Stool DNA Kit

Cat. No.: F1522829 | Pack size: 50T | Storage: Room temperature

Overview

This kit provides a method for extracting total DNA from fecal samples, enabling the extraction of total DNA from bacteria, parasites, and viruses present in the sample. It is also suitable for extracting DNA from samples containing high concentrations of PCR reaction inhibitors. The kit uses a unique buffer system that allows efficient binding of DNA from the lysate to the spin column. Inhibitors of PCR and enzymatic reactions, as well as residual impurities, are effectively removed through washing steps. Finally, high-purity DNA is eluted with a low-salt buffer (Buffer EB) or sterile water. The purified DNA can be directly used in downstream applications such as next-generation sequencing (16S amplicon and metagenomics), library construction, PCR, qPCR, Southern blot, and restriction enzyme-based molecular markers.

Kit Contents & Storage

Product No.	Component	Appearance	50T	Storage
F1522829A	Buffer MS1	Liquid	33 mL	RT
F1522829B	Buffer MS2	Liquid	10 mL	RT
F1522829C	Buffer NDS3	Liquid	10 mL	RT
F1522829D	Buffer SGB	Liquid	44 mL	RT
F1522829E	Buffer PW1	Liquid	17 mL	RT
F1522829F	Buffer PW2	Liquid	15 mL	RT
F1522829G	Buffer EB	Liquid	15 mL	RT
F1522829H	Proteinase K	Liquid	1.2 mL	RT
F1522829I	RNase A (10 mg/mL)	Liquid	550 µL	RT
F1522829J	PBF Spin Column (with 2 mL collection tube)	-	1 EA × 50	RT
F1522829K	Homogenization Tube A	-	1 EA × 50	RT

The kit can be stored under dry conditions at room temperature for 15 months.

Precautions

Freshly collected samples yield higher recovery. Before sampling, consult the appropriate optimal storage conditions for different sample types.

When pipetting supernatants, avoid sucking up any precipitate, as this may affect product purity.

Excess DNA may inhibit downstream PCR reactions. If this occurs, it is recommended to dilute the DNA template before use.

Before use, check Buffer MS2 for precipitation. If precipitation is present, heat at 37°C until completely dissolved before use.

Procedure

Before use, add 22 mL of absolute ethanol to Buffer PW1 and 60 mL of absolute ethanol to Buffer PW3.

1. Add 100-300 mg of fecal sample to Homogenization Tube A. For liquid fecal samples, transfer 200 µL to the tube.

2. Add 600 µL of Buffer MS1, 160 µL of Buffer MS2, and 20 µL of Proteinase K to the tube. For dry fecal samples, the amounts of Buffer MS1 and Buffer MS2 can be increased proportionally by a factor of 1.5-2.

3. Place the homogenization tube on a thermomixer at 70°C and 1500 rpm, and shake for 15 min. If using a bead-beating device, secure the tube in a 2-mL adapter and process according to the device instructions (see Appendix for details).

4. Centrifuge at 12,000 rpm (13,680 × g) for 2 min. Carefully transfer the supernatant to a new 2 mL centrifuge tube (avoiding any precipitate). Add 10 µL of RNase A, vortex briefly to mix, and let stand at room temperature for 5 min to remove RNA impurities.

5. Add 160 µL of Buffer NDS3 to the tube, mix thoroughly, vortex for 5 sec, and place at 4°C for 5 min to further remove PCR inhibitors.

6. Centrifuge at 12,000 rpm (13,680 × g) for 2 min to fully pellet impurities.

7. Transfer 550 µL of the supernatant to a new 2 mL centrifuge tube (if the supernatant volume is less than 550 µL, transfer all of it). Add 800 µL of Buffer SGB, vortex for 5 sec, then place the tube on a thermomixer at 25°C and 1500 rpm and shake for 5 min, or invert continuously for 5 min to promote DNA binding to Buffer SGB.

【Note】 Do not transfer any precipitate when removing the supernatant, as this may reduce DNA purity.

8. Transfer the solution and precipitate from step 7 into a PBF spin column (placed in the provided 2 mL collection tube). Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and

return the spin column to the collection tube. Add no more than 700 µL per transfer; if the volume exceeds, transfer in multiple portions until all liquid has been loaded.

9. Add 700 µL of Buffer PW1 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

10. Add 700 µL of Buffer PW2 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

11. Repeat step 10 (second wash) to further remove residual inhibitors, salts, and other impurities on the spin column, thereby increasing DNA purity.

12. Centrifuge the spin column together with the collection tube at 12,000 rpm (13,680 × g) for 2 min to completely remove residual wash buffer and impurities (this avoids residual ethanol affecting subsequent DNA elution and experiments).

13. DNA elution

Transfer the spin column to a new 1.5 mL centrifuge tube. Open the cap and let stand for 2 min to allow residual ethanol to evaporate completely. Slowly add 50 µL of Buffer EB dropwise to the center of the membrane without touching it. Let stand at room temperature for 1 min. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Collect the DNA solution in the centrifuge tube. The DNA can be used immediately for downstream experiments or stored at -20°C for long-term preservation.

Notes:

(1) If downstream applications are sensitive to pH or EDTA, sterile water can be used for elution. The pH of the elution buffer significantly affects elution efficiency. If water is used, ensure its pH is between 7.0 and 8.5 (adjust with NaOH). pH values below 7.0 will reduce elution efficiency.

(2) To increase the final DNA concentration, reload the collected DNA eluate from step 13 onto the same membrane, let stand at room temperature for 2 min, and centrifuge at 12,000 rpm (13,680 × g) for 30 sec.

(3) For long-term storage, it is recommended to elute with Buffer EB and store at -20°C to effectively prevent DNA degradation.

Appendix: Sample Grinding Methods

Choose one of the following methods to grind the sample, ensuring thorough disruption to improve DNA extraction efficiency:

- Vortex manually or automatically at maximum speed on a vortex mixer for 10 min.
- Using a vortex mixer equipped with a 1.5-2 mL horizontal centrifuge tube holder, vortex at maximum speed for 10 min (keep the tube horizontal). If more than 12 samples are processed,

extend the vortexing time by 5-10 min (e.g., using a Vortex-Genie 2 from Scientific Industries or Mobio).
- When using a Qiagen TissueLyser II, grind at 25 Hz for 10 min.
- When using a Qiagen PowerLyzer 24 Homogenizer, homogenize at 2000 rpm for 30 sec, pause for 30 sec, then homogenize again at 2000 rpm for 30 sec.
- When using an MP Biomedicals FastPrep-24, recommended speed is 6.0 for 40 sec.

DNA Concentration and Purity Assessment

The fragment size of the extracted genomic DNA is influenced by factors such as sample storage time and shear forces during handling. The purified DNA can be assessed for concentration and purity by agarose gel electrophoresis and UV spectrophotometry.

1. Concentration determination

Measure the absorbance of the sample at 260 nm (A_{260}) using a UV spectrophotometer to determine DNA concentration. If dilution is required, Buffer EB is recommended. Dilution with water may lead to inaccurate readings. The absorbance at 260 nm should be kept within 0.1-1.0 to ensure accuracy. Conversion: 1 unit of absorbance at 260 nm corresponds to 50 μg/mL of double-stranded DNA or 40 μg/mL of single-stranded DNA.

2. Purity assessment

Evaluate DNA purity by the A_{260}/ A_{280} ratio. For high-quality genomic DNA, the A_{260}/ A_{280} ratio should be between 1.7 and 1.9. If ddH_2O instead of Buffer EB is used for elution, the ratio may be lower due to the influence of pH and ions on light absorption; this does not necessarily indicate low DNA purity.

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