Magnetic Soil And Stool DNA Kit

Cat.No.: HYC274

Product Description

This kit delivers a simple, rapid and high-efficiency DNA extraction method optimized for soil and fecal samples. It adopts a unique inhibitor removal system to efficiently eliminate common PCR inhibitors. Supplied grinding beads enable thorough sample lysis and maximum nucleic acid release. In the presence of high salt, DNA binds to the surface of silica-coated magnetic beads. After washing steps, highly pure DNA is eluted in Buffer EBL or deionized water. The purified DNA features excellent purity and intact integrity, suitable for diverse downstream applications including short-read and long-read sequencing (amplification of 16S, 18S, ITS or target specific regions), metagenomic next-generation sequencing, quantitative PCR and microarray detection. This kit is compatible with 32-well and 96-well automated nucleic acid extractors for streamlined high-throughput extraction. It significantly boosts laboratory throughput and reduces manual experimental errors.

Components

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Buffer QSL	85 mL
Buffer RIL	20 mL
Buffer ML	18 mL
Buffer GW1*	80 mL
Buffer GW2*	50 mL
Buffer EBL	25 mL
Magbeads SN	2×1 mL
RNase A	450 μL
Lysis Tubes II	96 Tubes

Storage

Store Buffer RIL at 2–8 °C; all other reagents can be kept at room temperature (15–30 °C).

Required Reagents

- Anhydrous Ethanol
- Isopropanol

Instructions

Preparations

1. Before first use, add 104 mL anhydrous ethanol to Buffer GW1 as indicated on the bottle label and mark the bottle clearly.
2. Before first use, add 116 mL anhydrous ethanol to Buffer GW2 as indicated on the bottle label and mark the bottle clearly.
3. Vortex Magbeads SN thoroughly before each use until magnetic beads are fully homogenized.
4. Magbeads SN must not be frozen or subjected to centrifugation; such operations may cause irreversible damage to the magnetic beads.
5. Inspect Buffer QSL for crystals or precipitates. If solids form, warm the buffer to fully dissolve the precipitates.

Sample Lysis

1. Spin the Lysis Tube briefly to pellet grinding beads at the bottom.
2. a. Load 0.1–0.3 g soil or fecal sample into the Lysis Tube, add 740–820 µL Buffer QSL and 4 µL RNase A, tighten the cap, and mix by brief vortexing.
   b. For fecal samples preserved in non-lytic fecal storage solution: transfer 200–600 µL solid-liquid mixture to the Lysis Tube, centrifuge at 13000 rpm for 1 min and discard supernatant storage solution. Re-concentrate solids if the pellet mass is insufficient, with a maximum solid weight of 0.3 g. Add 620 µL Buffer QSL and 4 µL RNase A, tighten the tube cap and vortex briefly for mixing.
3. Secure the Lysis Tube in a bead homogenizer fitted with 2 mL adapters, and process under equipment-optimized lysis parameters (see Appendix).
4. Incubate the Lysis Tube on a thermomixer at 70 °C with shaking at 1200 rpm for 10 min. Centrifuge at 13000 rpm for 2 min to pellet solid debris, then transfer 540 µL supernatant to a new 2 mL centrifuge tube.

5. Add 180 µL Buffer RIL, vortex for 5 seconds, and centrifuge at 13000 rpm for 2 min.
   Note: Take Buffer RIL out immediately before use, and store at 2–8 °C right after use.

Manual Purification Workflow

1. Add reagents to the centrifuge tube in sequence: 160 µL Buffer ML, 480 µL supernatant obtained from Step 5 of sample lysis, 320 µL isopropanol and 20 µL Magbeads SN. Vortex for 5 sec, then incubate on a thermomixer at 25 °C with shaking at 1600 rpm for 10 min.
   Note: Vortex Magbeads SN vigorously for 20 seconds to fully homogenize before pipetting. Magbeads SN can be pre-mixed with isopropanol at the above ratio according to sample quantity prior to addition.
2. Place the tube on a magnetic rack and stand for 1 min until all Magbeads SN adhere to the tube wall. Discard supernatant completely while keeping the tube fixed on the rack.
3. Remove the tube from the magnetic rack, add 750 µL Buffer GW1 (confirm anhydrous ethanol has been added before use). Pulse-vortex for 1 min, or vortex briefly for 5 sec followed by incubation on a thermomixer at 25 °C, 1600 rpm for 2 min to maintain uniform bead suspension during shaking. Place the tube back on the magnetic rack for 1 min until full bead capture. Invert the rack gently to wash residual impurities off the tube cap, then discard all supernatant with the tube still secured on the rack.
4. Repeat Step 3 once.
5. Remove the tube from the magnetic rack, add 750 µL Buffer GW2 (confirm anhydrous ethanol has been added before use). Pulse-vortex for 1 min, or vortex briefly for 5 sec followed by incubation on a thermomixer at 25 °C, 1600 rpm for 2 min to keep beads evenly suspended during shaking. Set the tube on the magnetic rack for 1 min until all beads bind to the wall. Flip the rack gently to rinse contaminants from the cap, then fully discard supernatant with the tube fixed on the rack.
6. Repeat Step 5 once.
7. Keep the tube mounted on the magnetic rack. Pipette to remove residual liquid at the tube bottom and cap, then air-dry at room temperature for 5–10 min to completely evaporate ethanol. Stop drying when beads show a matte surface without cracking visible to the naked eye.

8. Take the tube off the magnetic rack and add 50–200 µL Buffer EBL. Vortex to fully suspend beads in eluate. Perform elution incubation either on a thermomixer at 56 °C, 1600 rpm for 10 min, or in a 56 °C water bath for 10 min with 10-second vortex mixing every 3 minutes.
9. Place the tube on the magnetic rack for 2 min until all Magbeads SN attach to the tube wall. Transfer the eluted DNA supernatant to a new centrifuge tube with a pipette, and store at −20 ± 5 °C for later use.

Appendix

Select one of the following methods for sample homogenization:

1. Manually vortex the sample at maximum speed on a vortex mixer for 10 minutes.
2. Use a vortex mixer equipped with a horizontal 1.5–2 mL tube adapter, run at maximum speed for 10 min with Lysis Tubes held horizontally. Extend homogenization time by 5–10 min if processing more than 12 samples. Compatible instruments include Vortex-Genie2 manufactured by Scientific Industries and Mobio.
3. For Qiagen TissueLyser II: Homogenize at 25 Hz for 10 min.
4. For Qiagen PowerLyzer 24 Homogenizer: Run homogenization at 2000 rpm for 30 sec, pause for 30 sec, then repeat homogenization at 2000 rpm for another 30 sec.
5. For MP Biomedicals FastPrep-24: Recommended speed setting of 6.0, homogenization duration of 40 sec.