Environmental DNA RNA Extraction Kit
(Silicone Membrane Centrifugal Column Method)

#Cat: NB-03-0600 Size:50preps

Kit Contents

Component	NB-03-0600
Buffer STL	40 ml
Buffer SL	15 ml
Buffer PCI	7 ml
Buffer GXP	50 ml
Buffer GWP	30 ml
Buffer RW1	30 ml
Buffer RW2	50 ml
Nuclease-free Water	15 ml
RNA Mini Column	50 pcs
DNA Mini Column	50 pcs
Collection Tube	100 pcs
Beads Tube	50 pcs

Description

This product utilizes bead milling and silica column purification techniques to rapidly and safely extract both RNA and DNA from soil samples, fecal samples, environmental samples, and aquatic samples simultaneously. The entire extraction process takes only 50 minutes. This product is suitable for the simultaneous extraction of RNA and DNA from soil samples of ≤500mg, fecal samples of ≤200mg, water body filters, sediment, or fermentation residue. The obtained RNA can be directly used for experiments such as RT-PCR, Northern Blot, Poly A purification, nucleic acid protection, and in vitro translation. The obtained DNA can be directly used for experiments such as PCR, Southern Blot, etc.

Storage

This product can be stored at room temperature (15~25° C) for 18 months.

Preparation

- Anhydrous Ethanol (96-100%)
- Add anhydrous ethanol to Buffer RW2 in a volume four times that of the buffer, and store at room temperature.

Protocol

1. In 2ml Bead Tubes, add approximately 0.5g of soil, 0.1-0.2g of feces, 0.3-0.5g of environmental samples, 0.3ml of fermentation suspension, 0.3ml of microbial suspension, and other samples.

2. Add 600µl of Buffer STL and 100µl of Buffer PCI to the sample, and tightly close the cap. For dry environmental samples, use a sieve to remove as much debris as possible, such as leaves, stones, or small twigs, before use. For very dry materials, if the sample material absorbs too much lysis buffer, control the amount of sample and increase the amount of Buffer STL accordingly. For very wet materials, centrifuge to remove excess liquid before adding the lysis buffer. Control the amount of sample to ensure there is enough space in the centrifuge tube for bead milling to lyse cell walls. Typically, reducing the starting material can also help improve lysis efficiency and increase the purity of RNA. When using a bead mill or a vortex mixer with a horizontal rotor, it is recommended to use screw-cap cryovials to prevent liquid leakage.

3. Transfer to the vortex mixer at the highest speed for vortex mixing for 10 minutes or to the bead mill at high speed for bead milling for 30-60 seconds.

- Vortex Mixer: It is recommended to use a vortex mixer equipped with a 2ml centrifuge tube rack. The lysis time should be as short as possible to avoid shearing time and minimize the release of humic acids. However, depending on the sample, increasing the bead milling lysis time to 15 minutes with the vortex mixer may be beneficial and can be adjusted based on the characteristics of the target microbial community and downstream applications.

- PowerLyzer Bead Mill: It is suggested to bead mill at 2000rpm for 30 seconds, pause for 30 seconds, and then bead mill at 2000rpm for another 30 seconds.

- FastPrep24 Bead Mill: It is suggested to bead mill at 5m/s for 30 seconds, pause for 30 seconds, and then bead mill at 5m/s for another 30 seconds.

- TissueLyser II Bead Mill: It is suggested to bead mill at 25Hz for 5 minutes, reposition the samples, and then bead mill at 25Hz for another 5 minutes.

4. Centrifuge at 12,000 x g for 5 minutes at room temperature.

5. Transfer the supernatant (~550µl) to a new centrifuge tube, add 200µl Buffer SL, vortex mix for 5-10 seconds. Centrifuge at 12,000 x g for 5 minutes.

6. Transfer the supernatant to a new centrifuge tube, add 700µl binding solution GXP, and invert 6-8 times.

Adsorbing DNA:

7. Place the DNA Mini Column in a 2ml collection tube and transfer half the volume of the mixture to the column. Centrifuge at 10,000 x g for 1 minute and transfer the filtrate to a 5ml centrifuge tube.

8. Put the DNA column back into the collection tube, transfer the remaining mixture to the column, and centrifuge at 10,000 x g for 1 minute. Transfer the filtrate back into the 5ml centrifuge tube, combine the filtrates from both steps, and proceed with RNA extraction as described in step 16.

9. Place the DNA column back into the collection tube, add 500µl of Buffer GWP to the column, and centrifuge at 10,000 x g for 1 minute.

10. Discard the filtrate, place the column back into the collection tube, and add 500µl of Buffer RW2 to the column. Centrifuge at 12,000 x g for 1 minute.

11. Repeat step 10 once.

12. Discard the filtrate, place the column back into the collection tube, and centrifuge at 12,000 x g for 2 minutes.

13. Place the DNA column in a 1.5ml centrifuge tube. Add 30-50µl of pre-warmed Nuclease-free Water at 65°C to the center of the column membrane, let it stand at room temperature for 5 minutes, then centrifuge at 12,000 x g for 1 minute.

14. Add another 30-50µl of pre-warmed Nuclease-free Water at 65°C to the center of the column membrane. Let it stand at room temperature for 5 minutes, then centrifuge at 12,000 x g for 1 minute.

15. Discard the DNA column and store the DNA at 2-8°C or-20°C.

Purifying RNA through RNA Mini Column:

16. Take the filtrate obtained from steps 7-8, add 0.5 times the volume of anhydrous ethanol, and invert to mix thoroughly 6-8 times.

17. Place the RNA Mini Column in a 2ml collection tube. Transfer up to 700µl of the mixture to the column. Centrifuge at 12,000 x g for 1 minute.

18. Discard the filtrate, place the column back into the collection tube. Transfer the remaining mixture to the column. Centrifuge at 12,000 x g for 1 minute. Repeat this step until all of the mixture has been transferred to the column and centrifuged.

19. Discard the filtrate, place the column back into the collection tube, add 500µl of Buffer RW1 to the column, and centrifuge at 12,000 x g for 1 minute.

20. Discard the filtrate, place the column back into the collection tube, and add 500µl of Buffer RW2 to the

column. Centrifuge at 12,000 x g for 1 minute.

21. Repeat step 20 once.

22. Discard the filtrate, place the column back into the collection tube, and centrifuge at 12,000 x g for 2 minutes.

23. Place the DNA column in a 1.5ml centrifuge tube. Add 30-100µl of Nuclease-free Water to the center of the column membrane, let it stand at room temperature for 2 minutes, then centrifuge at 12,000 x g for 1 minute.

24. Discard the column and store the RNA at-80°C