2. Pack the reagent in 96 deep hole plate according to the table below.

Position	Reagent	Volume
1, 7	Isopropanol	240 μL
2, 8	Buffer WB1	500 μL
3, 9	Buffer WB2	500 μL
3, 9	Magbeads PN	10 μL
6, 12	RNase-Free Water	70 μL

3. Add the supernatant sample in Step 1 and 20μL Proteinase K into the first and seventh columns of the 96-deep hole plate with the reagent packed.
4. Edit and run the extractor according to the following table:

Step	Position	Step name	Temperature	Release magbead	Speed	Time	Cycles	Magnetic frequency	Magnetic absorption time
1	1	Lysis	65°C	Y	Medium	10min	1	0	0
2	3	Collect magbead	0	N	Fast	5 s	1	2	3 s
3	1	Binding	0	Y	Medium	5 min	1	2	3 s
4	2	Washing	0	Y	Fast	2 min	1	2	3 s
5	3	Washing	0	Y	Fast	2 min	1	2	3 s
6	3	Drying	0			5 min			
7	6	Elution	56°C	Y	Medium	5 min	1	3	5 s
8	3	Release magbead	0	Y	Fast	5 s			

5. 5.After the program was finished, the 96-deep hole plate was taken out, and the eluent in columns 6 and 12 was transferred to a new centrifuge tube and stored at -20°C for a long time.

Version: 03/2022

Magbead Pathogenic Microbiome DNA/RNA Kit

Cat. No. : CW3061S (96 preps)

Storage Conditions: Room temperature (15-30°C)

Components

Component	CW3061S 96 preps
Buffer LBS	50 mL
Buffer WB1	50 mL
Buffer WB2	50 mL
RNase-Free Water	10 mL
Proteinase K	2×1.25 mL
Magbeads PN	1.5 mL
Lysis Tubes	96

Principle

This kit is suitable for purification and enrichment of DNA and RNA of pathogenic microorganisms such as viruses, fine bacteria and fungi from acellular body fluids such as plasma, serum and alveolar lavage. Microbial DNA and RNA purified with this kit are suitable for a variety of downstream applications, including whole-genome sequencing analysis, high-sensitivity 16srDNa-based microbiome analysis, and metagenomic shotgun sequencing analysis.

Self-provided instruments and reagents

1. 2/15mL magnetic stand or 32-channel nucleic acid extractor (CWE3200/CWE2100)
2. Isopropanol

Important things before the experiment

1. Before use, please check whether Buffer LBS is precipitated. If precipitated, please redissolve it in water bath at 56°C.
2. This kit is designed to isolate DNA and RNA from intact microbial cells. To ensure optimal recovery efficiency of microbial DNA and RNA, samples should be kept fresh. If storage or transportation is needed, it is best to do it at 2~8°C, and do not freeze-thaw, which will damage the integrity of microbial cells. To remove RNA, add 5μL of 100 mg/mL RNase A solution after step 2, shake and mix, and place for 10 minutes at 20-30°C. To remove DNA, add 80 μL DNase I premix after step 5 and incubate at 20-30°C for 15 min.
3. In order to avoid false results caused by contamination, please keep the working area clean and wear protective clothing, and set up reasonable reference products for quality control. Use appropriate measures to handle sample materials to reduce the risk of cross contamination. During extraction, DNA/RNA-free pipette heads and consumables are used and the bottle caps are closed immediately after reagent use to prevent contamination.

Operation

Manual operation

1. The Lysis Tubes were added with 300μL sample, 300μL Buffer LBS room temperature, and vortex for 10 min.
2. Centrifuge at 12000 rpm at room temperature for 1min, add 20 μL Proteinase K and 240 μL isopropyl alcohol to the supernatant, mix for 5 s, and incubate at 65℃ for 10 min.
3. After transient separation of the centrifuge tube to ensure that there is no liquid residue in the tube wall, add 10 μL Magbeads PN into the centrifuge tube and mix for 5 s. Let it stand at room temperature for 5 min.

   Note: The Magbeads PN should be vortex to ensure full suspension before use.
4. After the centrifuge tube is immediately separated and no liquid remains on the tube wall is ensured, the centrifuge tube is placed on the magnetic stand for 2 min or until the magbeads are completely absorbed, and all supernatants are carefully absorbed and discarded with a pipette.
5. Add 500 μL Buffer WB1 into the centrifuge tube, blow with pipette 5-10 times, place the centrifuge tube on the magnetic rack for 2 min or until the magbeads are completely absorbed, and carefully absorb and discard all supernatant with pipette.
6. Add 500 μL Buffer WB2 into the centrifuge tube, blow with pipette 5-10 times, place the centrifuge tube on the magnetic rack for 2 min or until the magbeads are completely absorbed, and carefully absorb and discard all supernatant with pipette.
7. The centrifuge tube was placed on the magnetic rack and left open to dry for 5 min.
8. Add 70 μL RNase-Free Water into the centrifuge tube, blow and mix with pipette, and incubate at 56℃ for 5 min. After the centrifuge tube is transient, the centrifuge tube is placed on the magnetic rack for 2 min or until the magnetic bead is completely absorbed, and all supernatant is transferred to the new centrifuge tube with a pipette. The obtained nucleic acid solution was stored at -20°C for a long time.

Matched with nucleic acid extractor CWE3200/CWE2100

1. The Lysis Tubes were added with 300μL sample and 300μLBuffer LBS at room temperature, vorticated for 10 min and centrifuged at 12000 rpm for 1min.