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Pathogen Nucleic Acid Extraction Kit Instructions

Composition

Pathogen Nucleic Acid Extraction Kit Cat. No.	50 Preps 4011050
Sample Lysis Tubes	50
Spin Columns	50
2 ml Collection Tubes	50
Proteinase K	1.2 ml×2
Buffer AT	40 ml
Buffer SL	12 ml
Buffer DX	500 µl
Buffer WA (concentrate)	12 ml
Buffer WB (concentrate)	10 ml
Buffer TE	6 ml
Instructions	1

Storage

1. Proteinase K should be stored at -20°C.
2. If other reagents and items are stored at room temperature (0~30°C), they can keep their performance unchanged for 2 years, and if the product is stored at 2~8°C, the validity period can be extended to more than 2 years.

Technical Support

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Introduction

This product is suitable for the extraction of nucleic acids from a variety of pathogens (bacteria, fungi, viruses) from a variety of samples such as whole blood, swabs, biological fluids, and culture cells. The nucleic acids in various pathogens are released by chemical and mechanical lysis methods, and the nucleic acids are adsorbed by column purification technology, and the nucleic acids are washed by Buffer WA and Buffer WB, and then eluted with Buffer TE, which can be used for various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml and 2 ml centrifuge tubes and pipette tips.
3. Disposable gloves and protective equipment and tissues.
4. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml tubes).
5. Water bath and vortexer.

Preparation Before Use

1. If the centrifuge has refrigeration function, set the temperature to 25°C.
2. Set the water bath temperature to 56°C and 70°C, incubate Buffer AT and Buffer TE to 56°C.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the reagent bottle and ticking the box on the label to mark "Ethanol Added".
4. While chemical lysis is sufficient for some gram-negative bacteria, the cell walls of gram-positive bacteria, as well as yeast and other fungi, need to be destroyed by other methods. To maximize the lysis efficiency of these cells, sample lysis tubes are provided for mechanical destruction. Simgen sample lysis tubes with L (Cat. No.: C-001-1）、M（Cat. No.: C-001-2) and S (Cat. No.: C-001-3) three specifications, this kit provides a universal sample lysis tube M for bacteria and fungi, if the pathogen detected is bacteria, you can purchase a more targeted sample lysis tube S separately.

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Protocol

For whole blood samples, please follow step 1a;
For swab samples, please follow step 1b;
For biological fluids or cultured cell samples, follow step 1c;

1a. Extraction and purification of pathogen nucleic acids from whole blood:

Pipette 400 µl whole blood sample directly into the sample lysis tube, add 100 µl Buffer AT, vortex vigorously for 10 min, spin down for a few seconds to allow the solution on the tube lid to settle to the bottom of the tube, and carefully transfer 400 µl supernatant into a clean 1.5 ml centrifuge tube (If a large amount of foam develops in the sample lysis tube, add 5 µl Buffer DX and gently invert until the foam disappears).

* Try to use freshly isolated or freeze-thaw samples no more than once for the extraction and purification of pathogen nucleic acids.
* Make sure to vortex vigorously to shake so that the pathogen is fully ground and lysed to improve the yield of nucleic acids.

1b. Extraction and purification of pathogen nucleic acids from eyes, nose, pharynx, or other swabs:

Cut the tip from the swab and place it in a 2 ml centrifuge tube, add 650 µl Buffer AT, incubate the centrifuge tube at 600 rpm for 10 min at 56°C, carefully transfer all the liquid to the sample lysis tube, vortex vigorously for 10 min, spin down for a few seconds to allow the solution on the tube lid to settle to the bottom of the tube, and carefully transfer 400 µl supernatant into a clean 1.5 ml centrifuge tube (If a large amount of foam develops in the sample lysis tube, add 5 µl Buffer DX and gently invert until the foam disappears).

1c. Extraction and purification of pathogen nucleic acids from biological or culture cells fluids:

Add up to 1.5 ml of biological or culture cells fluids to the sample lysis tube and centrifuge at full speed (>14,000 ×g) for 5 min. Discard the supernatant, be careful not to remove the porcelain beads. Add 500 µl Buffer AT, vortex vigorously for 10 min, spin down for a few seconds to allow the solution on the lid to settle to the bottom of the tube, carefully transfer 400 µl supernatant into a clean 1.5 ml centrifuge tube (If a large amount of foam develops in the sample lysis tube, add 5 µl Buffer DX and gently invert until the foam disappears).

* Biological fluids or liquid cultures can be collected repeatedly, if necessary.

2. **Add 40 µl Proteinase K, vortex for about 15 sec, and incubate at 56°C for 10 min.**
3. **Add 200 µl Buffer SL and vortex for about 15 sec, incubate at 70°C for 10 min. Spin down for a few seconds to allow the solution on the lid to settle to the bottom of the tube.**

* If DNA is extracted from a very fresh sample, some of the RNA may be extracted and purified together, but the presence of RNA does not affect PCR-related experiments. If RNA is to be completely removed, 5 µl RNase A (not provided, 50 mg/ml, Simgen Cat. No.: 8001001）。

4. **Add 300 µl absolute ethanol, close the lid, and gently invert 4~6 times to mix evenly.**
5. **transfer 600 µl mixture from step 4 into a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Be careful not to get the solution on the edge of the Spin Column orifice, so that subsequent washing steps fail to wash the Spin Column.

6. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, transfer the remaining mixture into the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the centrifuge tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, centrifuge at 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

9. Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, centrifuge at 14,000 rpm for 1 min.

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, otherwise the nucleic acid may be mixed with ethanol and the subsequent experimental results may be affected.

10. Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 50~100 µl Buffer TE incubated to 56°C in the Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm centrifugation for 1 min to avoid the tube lid coming off and damaging the centrifuge.

11. Discard the Spin Column, the eluted nucleic acid can be used for variety of molecular biology experiments immediately or stored at -20°C for later use.