma

EP<2.6.16> Tests for extraneous agents in viral vaccines for human use

EP<2.6.1> Sterility

USP<71> Sterility Tests

JP<4.06> Sterility Tests

Support & Contact

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