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Pathogenic Microbes DNA/RNA Kit

Product Number: DNK303

Shipping and Storage

Room temperature (15-30℃)

Components

Component	DNK303 50 preps
Buffer SL-A	20 mL
Buffer GL	20 mL
Buffer GW1(concentrate)	25 mL
Buffer GW2(concentrate)	20 mL
RNase-Free Water	3.5 mL
Proteinase K	1 mL
Lysis Tubes	50
Spin Columns DM with Collection Tubes	50

Description

This kit is a specialized sample preparation solution for microbiome analysis, suitable for purifying and enriching DNA/RNA of pathogenic microorganisms such as bacteria and fungi from mixed samples such as swabs, blood, sputum, and bronchoalveolar lavage fluid. The microbial DNA/RNA purified using this kit is suitable for various downstream applications, including whole genome sequencing analysis, high-sensitivity microbial community analysis based on 16S rDNA, and metagenomic shotgun sequencing analysis.

Self provided reagents and consumables

1. Aseptic pipette tip with aerosol barrier to prevent cross contamination
2. Micro centrifuge tube (2 mL/1.5 mL)
3. PBS buffer (only required for certain samples)
4. Isopropanol

Preparation and important precautions before the experiment

1. This kit is designed to isolate DNA/RNA from intact microbial cells. To ensure optimal recovery efficiency of microbial DNA/RNA, the samples should be kept fresh.
2. To avoid erroneous results caused by pollution, please keep the work area clean and wear protective clothing, and set up control samples for quality control reasonably. Take appropriate measures to handle sample materials and reduce the risk of cross contamination.
3. Buffer GW1 (concentrate) and Buffer GW2 (concentrate) add corresponding amounts of ethanol based on the bottle information.

Protocol

1. Sample pre-processing:
   1.1. Urine, pleural and peritoneal fluid, cerebrospinal fluid and other non viscous body fluid samples
   Take 400μL of sample directly and perform the second step.
   1.2. Swab samples (such as nasal, pharyngeal, and anal swabs)

After vortex oscillation mixing, directly take 400μL for the second step of the experiment.

1.3. Sputum and bronchoalveolar lavage fluid samples

Take an appropriate amount of liquefied sputum sample into a 1.5 mL centrifuge tube (the recommended liquefaction method is to use 1.5 times the volume of Buffer GB1, which is not provided in this kit), centrifuge at 12000 rpm for 5 minutes, discard the supernatant, resuspend and precipitate with 400μL PBS, and extract. For bronchoalveolar lavage fluid containing a small amount of viscous sputum, centrifuge as many bronchoalveolar lavage fluid samples as possible, carefully remove the supernatant, and retain the lower layer of viscous part (including sputum, cells, and bacteria) for liquefaction treatment according to the sputum sample.

1.4. Blood samples

Serum, plasma, and small amounts of whole blood samples (less than 200μL) can be directly taken in 400μL (a small amount of whole blood can be supplemented with PBS) for the second step of the experiment. For large volume whole blood samples, it is recommended to use RBC Lysis Buffer(DNK0613)for processing before extraction.

2. Add 400μL of sample, 20μL of Proteinase K, and 400μL of Buffer SL-A to Lysis Tube, and incubate on a constant temperature mixer at a maximum vibration speed of 65℃ (2500-2900 rpm) for 10 minutes. After a brief centrifugation, add 400μL Buffer GL and continue shaking at a maximum speed of 65℃ (2500-2900 rpm) for 10 minutes.
3. Centrifuge at room temperature of 12000 rpm for 1 minute, carefully transfer all the supernatant to a new centrifuge tube, add 400μL isopropanol, vortex and mix well, and centrifuge instantly to collect the solution to the bottom of the tube.
4. Add all the solution obtained in step 3 (including the precipitate formed) to the adsorption column already loaded into the collection tube. If the solution cannot be added at once, it can be transferred in multiple times (not exceeding 700μL each time). Centrifuge at 12000 rpm for 1 minute, discard the waste liquid from the collection tube, and place the adsorption column back into the recovery manifold.
5. Add 500μL of Buffer GW1 to the adsorption column (check if anhydrous ethanol has been added before use), centrifuge at 12000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the recovery header. Repeat this step once.
6. Add 500μL of Buffer GW2 to the adsorption column (check if anhydrous ethanol has been added before use), centrifuge at 12000rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the recovery header. Repeat this step once.
7. Centrifuge at 12000rpm for 2 minutes and discard the waste liquid from the collection tube. Place the adsorption column at room temperature for a few minutes and thoroughly air dry (5-10 minutes).
8. Place the adsorption column in a new centrifuge tube (self provided), suspend 70μL RNase Free Water in the middle of the adsorption column, let it stand at room temperature for 5 minutes, centrifuge at 12000 rpm for 1 minute, collect the nucleic acid solution, and store it at -20℃.