Pathogenic Microbiome DNA Kit

Pathogenic microorganism genomic DNA extraction kit

Catalog number: P666038 (50 preps)

Storage condition: Box Ⅰ is stored at room temperature (15-30℃), Box Ⅱ is stored at -20℃.

Products content

Box Ⅰ.

Component	50 preps
Buffer GL	15 ml
Buffer GW1 (concentrate)	13 ml
Buffer GW2 (concentrate)	15 ml
Buffer GE	15 ml
Enzymatic Lysis Buffer	10 ml
Proteinase K Storage Buffer	1.25 ml
Lysis Tubes	50
Spin Columns DM with Collection Tubes	50

Box II.

Component	50 preps
Buffer GB1	2×20 ml
Buffer GB2	4×25 ml
Benzonase (250 U/μl)	100 μl
Lysozyme	100 mg
Proteinase K	25 mg

Products Introduction

This kit is a dedicated sample preparation solution for microbiome analysis and is suitable for the purification and enrichment of genomic DNA of pathogenic microorganisms such as bacteria and fungi from mixed samples such as swabs, blood, sputum, alveolar lavage, etc. During the purification process, differential lysis of the host cells and subsequent enzymatic digestion can effectively remove most of the host DNA while providing a comprehensive coverage of the bacterial and fungal DNA loci to a higher level. By differential lysis of host cells and subsequent enzymatic digestion, this kit can effectively remove most of the host DNA while maximizing the full coverage of bacterial, fungal and other pathogenic microbial DNA sites, thus obtaining microbiome DNA enrichment products with a higher coverage. Microbial DNA purified with this kit is suitable for a variety of downstream applications, including whole genome sequencing analysis, 16S rDNA-based high sensitivity microbiome analysis, and macrogenomic birdshot sequencing analysis.

Self-contained reagents and consumables

Sterile pipette tips with aerosol barrier to prevent cross-contamination

anhydrous ethanol

Microcentrifuge tubes (2 ml/1.5 ml)

PBS buffer (required for some samples only)

Pre-experiment Preparation and Important Notes

1. Add 1.25 ml Proteinase K Storage Buffer to Proteinase K and store at -20℃. Do not leave the prepared Proteinase K (20 mg/ml) at room temperature for a long time, and avoid repeated freezing and thawing to avoid affecting its activity.
2. Dissolve Lysozyme (100 mg) in 10 ml Enzymatic Lysis Buffer to a final concentration of 10 mg/ml, dispense into sterile tubes and store at -20℃. Do not leave the prepared Lysozyme (10 mg/ml) at room temperature for a long time and avoid repeated freezing and thawing to avoid affecting its activity.
3. Thaw Buffer GB1 and Buffer GB2 at room temperature or 2-8°C before use and mix thoroughly. Thawed Buffer GB1 and Buffer GB2 can be left at 2-8°C for 1-2 weeks without affecting their activity, and should be stored at -20°C for long term storage. To ensure optimal performance, do not freeze or thaw more than three times. If less than one bottle of Buffer GB1 and Buffer GB2 is required for a single extraction, ensure that it is used under sterile conditions such as an ultra-clean bench and avoid microbial contamination and growth in the remaining buffer.
4. Before first use, anhydrous ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions on the vial label and labeled.
5. Check Buffer GL for crystallization or precipitation before use, and if crystallization or precipitation occurs, redissolve Buffer GL in a 56°C water bath.
6. If the downstream experiments are sensitive to RNA contamination, 4 µl of DNase-Free RNase A (100 mg/ml) can be added before adding Buffer GL. RNase A is not provided in the kit, but can be ordered separately from CW0601S.
7. This kit is designed for the isolation of DNA from intact microbial cells. To ensure optimal recovery of microbial DNA, samples should be fresh. If storage or transportation is required, this should preferably be done at 2-8°C and not frozen or thawed, as freezing and thawing can damage the integrity of the microbial cells and therefore result in the loss of exposed microbial DNA during host DNA removal.
8. To avoid false results due to contamination, keep the work area clean, wear protective clothing, and set up controls for quality control. Use appropriate measures to handle sample materials to minimize the risk of cross-contamination. During the extraction process, use DNA-free pipette tips and consumables, and cap reagents immediately after use to prevent contamination.

procedure

1. Sample pre-treatment: 1a: For swab samples, swirl the swab portion of the swab in 0.5 ml PBS for at least 20 s. Squeeze the swab several times against the wall of the tube before removing it so that as much of the bacterial fluid as possible can be squeezed out of the swab to minimize sample loss. 1b: For viscous samples, e.g. sputum, take ~500 µl of sample, add 1.5 times the volume (~750 µl) of Buffer GB1 and incubate at 37°C, 600 rpm for 15-30 min until the sample is completely liquefied.

Note: The sample volume can be increased or decreased appropriately and the amount of

Buffer GB1 added adjusted accordingly.

1c: For alveolar lavage fluid containing a small amount of viscous sputum, centrifuge as much of the alveolar lavage fluid as possible, carefully remove the supernatant, and retain the lower viscous fraction (containing sputum, cells, and organisms), add 1.5 times the volume of Buffer GB1, and incubate for 15-30 min at 37°C, 600 rpm until the sample is completely liquefied.

1d: For non-viscous body fluid samples such as blood and cerebrospinal fluid, liquefaction treatment is not required, and an appropriate amount of sample is taken directly, the operation of step 2 is carried out, and the cell precipitate is collected by centrifugation.

2. Centrifuge at 10000 rpm for 5-10 min at room temperature and carefully discard the supernatant.

Note: Do not disturb the lower cell sediment to avoid sample loss.

3. Add 500 µl Buffer GB2, vortex to mix, and incubate at room temperature, 600 rpm for 10 min.

4. Centrifuge at 12000 rpm for 2 min and carefully remove the supernatant.

Note: Do not disturb the bacterial precipitate when removing the supernatant to avoid sample loss.

5. Add 200 µl of Buffer GB2 to the precipitate, add 2 µl of Benzonase and incubate for 30 min at 37°C, 600 rpm.

6. Centrifuge at 12000 rpm for 2 min, discard the supernatant, add 500 µl of Buffer GB2, vortex and wash the precipitate. Repeat the procedure once.

7. Centrifuge at 12000 rpm for 2 min, discard the supernatant, and finally aspirate the residual Buffer GB2 with a small-volume tip.

8. Add 180 µl Lysozyme (10 mg/ml), resuspend the bacterial precipitate and transfer the bacterial resuspension to a Lysis Tube.

9. The Lysis Tube is incubated at 37°C, 600 rpm for 20-30 min, then vortexed for 10 min or processed on a thermostatic homogenizer for 10 min at maximum vibration speed (2500-2900 rpm).

10. Centrifuge briefly, add 20 µl proteinase K, vortex to mix, add 200 µl buffer GL, vortex to mix, and incubate for 30 min at 56°C, 600 rpm.

Note: 1) Do not add Proteinase K directly to Buffer GL.

2）For RNA removal, add 4 µl DNase-Free RNase A (100 mg/ml) before adding Buffer GL, shake to mix, and let stand at room temperature for 5-10 minutes.

11. Centrifuge at 12000 rpm for 1 min and carefully aspirate the supernatant into a new centrifuge tube. Note: Do not aspirate the glass beads.

12. Add 200 µl of anhydrous ethanol, vortex to mix, and centrifuge momentarily to collect the solution to the bottom of the tube. Note: The addition of anhydrous ethanol may produce a white precipitate that will not affect subsequent experiments.

13. Add all of the solution from step 12, including the precipitate, to the Spin Columns DM in the collection tube, or transfer the solution several times if it cannot be added all at once. centrifuge at 12,000 rpm for 1 minute, pour off the waste from the collection tube, and return the column to the collection tube.

14. Add 500 µl Buffer GW1 to the adsorbent column (check that anhydrous ethanol has been

added before use), centrifuge at 12,000 rpm for 1 min, pour off the waste liquid from the collection tube, and put the adsorbent column back into the collection tube.

15. Add 500 µl Buffer GW2 to the adsorbent column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube. Note: Step 15 can be repeated once if further improvement of DNA purity is required.

16. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the column at room temperature for a few minutes and dry thoroughly. Note: The purpose of this step is to remove residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).

17. Place the adsorbent column in a new centrifuge tube (supplied), add 50 µl of Buffer GE to the center of the adsorbent column overhang, let stand at room temperature for 5 minutes, centrifuge at 12,000 rpm for 1 minute, collect the DNA solution, and store the DNA at -20 °C.

Attention:

1）If the downstream experiments are sensitive to pH or EDTA, sterilized water can be used for elution. The pH value of the eluent has a great influence on the elution efficiency. If the eluent is made of water, the pH value should be 7.0-8.5 (the pH value of water can be adjusted to this range with NaOH), and the elution efficiency is not high when the pH value is lower than 7.0.

2）Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

3）If the final concentration of DNA is to be increased, the DNA eluate obtained in step 17 can be re-spiked onto the adsorbent membrane and step 17 repeated.

4）DNA stored in water will be affected by acidic hydrolysis. For long-term storage, it is recommended to elute with Buffer GE and store at -20℃.