

Soil and Fecal DNA Extraction Kit by Magnetic Bead Method

Project number: M669928

Storage conditions: 2–8° C.

Product Content:

Component	M669928 96preps
BufferQSL	85mL
BufferRIL	20mL
BufferML	18mL
BufferGW1 (concentrate)	80mL
BufferGW2 (concentrate)	50mL
BufferEBL	25mL
MagbeadsSN	2×1mL
RNaseA	450μL
LysisTubes II	96

Product Introduction

The kit provides a simple, fast and efficient method of extracting DNA for soil and fecal samples. In the presence of high salt, DNA is bound to the surface of silica-coated Magbeads. After rinsing, the highly pure DNA is eluted in BufferEBL or deionized water. The DNA obtained from purification has good purity (A260/280 ratio between 1.7–1.9) and high integrity (>15kb), and can be used for downstream experiments such as second-generation sequencing, quantitative PCR, and microarray detection.

The kit can be matched and used with CWE2100 or CWE3200 32-channel Nucleic Acid Extractor and CWE9600 or CWE96096-channel Nucleic Acid Extractor, which can be used to carry out high-throughput extraction in a simple and rapid way, greatly reducing the workload of the experimenter and the human error in the experiment.

Bring your own instruments and reagents

1. Thermostatic mixer
2. 2/15mL Magnetic Holder
3. 32 Channel Nucleic Acid Extractor
4. 96 Channel Nucleic Acid Extractor
5. 96DWPlate
6. 8 channelComb
7. Spintipspack
8. Anhydrous ethanol, isopropanol
9. Vortex oscillator or tissue grinder

caveat

1. Add anhydrous ethanol to BufferGW1 and BufferGW2 according to the labels of the reagent bottles and label them before the first use.
2. Freezing and centrifugation of Magbeads is strictly prohibited. Freezing and centrifugation may cause irreversible damage to Magbeads.

procedure**1. Sample pre-treatment**

- 1.1 Centrifuge the LysisTube briefly to allow the beads to settle to the bottom.
- 1.2A. Add 0.1–0.3 g of soil or fecal sample to LysisTube, add 740–820 μ L of BufferQSL with 4 μ L of LRNaseA, screw the cap on the tube tightly and vortex briefly to mix.

B. For fecal samples preserved in non-lytic fecal preservation solution, add 200 μ L–600 μ L of solid–liquid mixture to LysisTube, centrifuge at 13,000 rpm for 1 min, and discard the preservation solution (if the amount of solids after centrifugation is too low, it can be enriched again, but should not be more than 0.3 g). Add 620 μ L of BufferQSL and 4 μ L of RNaseA, screw the cap on the tube tightly and vortex briefly to mix.
- 1.3 Secure the LysisTube in an oscillating milling device fitted with a 2mL adapter and process using milling conditions optimized for your facility (see Appendix).
- 1.4 Shake the LysisTube on a thermostatic mixer at 70° C, 1200 rpm for 10 min. followed by centrifugation at 13,000 rpm for 2 min to precipitate the solid pellet. Transfer 540 μ L of supernatant to a new 2 mL centrifuge tube.
- 1.5 Add 180 μ L BufferRIL, vortex for 5 sec, and centrifuge at 13,000 rpm for 2 min.

Note: BufferRIL should be removed before use and stored at 2–8° C immediately after use.

- 1.6 Manual extraction is operated in accordance with Part 2 and fully automated nucleic acid extractors are operated in accordance with Part 3 or 4.

2. Manual extraction

- 2.1 Add 160 μ L BufferML, 480 μ L of supernatant from step 1.5, 320 μ L of isopropanol, and 20 μ L of MagbeadsSN sequentially to the centrifuge tube, vortex and oscillate to mix for 5sec, and then place the tube on a thermostatic mixer at 25° C and 1600 rpm to oscillate and mix for 10min.

Note: MagbeadsSN should be vortexed and shaken for 20 seconds prior to addition to allow for thorough mixing. MagbeadsSN can be premixed with isopropanol in the volume described above depending on the number of samples and then added.

- 2.2 Place the centrifuge tube on the magnetic rack and let it stand for 1 min. Discard the solution thoroughly after the Magbeads are completely adsorbed on the sidewalls of the centrifuge tube (keep the centrifuge tube fixed on the magnetic rack).
- 2.3 Remove the centrifuge tube from the magnetic rack, add 750 μ L of BufferGW1 (please check that anhydrous ethanol has been added before use) and vortex for 1 min or vortex for 5 sec, and then place the tube in a thermostatic mixer at

25°C and 1600 rpm for 2 min (make sure that the Magbeads are in the mixing state during the oscillation). Afterwards, the centrifuge tube was placed on a magnetic rack for 1 min, and after the Magbeads were completely adsorbed on the side wall of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and then the solution was completely discarded (keep the centrifuge tube fixed on the magnetic rack).

2.4 Repeat step 2.3.

2.5 Remove the centrifuge tube from the magnetic rack, add 750 μ L of BufferGW2 (check that anhydrous ethanol has been added before use) and vortex for 1 min or vortex for 5 seconds, then place the tube on a thermostatic mixer at 25°C and 1600 rpm for 2 min (make sure that the Magbeads are in the mixing state during oscillation). Afterwards, the centrifuge tube was placed on a magnetic rack for 1 min, and after the Magbeads were completely adsorbed on the side wall of the centrifuge tube, the magnetic rack was gently inverted to wash the impurities from the cap of the centrifuge tube, and then the solution was completely discarded (keep the centrifuge tube fixed on the magnetic rack).

2.6 Repeat step 2.5.

2.7 Keeping the centrifuge tube fixed on the magnetic rack, use a pipette to further remove the solution from the bottom and cap of the centrifuge tube, after which it is left at room temperature for 5–10 minutes to allow the ethanol to evaporate cleanly (the surface of the magnetic beads becomes matte to the naked eye and the beads are not dry-cracked).

2.8 Remove the centrifuge tube from the magnetic rack and add 50–200 μ L BufferEBL, vortex and oscillate to completely suspend the magnetic beads in the eluent and then place it on a thermostatic mixer at 56° C, 1600 rpm to oscillate the eluent for 10 min, or place the centrifuge tube in a water bath at 56° C for 10 min, during which time the tube was vortexed and oscillated for 10 sec at 3-min intervals.

2.9 Place the centrifuge tube on a magnetic rack and let it stand for 2 min. After the Magbeads are completely adsorbed on the side wall of the centrifuge tube, use a pipette to transfer the eluate to a new centrifuge tube and store it at –20° C for later use.

3. Match with 32-channel nucleic acid extractor

3.1 Add the appropriate reagents to the 96DW deep well plate according to the table below.

Position	Reagent
1&7Colume	BufferML:160 μ LLL ysate:480 μ L Isopropanol:320 μ L
2&8Colume	BufferGW1:750 μ L
3&9Colume	BufferGW1:750 μ L
4&10Colume	BufferGW2:750 μ L MagbeadsSN: 20 μ L
5&11Colume	BufferGW2:750 μ L
6&12Colume	BufferEBL:100 μ L

Note: Reagents in 1.1&7Colume are added sequentially in order.

2. Please check whether anhydrous ethanol has been added before using GW1 and GW2.

3. BufferGW2 and MagbeadsSN can be premixed according to the above volume depending on the number of samples and vortexed for 10sec to mix before use.

3.2 Place the reagent-added deep-well plate and magnetic sleeve in the corresponding position of the 32-channel nucleic acid extractor and run the extraction program. The program will end after about 40 min.

3.3 Transfer the elution products from columns 6 & 12 of the deep well plate to a 1.5mL centrifuge tube and store at -20° C.

4. Match with 96-channel nucleic acid extractor

4.1 Add the appropriate reagents to the 96DW deep well plate according to the table below.

Position	Reagent
Plate1	BufferML:160 μ L Lysate:480 μ l Isopropyl alcohol:320 μ L
Plate2	BufferGW1:750 μ L
Plate3	BufferGW1:750 μ L
Plate4	BufferGW2:750 μ L MagbeadsSN: 20 μ L
Plate5	BufferGW2:750 μ L
Plate6	BufferEBL:100 μ L

Note: 1. Reagents in Plate1 are added sequentially in order.

2. Please check whether anhydrous ethanol has been added before using GW1 and GW2.

3. BufferGW2 and MagbeadsSN can be premixed according to the above volume based on the number of samples and vortexed for 10sec to mix before use.

4.2 Place the reagent-added deep-well plate and magnetic sleeve in the corresponding position of the CWE960 (magnetic sleeve placed in plate position 4) or CWE9600 (magnetic sleeve placed in plate position 8), run the extraction program, and the program will end after about 50 min.

4.3 Transfer the elution product from the deep-well plate Plate6 to a 1.5mL centrifuge tube and store at -20° C.

Appendix:

Grind the sample using one of the following methods:

1. Manually vortex oscillate on a vortex oscillator at maximum speed for 10min.
2. Oscillate for 10 min at maximum speed on a vortex oscillator with a 1.5-2mL horizontal centrifuge tube holder (keep the LysisTube horizontal). If the number of samples exceeds 12, extend for 5-10min.

For example, use the Vortex-Genie2 vortex oscillator from ScientificIndustries or Mobio.

3. When using Qiagen's TissueLyserII, grind at 25Hz for 10min.
4. When using Qiagen's PowerLyzer24 Homogenizer, homogenize for 30sec at 2000rpm, pause for 30sec, and then homogenize again for 30sec at 2000rpm.
5. When using MPBiomedicals FastPrep-24, the recommended speed is 6.0 and the time is 40sec.

This product is for scientific research only, please do not use for clinical diagnosis and other purposes.