SHENTEK

Mycobacteria DNA Extraction Kit

User Guide

Version: A/1
For Research Use Only
Product No.: 1503601
Reagents for 50 Extractions

Huzhou Shenke Biotechnology Co., Ltd

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

1. Product information

■ Product description

MycoSHENTEK® Mycobacteria DNA Extraction Kit is used for extraction of trace Mycobacteria DNA from a variety of biologics. It works with MycoSHENTEK® Mycobacteria DNA Detection Kit for an integrated workflow from sample preparation to detection assay.

For sample volume that is less than 400 μL, test sample DNA can be extracted directly using this kit. If sample volume is more than 400 μL, or it is necessary to increase the sample volume to achieve a higher detection sensitivity, the sample should be concentrated by centrifugation to a final volume of approximately 400 μL before using this extraction kit.

The kit is compatible with manual sample preparation, or 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

Reagent	Part No.	Quantity	Storage
Lysis buffer	NND028	5 mL × 1 bottle	room temperature
Binding solution	NND017	10 mL × 1 bottle	
Wash buffer A	NND015	15 mL × 1 bottle	
Elution buffer	NND019	5 mL × 1 bottle	
Dilution buffer	NND022	5 mL × 1 bottle	
MB Cell lysis buffer	NND039	1.25 mL × 2 tubes	2-8℃
5M NaCl	NND040	500 μL × 1 tube	
Pretreatment buffer	NND041	1.25 mL × 2 tubes	
Magnetic particles	NND034	1.25 mL × 2 tubes	
Proteinase K	NND024	1 mL × 1 tube	

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

- Anhydrous Ethanol (AR)
- 100% Isopropanol (AR)
- Nonstick, Low Retention Tips, 1000μL，100μL，10μL
- Nonstick, RNase-free Microfuge Tubes (1.5mL or 2mL, 50mL)

■ Related equipment

- Benchtop microcentrifuge
- Magnetic stand or rHCDpurify
- Vortex mixer
- Thermostat dry bath
- Pipettors, 1000μL, 100μL, 10μL
- Real-Time PCR System
- High-speed refrigerated centrifuge, for use with 50mL tubes

2. Methods

■ Experiment preparation

Before first use of the kit:

- Add 20mL of Anhydrous Ethanol to Wash buffer A.
- Prepare a 70% Anhydrous Ethanol buffer in a clean tube, label it “Wash buffer B”.
- Store Wash buffer at room temperature.

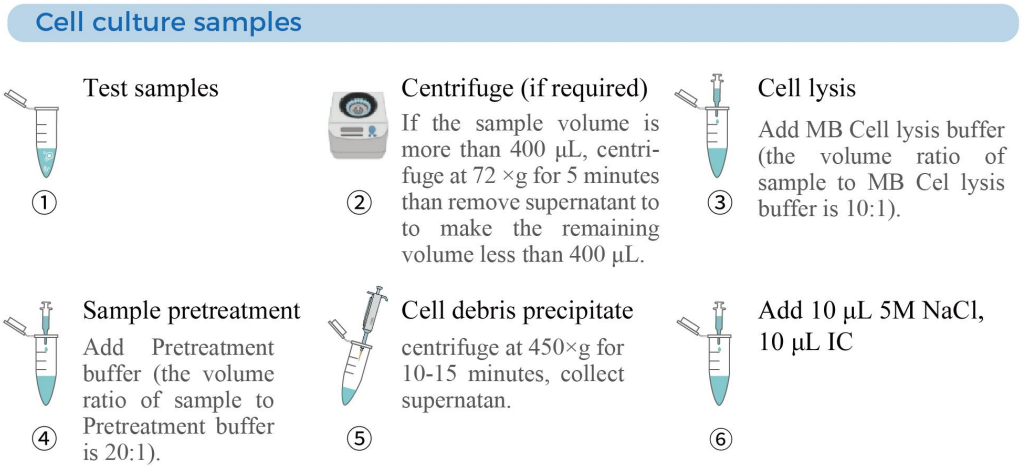
Before each use of the kit:

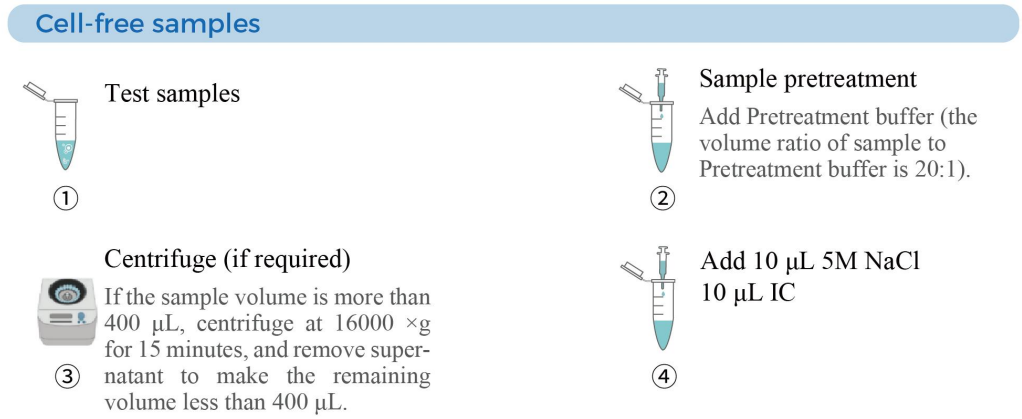
- Prepare 100% Isopropanol.
- Prepare 2 dry bath temperatures, 55°C and 70°C.

  Note: If the Lysis Buffer or Binding Solution is cloudy or contains precipitates, heat at 37°C until it clears.

➢ Balance the Magnetic Particles at room temperature for 10 minutes, and vortex to mix well before use.

■ Sample preparation





➢ **Cell-free samples**

1) Add Pretreatment buffer (the volume ratio of sample to Pretreatment buffer is 20:1), vortex and mix well.

2) If the sample volume is less than 400 µL, go direct to step 3); If the sample volume is more than 400 µL, centrifuge at 16000×g for 15 minutes, and remove supernatant to make the remaining volume less than 400µL.

3) Add 10 µL 5M NaCl, 10 µL IC, vortex for 10s, and spin for 3s.

➢ **Cell culture samples**

1) If the sample volume is less than 400 μL, go directly to step 5); If the sample volume is more than 400 μL, centrifuge at 72×g for 5 minutes to pellet the cells and remove supernatant to make the remaining volume less than 400 μL.

2) Resuspend the cells and add MB Cell lysis buffer (the volume ratio of sample to MB Cell lysis buffer is 10:1), vortex for 10s, and spin for 3s.

3) Add Pretreatment buffer (the volume ratio of sample to the pretreatment buffer is 20:1), vortex and mix well.

4) Incubate at room temperature for 5 minutes, centrifuge at 450×g for 10-15 minutes to precipitate the cell debris. Collect as much supernatant as possible.

5) Add 10 μL 5M NaCl, 10 μL IC, vortex for 10s, and spin for 3s.

- **Negative control sample (NCS)**

1) Add 100-400 μL Dilution buffer (the volume can be consistent with the volume of test samples).

2) Add Pretreatment buffer (the volume ratio of sample to Pretreatment buffer is 20:1), vortex to mix well.

3) Add 10 μL 5M NaCl, 10 μL IC, vortex for 10s, and spin for 3s.

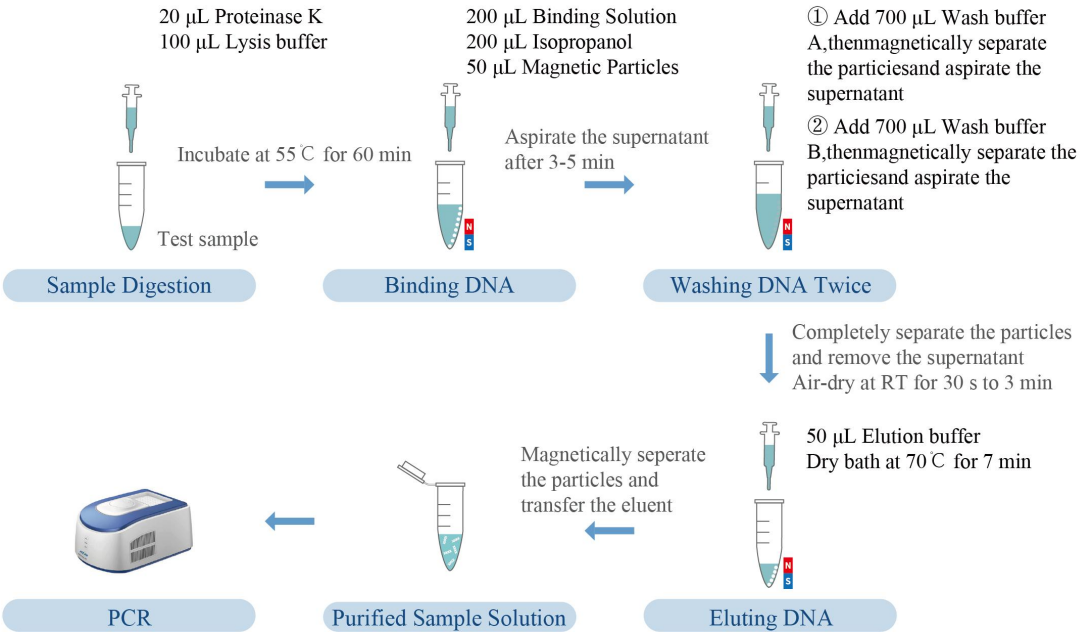
■ Sample digestion

1. Add 20 μL Proteinase K to each sample, vortex to mix well.
2. Add 100μL Lysis buffer, vortex to mix well, incubate at 55℃ for 60min.

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

■ Mycobacteria DNA extraction

1. Manual procedure



Binding DNA

1. Balance the Magnetic Particles at room temperature for 10min， vortex until the particles are completely suspended.
2. Spin the samples for 3 seconds , add 200 μL of Binding solution, and add 200 μL of Isopropanol and 50 μL of Magnetic particles.
3. Place the centrifuge tube of the mixture on a vortex adaptor and shake for 5 min, then place it on a Magnetic stand after a 3s spinning in a microcentrifuge.
4. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.

   Note: The magnetic particles should be resuspended before added to the sample, to ensure the consistency of the amount of magnetic particles added to each sample.

   The time for complete separation is about 3-5 minutes. When removing the supernatant, avoid removing the Magnetic particles together with the supernatant.

Washing DNA

1. Take each tube of Magnetic particles pellet from the Magnetic stand, add 700 μL of Wash buffer A, vortex for 10s and spin for 3s. Place the tubes in the Magnetic stand till the supernatant clears and particles completely separate. Aspirate the supernatant without disturbing the Magnetic particles, then discard the supernatant to complete the first washing.
2. Take each tube of Magnetic particles pellet from the Magnetic stand, add 700 μL of Wash buffer B, vortex for 40s and spin for 3s in .Place the tube in the Magnetic stand till the supernatant clears and particles completely separate. Aspirate the supernatant without disturbing the Magnetic particles, then discard the supernatant to complete the second washing.
3. To remove supernatant completely, spin in a microcentrifuge for 3 seconds and place each tube in the Magnetic stand. Wait until the particles completely separate, use a 10 μL volume pipette to carefully remove the residual supernatant.
4. With the cap open, air-dry the Magnetic Particles pellet at room temperature for 30s-3min to remove any residual ethanol.

   Note:When removing the supernatant, avoid removing the Magnetic particles together with the supernatant.

   The drying time depends on the specific environment. It could be shorter in higher temperature or lower humidity condition, while slightly longer in lower temperature or higher humidity condition.

Eluting DNA

1. Add 50 μL Elution buffer to each sample, vortex for 5 seconds, Incubate at 70℃ for 7 min. Vortex 2 – 3 times during incubation to ensure complete resuspension of the Magnetic particles.

   Note: Vortex to shake the magnetic particles and eluent to the bottom of the tube. If centrifuge is needed to spin the particles and eluent from the cap to the bottom of the tube, vortex it again to mix well.
2. Quickly spin the tube for 3 seconds, and then place the tubes in the magnetic

stand. Carefully transfer the eluate to a nonstick 1.5mL centrifuge tube.

3. Quickly spin the tube for 3 seconds again, and then place it in the magnetic stand again. Carefully transfer at least 40 µL eluent to a new nonstick 1.5mL centrifuge tube .

Note: Transfer the eluate completely and avoid leaving any residuals behind.

2. Automated procedure with rDNApurify

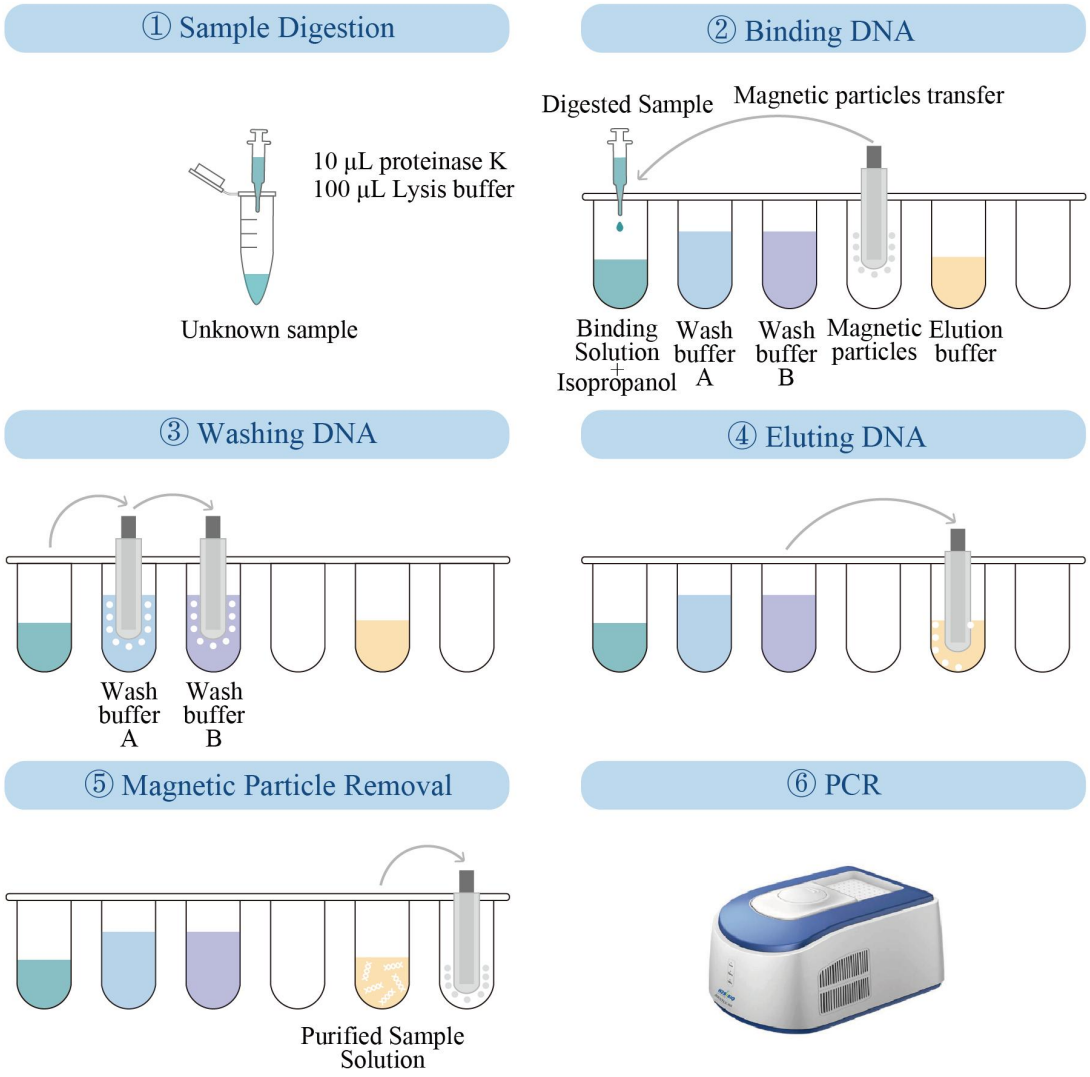


Plate preparation

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

Table 2. Example of 96-well Plate layout

Group 1						Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1											
S2											
S3											
S4											
S5											
S6											
S7											
S8						NCS					

Column 1 or 7: Binding solution 200 µL/well, Isopropanol 200 µL/well, 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, 50 µL/well

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

Note: The samples should be added after all reagents are transferred.

Program Processing

Start the program before plate preparation:

Power on—self-test—  .

1. Use 75% alcohol wipes to clean the insider walls.
2.  —UV—  —  15min—  .

After plate is prepared, continue with the following steps:

3. Put the 96 deep well plate into the instrument and insert the plastic sleeve into the corresponding position.
4. Run—  —Myco-601—  , run about 50min.
5. After extraction, immediately take out the deep-well plate, and transfer all the

sample purification solution to a nonstick 1.5mL 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 has separate sets of equipment and supplies to avoid intermixing. Experimental reagents, test samples, and PCR amplification products should be stored separately and not in the same storage place. Eliminate unnecessary walk in the experimental area to reduce the contamination risks.*
2. *Ensure that the ambient temperature is not lower than 22°C before the experiment begins.*
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. *Centrifuge the reagent tubes before opening to avoid aerosol production or liquid splashing, as well as to avoid contamination to the gloves or pipettes. The liquid on the caps or walls should be spun down to the bottom of the tube.*
5. *Used tips and liquid waste must be disinfected, and then discarded in a designated place, and if necessary, shipped off-site.*
6. *After PCR amplification, wear disposable gloves to take the PCR tube or plate out, and check whether the caps or seals are tightly closed, and whether the walls are cracked. Ensure that the reaction mixture does not leak. Discard it in the designated place, and the caps or seals should not be removed.*
7. *Place the tubes in the magnetic stand with the pellet against the magnet, and rotate the tubes slowly during the process to accelerate the magnetic particle aggregation.*
8. *During DNA washing and elution, centrifugation should be performed right after vortex to ensure that no magnetic particles or liquid on the tube caps or walls.*

9. Do not over dry the pellet when removing the residual ethanol, over drying will make the pellets difficult to resuspend in the Elution buffer in the next step.

10. Please perform mycoplasma DNA detection assays on the same day after mycoplasma DNA extraction to ensure the accurate results.

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

12. Before and after rHCDpurify program running, UV sterilization of the machine is required for at least 15 min, and use 75% alcohol wipes to clean the insider wall. The minimum interval between two extractions is 30 min.

13. After rHCDpurify programed, immediately transfer each sample solution to a new centrifuge tube. Condensed 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 40 µL as required for the assay.

Effective date: 08 Jul. 2024

Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189