Soil and Fecal DNA Extraction Kit

Project number: S665546

Storage conditions: room temperature.

Products content

Component	S665546 50preps
BufferQSL	45mL
BufferRIL	11mL
BufferML	10mL
BufferGW1 (concentrate)	13mL
BufferGW2 (concentrate)	26mL
BufferEBL	13mL
RNaseA	240μL
LysisTubes II	50
SpinColumnsDM WithCollectionTubes	50

Product Introduction

This kit provides a method for the extraction of total DNA from soil or fecal samples, including the total DNA of cells, bacteria, parasites, and viruses in the sample, and is also suitable for the extraction of DNA from samples containing high concentrations of PCR reaction inhibitors. The kit uses a unique buffer system to make the DNA in the lysate efficiently bind to the adsorption column, the inhibitors of PCR and enzyme reactions as well as residual impurities can be effectively removed through the washing step, and finally eluted with low-salt buffer or water to obtain high-purity DNA. purified DNA can be directly used for second-generation sequencing (16S amplicon and macro genome), library construction, PCR. qPCR, SouthernBio, and other DNA extraction methods are also available in the kit, qPCR, SouthernBlot, enzymatic molecular labeling and other downstream experiments.

Self-contained reagents

1. Thermostatic mixer
2. Anhydrous ethanol, isopropanol
3. Vortex oscillator or tissue grinder

Pre-experiment Preparation and Important Notes

1. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller DNA fragments and reduced extraction.

2. Anhydrous ethanol should be added to BufferGW1 (concentrate) and BufferGW2 (concentrate) before first use according to the instructions on the label of the reagent bottle.
3. BufferRIL should be removed before use and stored at 2-8° C immediately after use.

procedure

1. Centrifuge the LysisTube briefly to allow the beads to settle to the bottom.
2. a. Add 0.1-0.3 g of soil or fecal sample to LysisTube, add 740-820 μL of BufferQSL with 4 μL of LRNaseA, screw the cap on the tube tightly, and vortex briefly to mix.
b. For fecal samples preserved in non-lytic fecal preservation solutions (e.g., CWY041S and CWY041M), add 200 μL-600 μL of the solid-liquid mixture to the LysisTube, centrifuge at 13,000 rpm for 1 min, and discard the preservation solution (if the amount of solids from centrifugation is too low, it can be enriched again but should not be more than 0.3 g). Add 620 μL of BufferQSL and 4 μL of RNaseA, screw the cap on the tube tightly and vortex briefly to mix.
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).
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 540μL of supernatant to a new 2mL centrifuge tube.
5. Add 180μLBufferRIL, vortex for 5sec and centrifuge at 13000rpm for 2min.
Note: BufferRIL should be removed before use and stored at 2-8° C immediately after use.
6. In a new centrifuge tube, sequentially add 160 μL of BufferML, 480 μL of supernatant from step 5, 320 μL of isopropanol and vortex for 5 seconds.
7. Transfer 650 μL of the solution from the previous step to an adsorbent column (SpinColumnsDM) that has been loaded into a collection tube, and centrifuge at 12,000 rpm (~ 13,400 × g) for 1 min.
8. Pour off the waste solution in the collection tube and place the adsorption column back into the collection tube. Repeat step 7 until all of the solution has been transferred.
9. Add 500 μLBufferGW1 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.
10. Add 500 μLBufferGW2 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 place the adsorbent column back into the collection tube. 11. Repeat step 10.
12. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
13 Place the adsorbent column in a new centrifuge tube (self-provided), add 50-200 μL of BufferEBL or sterilized water dropwise to the middle of the adsorbent column in suspension, leave at room temperature for 2-5 min, centrifuge at 12,000 rpm for 1 min, collect the DNA solution, and store the DNA at -20° C.
Note: 1) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

2) Re-elution with an additional 50-100 μL of elution buffer or sterilized water can increase the yield.

3) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 13 can be re-spiked onto the adsorbent membrane and step 13 can be repeated, but the total yield may be reduced.

4) The elution buffer does not contain chelating agents, please store the DNA at -20° C.

(5) Trace amounts of PCR inhibitors remaining in the genomic DNA template may adversely affect the PCR reaction, which is usually resolved by diluting the DNA 2-10 times.

Appendix: Grinding of samples using one of the following methods

1. Manually vortex and oscillate on a vortex oscillator at maximum speed for 10 minutes.

2. Oscillate for 10 minutes at maximum speed on a vortex oscillator with a 1.5-2mL horizontal centrifuge tube holder (keep the LysisTube horizontal). If sample size exceeds 12, extend by 5-10 minutes. For example, use a ScientificIndustries or Mobio Vortex-Genie2 vortex oscillator.

3. When using Qiagen's TissueLyserII, grind at 25Hz for 10 minutes.

4. When using Qiagen's PowerLyzer24 Homogenizer, homogenize at 2000 rpm for 30 seconds, pause for 30 seconds, and then homogenize again at 2000 rpm for 30 seconds.

5. When using MPBiomedicals FastPrep-24, the recommended speed is 6.0 and the time is 40 seconds.