Spin Soil and Stool DNA Kit

Cat.No.: HYC273

Product Description

This kit provides a method for extracting total DNA from soil or stool samples, including total DNA of cells, bacteria, parasites and viruses in samples. It is also applicable to DNA extraction from samples containing high concentrations of PCR inhibitors. Adopting a unique buffer system, this kit enables efficient binding of DNA in lysate to adsorption columns. PCR inhibitors, enzymatic reaction inhibitors and residual impurities can be effectively removed through washing steps. Finally, high-purity DNA is obtained by elution with low-salt buffer or nuclease-free water. The purified DNA can be directly used for downstream experiments such as next-generation sequencing (16S amplicon and metagenome sequencing), library construction, PCR, qPCR, Southern Blot, restriction digestion molecular marker assays.

Components

Components	50T
Buffer QSL	45 mL
Buffer RIL	11 mL
Buffer ML	10 mL
Buffer GW1*	13 mL
Buffer GW2*	26 mL
Buffer EBL	13 mL
RNase A	240 µL
Lysis Tubes II	50
Spin Columns DM With Collection Tubes	50

Storage

Buffer RIL at 2-8℃, other components at room temperature (10-30℃).

Required Reagents and Equipment

- Thermomixer
- Absolute ethanol, isopropanol
- Vortex mixer or tissue homogenizer

Instructions

Preparations

- Avoid repeated freeze-thaw cycles of samples, as this will result in shorter DNA fragments and reduced yield.
- Before first use, add absolute ethanol to Buffer GW1 and Buffer GW2 as indicated on the respective reagent bottle labels.
- Remove Buffer RIL only immediately before use, and return it to 2-8°C storage immediately after use.

Protocol

1. Briefly centrifuge the Lysis Tube to pellet the beads at the bottom.
2. Sample processing

- Add 0.1–0.3 g of soil or stool sample to the Lysis Tube, then add 740–820 µL of Buffer QSL and 4 µL of RNase A. Tightly cap the tube and briefly vortex to mix.
- For stool samples preserved in non-lytic stool preservative: Add 200–600 µL of the solid-liquid mixture to the Lysis Tube, centrifuge at 13,000 rpm for 1 min, and discard the preservative. (If the pellet after centrifugation is insufficient, repeat the enrichment, but the total sample amount should not exceed 0.3 g.) Add 620 µL of Buffer QSL and 4 µL of RNase A, tightly cap the tube, and briefly vortex to mix.

3. Secure the Lysis Tube in a bead beating homogenizer equipped with 2 mL adapters, and process using optimized bead beating conditions (see Appendix).
4. Incubate the Lysis Tube at 70°C with shaking at 1200 rpm for 10 min in a thermomixer. Centrifuge at 13,000 rpm for 2 min to pellet debris, then transfer 540 µL of the supernatant to a new 2 mL centrifuge tube.
5. Add 180 µL of Buffer RIL, vortex for 5 sec, then centrifuge at 13,000 rpm for 2 min. *Remove Buffer RIL only immediately before use, and return it to 2–8°C storage immediately after use.*

6. In a new centrifuge tube, add 160 µL Buffer ML, 480 µL supernatant from Step 5, and 320 µL isopropanol in sequence, then vortex for 5 seconds to mix well.
7. Transfer 650 µL of the solution from the previous step to a Spin Columns DM already placed in a collection tube, and centrifuge at 12,000 rpm (~13,400×g) for 1 minute.
8. Discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. Repeat Step 7 until all the solution is transferred.
9. Add 500 µL Buffer GW1 to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.
10. Add 500 µL Buffer GW2 to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube.
11. Repeat Step 10.
12. Centrifuge at 12,000 rpm for 2 minutes, discard the waste liquid in the collection tube. Place the adsorption column at room temperature for several minutes to dry the adsorption membrane completely.

    The purpose of this step is to remove residual ethanol in the adsorption column; residual ethanol will affect subsequent enzymatic reactions (such as restriction digestion, PCR, etc.).
13. Place the adsorption column into a new centrifuge tube (provided by the user), suspend and add 50-200 µL Buffer EBL or sterile water to the middle of the adsorption column, incubate at room temperature for 2-5 minutes, centrifuge at 12,000 rpm for 1 minute to collect the DNA solution, and store the DNA at -20℃.

    1) Incubating at room temperature for 5 minutes before centrifugation can increase DNA yield.

    2) Re-eluting with an additional 50-100 µL elution buffer (Buffer EBL) or sterile water can further increase DNA yield.

    3) If it is necessary to increase the final concentration of DNA, the DNA eluate obtained in Step 13 can be added back to the adsorption membrane and Step 13 can be repeated, but this may reduce the total DNA yield.

    4) The elution buffer (Buffer EBL) does not contain chelating agents; store DNA at -20℃ to prevent degradation.

    5) Trace residual PCR inhibitors in the genomic DNA template may have an adverse effect on the PCR reaction; usually, diluting the DNA 2-10 times can solve this problem.

Appendix: Grind the sample using one of the following methods

1. Manually vortex at maximum speed on a vortex mixer for 10 minutes.
2. Vortex at maximum speed for 10 minutes on a vortex mixer equipped with a 1.5-2 mL horizontal centrifuge tube holder (keep the Lysis Tube in a horizontal position). If the number of samples exceeds 12, extend the vortex time by 5-10 minutes. For example, use a Vortex-Genie2 vortex mixer from Scientific Industries or Mobio.
3. When using a TissueLyser II from Qiagen, grind at 25 Hz for 10 minutes.
4. When using a PowerLyzer 24 Homogenizer from Qiagen, homogenize at 2000 rpm for 30 seconds, pause for 30 seconds, then homogenize again at 2000 rpm for 30 seconds.
5. When using a FastPrep-24 from MP Biomedicals, the recommended speed is 6.0 and the time is 40 seconds.