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Magbead Pathogenic Microbiome DNA/RNA Kit

Catalog No.	Specification	Storage/Shelf life
EP027	96T	Room temperature/1 year

Introduction

This kit is designed for purifying and enriching DNA and RNA from pathogenic microorganisms, such as viruses, bacteria, and fungi, present in biological fluid samples like sputum, bronchoalveolar lavage fluid, blood, and ascites. The purified microbial DNA and RNA are suitable for a variety of downstream applications, including PCR, RT-PCR, qPCR, NGS, and other molecular biology experiments.

Storage Condition: Room temperature (10-30℃)

Components

Component	96 preps
Buffer LBS-A	20 mL
Buffer ML	25 mL
Buffer GW1	180 mL
Buffer GW2	180 mL
RNase-Free Water	10 mL
Proteinase K	4×1.25 mL
Magbeads PN	2×1.5 mL
Lysis Tubes II (96)	1

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Reagents and Equipment to be Supplied by the User

1. 2/15 mL magnetic rack or 32-channel nucleic acid extractor
2. 96 DW Plate/8 channel Comb
3. Isopropanol
4. PBS Buffer

Precautions

1. Inspect Buffer LBS-A and Buffer ML for any precipitate before use. If precipitate is present, dissolve it by incubating the buffer in a 56°C water bath.

2. Ensure samples are fresh for optimal DNA and RNA recovery. If necessary, short-term store or transport samples at 2-8°C, and long-term storage can be placed at -20 °C or -80 °C. Avoid repeated freezing and thawing, as this can damage microbial cells.

To prevent erroneous results due to contamination, maintain a clean work area and wear protective clothing. Establish appropriate controls for quality assurance. Implement proper measures to handle sample materials and reduce the risk of cross-contamination. During extraction, useDNA/RNA-free pipette tips and consumables, and promptly cover reagents after use. It is recommended to wipe with 75% ethanol and turn on UV irradiation before and after using nucleic acid extractor.

Sample Preprocessing

1. Non-viscous liquid samples (urine, pleural effusion, cerebrospinal fluid, etc.) Take 400 μL sample directly for extraction.

2. Swab samples (nasal, throat, anal swabs, etc.)

Wet swab sample (with preservation solution): After vortexing and shaking, take 400 μL sample directly for extraction. Dry swab sample: Rotate the swab cotton swab in 0.5 mL PBS for at least 20 seconds. Before removing the swab, squeeze it multiple times on the tube wall to squeeze out the bacterial solution as much as possible, reducing sample loss. Then, take 400 μ L for extraction.

3. Sputum Take about 500 μL sample, add 2 times the volume (about 1 mL) of

liquefaction reagent Buffer GB1, incubate at 37 ℃ and 600 rpm for 15-30 minutes. For thick sputum samples, it is recommended to vortex and mix every 5 minutes until the sample is completely liquefied. Take an appropriate amount of liquefied sputum sample into a centrifuge tube, centrifuge at 12000 rpm for 5 minutes, discard the supernatant, andresuspend the sediment with 400 μL of sputum supernatant.

Note: The sample volume can be increased or decreased appropriately, and the amount of Buffer GB1 can be adjusted accordingly, which is not provided in this kit. 10-15min can be selected for thin sputum samples, and 30min is recommended for thick sputum samples, depending on the specific degree of liquefaction.

4. Bronchoalveolar Lavage Samples Clarified sample: Take 400 μL sample directly for extraction without liquefaction. For samples containing a small amount of viscous sputum, centrifuge as much of the lavage fluid sample as possible. Carefully remove the supernatant and retain the lower viscous portion (containing sputum, cells, and microbial bodies). Follow the same liquefaction method described for sputum samples, andresuspend the pellet with 400 μL of sample supernatant.

5. Blood For serum, plasma, and small volume whole blood samples (<200 μL): Take 400 μL (use PBS to make up the volume for small volume whole blood) for extraction. For large-volume whole blood samples: It is recommended to use red blood cell lysis buffer before extraction.

6. Microbial culture samples Bacteria (≤ 1 × 109 cells), fungi (≤ 1 × 109 cells)

Protocol

Manual Operation

1. Add 400 μL of sample, 200 μL Buffer LBS-A and 20 μL Proteinase K to the Lysis Tubes II. Process the samples using one of the following methods:

1.1 Place the Lysis Tube II at room temperature and vortex for 10 minutes.

1.2 Place the Lysis Tube II in a thermostatic mixer at 65°C at 2500 rpm for 10 min (without heating when the temperature could not be set).

1.3 Place the Lysis Tube II in a sample homogenizer and select the appropriate

program according to the brand of the instrument. For example, if using the MP FastPrep-24-5G, shake at a speed of 6 M/S for 30 seconds, with 30-second intervals, for 6 cycles.

2. Add 250 μL of Buffer ML and 20 μL of Proteinase K to the shaken Lysis Tubes II, vortex and mix well, and incubate in a 56°C water bath for 10 minutes, or place on a thermomixer at 56°C at 0 rpm for 10 minutes, then centrifuge at room temperature at 12,000 rpm for 1 minute, and transfer all supernatants to new centrifuge tubes.

3. Add 400uL of isopropanol to the tube, vortex to mix, and centrifuge for 1-3 seconds to collect the liquid on the inner wall of the tube.

4. Add 30μL of Magbeads PN to the centrifuge tube (mix thoroughly before loading), vortex to mix, place the centrifuge tube on a thermomixer at 1500 rpm for 3 min. And then let stand for 1 min. Centrifuge instantaneously for 1-3 seconds, place the centrifuge tube on a magnetic stand, and let stand for 1 min. Carefully aspirate all supernatant with a pipette.

5. Remove the centrifuge tube from the magnetic stand, add 800 μL Buffer GW1 to the centrifuge tube, vortex at room temperature for 1 min (or thermomixer 2500 rpm for 1 min, the same below), let stand for 1 min, and centrifuge instantly for 1-3 seconds. Place the centrifuge tube on the magnetic stand for 1 min or until the beads are completely adsorbed, and carefully aspirate all the supernatant with a pipette.

6. Repeat Step 5 once.

7. Remove the centrifuge tube from the magnetic stand, add 800 μL Buffer GW2 to the centrifuge tube, vortex at room temperature for 1 minute, let it stand for 1 minute, and centrifuge instantly for 1-3 seconds. Place the centrifuge tube on the magnetic stand and let it stand for 1 min or until the beads are completely adsorbed, and carefully aspirate all the supernatant with a pipette.

8. Repeat Step 7 once.

9. Open the lid and dry until the surface of the beads is matte (about 3 min), add 70μL of RNase-Free Water, vortex and mix well for 1 min, centrifuge instantaneously for 1-3 seconds. Place the centrifuge tube on a magnetic stand, and let it stand for 3-5 min until the beads are completely adsorbed.

10. Transfer the elution product to a new 1.5 mL centrifuge tube, where it can be used directly for downstream experiments or stored at -20°C for long-term storage.

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Operation with Automatic Nucleic Acid Extractor

1. Add 400 μL of sample, 200 μL Buffer LBS-A and 20 μL Proteinase K to the Lysis Tubes II. Vortex and mix well, following the same shaking method as described in Step 1 of the manual operation. Centrifuge at 12,000 rpm for 1 minute at room temperature

2. Add reagents into the 96 deep-well plate according to the table below.

Position	Reagent	Volume
Column 1, 7	Buffer ML	200 μL
Column 1, 7	Isopropanol	350 μL
Column 2, 8	Buffer GW1	800 μL
Column 3, 9	Buffer GW1	800 μL
Column 4, 10	Buffer GW2	800 μL
Column 5, 11	Buffer GW2	800 μL
Column 3, 9	Magbeads PN	30 μL
Column 6, 12	RNase-Free Water	70 μL

3. Add all the supernatant samples from step 1 (approximately 480-500 μL) and 20 μL Proteinase K to columns 1 and 7 of the 96-well plate with the aliquoted reagents.

4. Edit and run the extractor according to the following table

Step	Magnetic Rod Position	Step Title	Temperature	Release Magbeads	Speed	Time	Cycles	Magbeads Attraction Frequency	Magbeads Attraction Time
1	1	Lysis	56℃	No	slow	10min	1	0	0
2	3	Collect magbeads	0	No	Fast	5s	1	2	30s
3	1	Binding	0	Yes	Medium	5min	1	2	10s
4	2	Washing 1	0	Yes	Fast	2min	1	2	10s

5	3	Washing 2	0	Yes	Fast	2min	1	2	10s
6	4	Washing 3	0	Yes	Fast	2min	1	2	10s
7	5	Washing 4	0	Yes	Fast	2min	1	2	10s
8	5	Drying	0			5min			
9	6	Elution	56℃	Yes	Fast	3min	1	0	0
10	6	standing	0			3min			
11	6	Collect Magbeads	0	No	Fast	5s	1	2	30s
12	2	Release Magbeads	0	Yes	Fast	5s	1	0	0