SHENTEK

Fungi & Bacteria DNA Extraction Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1504633
Reagents for 50 Extractions

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

1. Product information

■ Product description

MicroSHENTEK® Fungi & Bacteria DNA Extraction Kit is used for the extraction of trace Fungi & Bacteria DNA from master cell banks, working cell banks, vaccines and cellular therapeutic products, and other complex matrices (≤ 10^6 cells). The extraction kit works together with MicroSHENTEK® Fungi DNA Detection Kit and MicroSHENTEK® Bacteria DNA Detection Kit for an integrated workflow from sample preparation to detection assay. The recommended system extraction performance can reach 50-100CFU/tube and the detection limit of the corresponding test kit is not greater than 35CFU/reaction.

The kit is compatible with automated extraction using rHCDpurify instrument.

■ Kit contents and storage

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

Table 1. Kit component and storage

No.	Reagent	Part No.	Quantity	Storage
I	Lysis buffer	NND046	2.5 mL × 2 bottles	room temperature
	Binding solution	NND047	5 mL × 2 bottles	
	Wash buffer A	NND048	7.5 mL × 2 bottles	
	Elution buffer	NND049	2.5 mL × 2 bottles	
	Dilution buffer	NND021	10 mL × 2 bottles	
	Sample treatment tube	NND005	50 tubes	
II	5M NaCl	NND040	500 μL × 2 tubes	2-8℃
	Magnetic particles	NND033	750 μL × 2 tubes	
III	Precipitation solution I	NND003	25 μL × 2 tubes	-20℃
	Precipitation solution II	NND004	500 μL × 2 tubes	
	Proteinase K	NND023	500 μL × 2 tubes	

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

■ Required materials not included in the kit

- Sterile ultra-pure water
- Anhydrous Ethanol (AR)
- 100% Isopropanol(AR)
- 75% Ethanol
- Sterile，Low Retention tips 1000 µL, 100 µL and 10 µL
- Sterile, Low Retention tubes 1.5 mL, 2.0 mL and 50 mL
- Bacteria positive control strain, contact us to order
- Fungi positive control strain, contact us to order
- Disposable sharp weapon box for medical use
- Nucleic acid scavengers

■ Related equipment

- Cell wall breaking system(Lyse-S)
- Single sterile desktop
- rHCDpurify System
- Benchtop microcentrifuge
- High-speed refrigerated centrifuge
- Vortex mixer
- Dry bath incubator
- Micropipettes,1000 µL, 200 µL, 100 µL and 10 µL

2. Methods

■ Experiment preparation

Experimental areas:

- The experimental areas are recommended to be divided into Negative, Sample and Positive areas according to the type of sample.
- Operate in Single sterile desktop in each area.

Negative: Negative control samples preparation area & reagent preparation area.

Sample: Samples processing area.

Positive: Positive control samples preparation area.

Environmental control:

- Working area and environment are properly disinfected to remove residual nucleic acid. At least use nucleic acid scavenger and 75% alcohol to fully wipe and disinfect the ultra-clean bench in each area.
- Turn on the ultraviolet lamp of the clean bench and ensure that the irradiation time is not less than 1 hour, then open the ultraviolet facilities in each area and ensure that the irradiation time is not less than 30 minutes.

Reagent preparation:

Note: All reagent dilution and preparation are operated in the negative area!

- Add 10 mL of Anhydrous Ethanol to Wash buffer A(NND048).
- Prepare a 70% Anhydrous Ethanol buffer in a clean tube, label as “Wash buffer B”, expiry date is 2 weeks.
- Dilute Precipitation solution I(NND003) for 100 times in a 10 fold gradient according to the volume needed.
- Store Wash buffer at room temperature and seal to prevent ethanol evaporation (please pay attention to the expiry date).
- Prepare 100% Isopropanol.
- If the Lysis buffer(NND046) or Binding solution(NND047) are cloudy or contains, heat at 37℃ until it clears and mixed thoroughly.
- The Magnetic particles should be equilibrated at room temperature for 10 minutes before use, and vortex to mix well for 10 seconds.

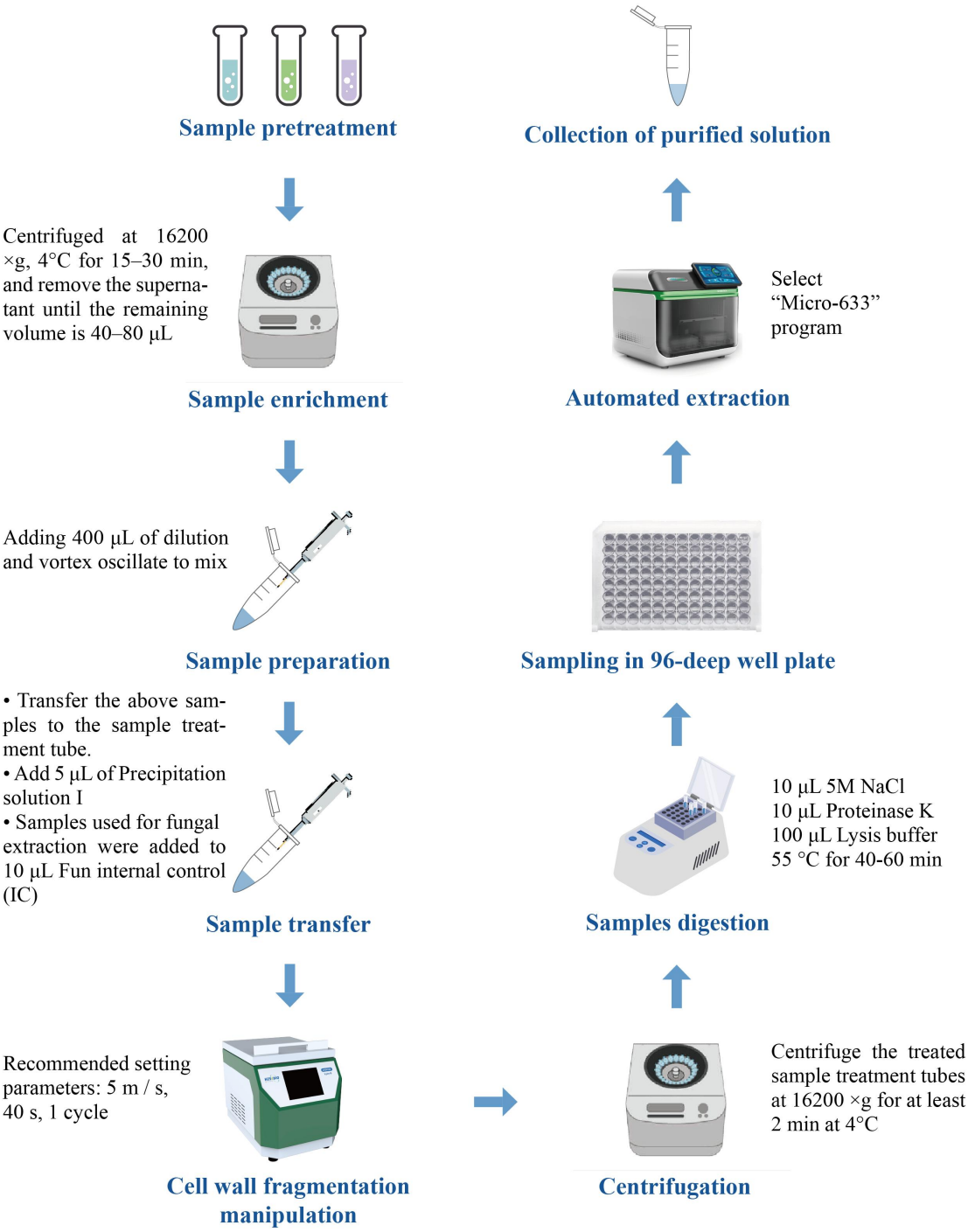
Equipment preparation:

- Set the dry bath temperatures to 55°C.
- Cell wall breaking system: All-around wipe disinfection treatment with nucleic acid scavenger and 75% ethanol.
- rHCDpurify System:

  Open the power button, click "Login" to enter the account and password, and enter the main interface.

Wipe the surface with nucleic acid scavenger and 75% ethanol respectively, and insert the plastic sleeve followed by UV-sterilization for no less than 1 hour.

■ Sample pretreatment



Note: If the sample is a cellular matrix, first centrifuge for 5 minutes at 70-75×g and absorb the supernatant.

Sample enrichment：

1) Divide the samples into two tubes for bacterial extraction and fungal extraction. The samples were then centrifuged at 16200×g, 4℃ for 15-30 min, and remove the supernatant until the remaining volume is 40–80 μL, adding 400 μL of Dilution buffer(NND021) and vortex to mix well.

Sample preparation：

1) Transfer the above samples to the Sample treatment tube (NND005).
2) Add 5 μL of diluted precipitation solution I.
3) Additional step for fungal extraction, add 10 μL Fun Internal Control (IC) to each sample tube (reagent from MicroSHENTEK® Fungi DNA Detection Kit).

Cell wall fragmentation:

1) Place the sample treatment tubes symmetrically into the instrument according to the instruction of Cell wall breaking system, tighten the cover fastener and close the top cover until it locks automatically.
2) Select the custom parameter setting interface, and choose the speed, time and cycle number according to the prompts. Recommended setting parameters: 5 m/s, 40 s per cycle.

Centrifugation:

1) Centrifuge the treated sample treatment tubes at ≥ 16200×g for at least 2 minutes at 4℃.
2) Transfer the supernatant to a new 1.5 mL centrifuge tube (avoid to aspirate white particles at the bottom).

Control samples：

1) Negative Control Sample(NCS): Take equal amout of the Diluent buffer (NND049) to the test sample and follow the corresponding procedure above.
2) Positive Control Sample(PCS): Take the Fungi & Bacteria positive control, and add the test sample (recommended) or Diluent buffer(NND049) (please keep the total volume the same as the preapared test samples), and proceed as described above.

■ Samples digestion

1) Add 10 µL of 5M NaCl(NND040), 10 µL of Proteinase K(NND023) and 100 µL of Lysis buffer(NND046) to each sample, vortex to mix well and incubate at 55℃ for 40-60 minutes.

Note: To ensure complete digestion, incubate the sample for 20-30 minutes and vortex to mix well. The incubation time is determined by the complexity of the sample matrix.

After sample digestion, the following DNA extraction experiment should be performed as soon as possible.

■ DNA extraction

Automated procedure with rHCDpurify System

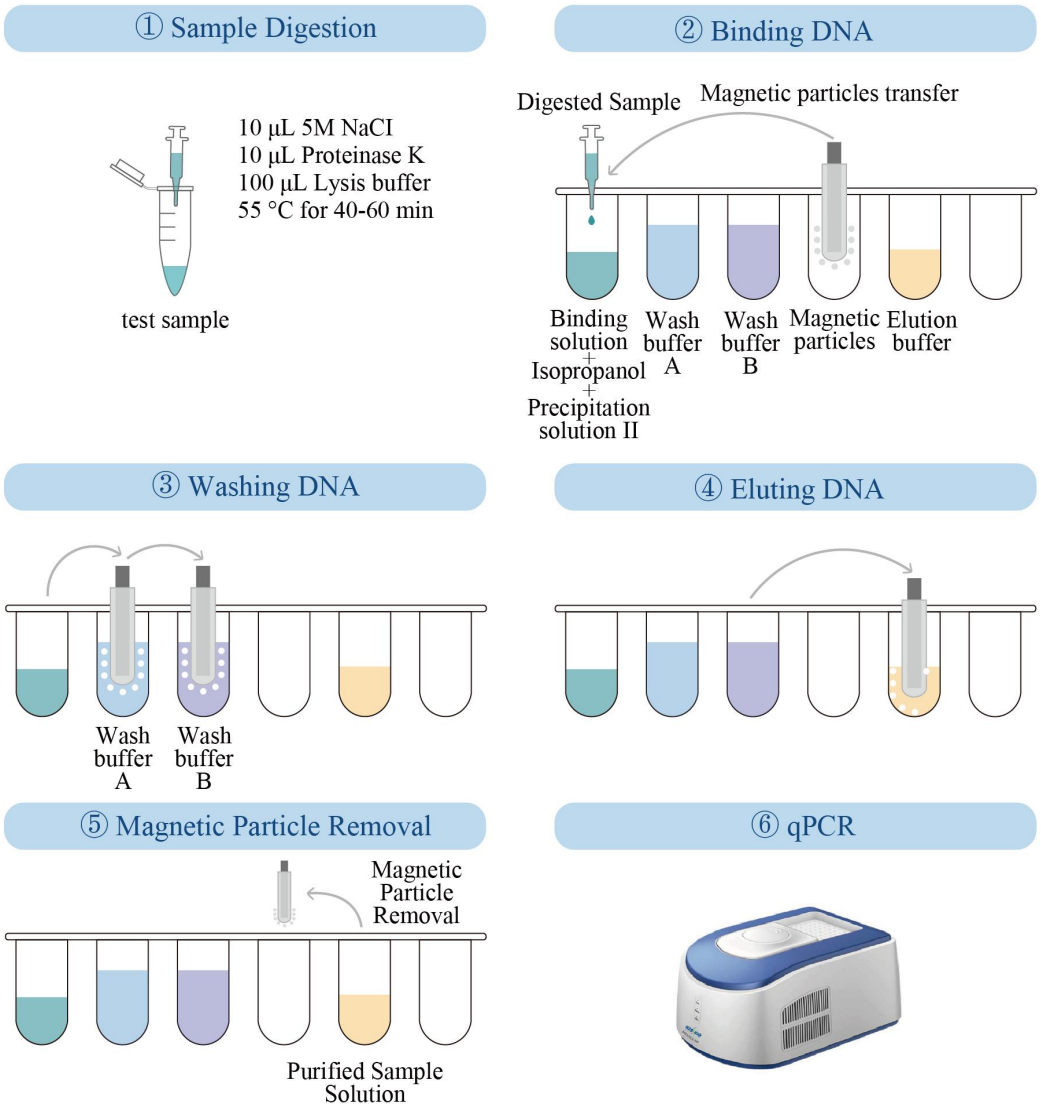


Plate preparation

Add the corresponding solution to each well according to the following 96 well plate layout:

Table 2. Example of samples and NCS layout-1

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1-Bac						NCS-Bac					
S2-Bac											
S3-Bac											
S4-Bac											
S1-Fun											
S2-Fun											
S3-Fun											
S4-Fun						NCS-Fun					

Table 3. Example of PCS layout-2

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
PCS-Bac											
PCS-Fun											

Column 1 or 7: Binding solution 200 µL/well, Isopropanol 200 - 400 µL/well, Precipitation solution II 9 µL/well and the digested sample solutions.

Column 2 or 8: Wash buffer A, 700 µL/well

Column 3 or 9: Wash buffer B, 700 µL/well

Column 4 or 10: Magnetic particles, 30 µL/well

Column 5 or 11: Elution buffer, 65 µL/well

Note: PCS and other samples are recommended to be placed in separate 96-well plates as shown in above plate layout.

The tested samples, NCS and PCS labeled with "-Bac" indicates bacteria extraction step, and the wellls labeled with "-Fun" indicates fungi in the extraction step.

Program Processing

Start the program before plate preparation:

Power on—self-test—  .

After plate is prepared, continue with the following steps:

1. Put the 96-deep well plate into the instrument and insert the plastic sleeve into the corresponding position. 96 deep well plate-1 is placed on the right side of the instrument, and plate-2 is placed on the left of the instrument.
2. Run—  —Micro-633—  , run about 52 min.
3. After extraction, immediately take out the deep-well plate, and transfer each purified sample solution to a nonstick centrifuge tube.

Precautions

1. It is recommended to separate molecular laboratory spaces for reagent preparation area (negative control sample preparation, PCR reagent preparation, negative test control preparation), sample preparation area (sample preparations), amplification area, etc. Each area is clearly marked with a fixed sign and provide separate sets of equipment and supplies to avoid cross-contamination. Experimental reagents, test samples, and PCR amplification products should be stored separately and not in the same storage place. Avoid every unnecessary walk in the experimental area to reduce the risk of contamination.

2. Ensure that the ambient environment temperature is not lower than 22°C during the experiment begins and humidity is not higher than 70%.

3. During the experiment, choose suitable gloves and change them regularly. Also use different lab coats, masks, hair covers and gloves in different experimental areas to avoid cross contamination.

4. Before and after rHCDpurify running, UV sterilization is required for at least 15 min, and use 75% ethanol wipes to clean the insider walls. The minimum interval between two extractions is 30 min.

5. Before rHCDpurify program starts, ensure that the PCR plate and plastic sleeves are loaded appropriately.

6. After rHCDpurify program, immediately transfer each sample solution to a new centrifuge tube. Condensated water may appear on the walls of Row 5th or 11th wells, which does not affect the DNA extraction, just simply transfer the bottom eluate to a new tube and guarantee more than 40μL as required for the assay.

7. To ensure the accuracy of test results, it is recommended that subsequent qPCR be performed the day the sample completes purification treatment,

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Support & Contact

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