ARPENspin fast Soil/Stool Genomic DNA/ RNA Extraction Kit

Cat# ARP-FSSDRS50

Kit Components

COMPONENTS	50 tests
SP Buffer	30 mL
Lysis S Buffer	12.5mL
DA Buffer	7.5mL
Binding Buffer	70mL
PW Buffer	12mL (add 18mL ethanol before use)
DNA Wash Buffer	24mL (add 36mL ethanol before use)
RNA Wash Buffer	24mL (add 36mL ethanol before use)
Elution Buffer	10ml
RElution Buffer	10mL
Grind Tube	50 tubes
DNA Spin Columns	50 Tubes
RNA Spin Columns	50 Tubes
Handbook	1 copy

Storage

The kit has shown stability for 18 months when stored at temperatures between 2°C and 30°C.

Introduction

The kit offers a simple, fast, and cost-effective method for isolating PCR-ready genomic DNA and RNA from the same soil and stool sample. The purification procedure is designed to simultaneously extract microbial DNA and RNA from the same stool sample while separating them into distinct eluate fractions. This process does not require expensive equipment, involves only a few straightforward steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform.

The DNA in the sample is first liberated. After centrifugation, impurities are discarded. The released DNA binds exclusively and specifically to the Biospin membrane in the presence of a Binding Buffer, under the appropriate conditions of salt concentration, ionic strength, and pH. Denatured proteins and other contaminants are removed through several washing steps. The DNA is then eluted from the membrane using the Elution Buffer.

Next, ethanol is added to the flow-through from the DNA Spin Column to create suitable binding conditions for RNA. The sample is then transferred to an RNA Spin Column, where the total RNA binds to the membrane. After washing, the RNA is eluted in RNase-Free Water, making it ready for downstream applications, including PCR, qPCR, and NGS.

Additional apparatus and materials required but not supplied

- Sterile 1.5mL microcentrifuge tubes
- 10µL/100µL/1000µL tips
- Microcentrifuge capable of 14,000g
- Absolute ethanol
- 2.0mL microcentrifuge tubes

Important Notes

Note: When handling large liquid volumes, you may divide them into smaller portions to pass through the Spin Column more easily.

1. Before the first use, add absolute ethanol to the PW Buffer, DNA Wash Buffer, and RNA Wash Buffer, and mix thoroughly.

2. The Lysis S Buffer may form precipitates during storage. If this occurs, incubate the buffer at a temperature between 37°C and 55°C until the precipitate has completely dissolved.

Protocol

1. Place 100 to 200 mg of soil or stool into the Grind Tube (provided).
2. Add 600 µL of SP Buffer and 250 µL of Lysis S Buffer.
3. Homogenize the mixture in the lysis instrument for 40 seconds at a speed setting of 6.0 m/s, or vortex for 5 minutes at maximum speed using a vortex generator.
4. Centrifuge the sample at 14,000 g for 10 minutes to pellet debris.
5. Transfer the supernatant to a 1.5 mL microcentrifuge tube.
6. Add 150 µL of DA Buffer and mix thoroughly.
7. Incubate the mixture on ice for 5 minutes, then centrifuge at 14,000 g for 5 minutes.
8. Transfer the supernatant to a 2.0 mL microcentrifuge tube.
9. Add 350 µL of Binding Buffer and mix thoroughly.

Genomic DNA purification

10. Transfer the mixture to the Spin Column and centrifuge at 14,000 g for 1 minute. Next, transfer the flow-through into a new 2.0 mL centrifuge tube. Incubate this tube on ice or at 4°C for the upcoming RNA purification steps.
11. Add 500µL of PW buffer to the DNA Spin Column and centrifuge at 14,000 g for 30 seconds. Discard the flow-through.
12. Add 500µL of DNA Wash Buffer to the DNA Spin Column and centrifuge at 14,000 g for 30 seconds. Discard the flow-through.
13. Repeat the previous step.
14. Place the DNA Spin Column back in the collection tube and centrifuge at 14,000 g for 1 minute. Afterward, transfer the column to a clean 1.5 mL microcentrifuge tube.
15. Add 50–100 µL of Elution Buffer to the center of the membrane, incubate for 1 minute at room temperature, and then centrifuge at 14,000 g for 1 minute. The DNA will now be in the 1.5 mL centrifuge tube. If you do not plan to use it immediately, store it at -20°C.

Total RNA Purification

16. Add an equal volume of ethanol to the flow-through and mix well. Transfer the mixture to the RNA Spin Column, then centrifuge at 14,000 g for 30 seconds and discard the flow-through.
17. Add 500 µL of RNA Wash Buffer to the RNA Spin Column, centrifuge at 14,000 g for 30 seconds, and discard the flow-through.
18. Repeat step 17.
19. Place the RNA Spin Column back into the collection tube and centrifuge at 14,000 g for 1 minute. Then transfer the column to a clean 1.5 mL microcentrifuge tube.
20. Add 50–100 µL of RElution Buffer to the center of the membrane. Incubate for 1 minute at room temperature, then centrifuge at 14,000 g for 1 minute. The RNA will now be in the 1.5 mL centrifuge tube. If not used immediately, please store at -80°C.

FAQ:

Q1: What is the optimal quantity for extracting stool samples?

A: The optimal extraction quantity for stool samples ranges from 100 to 200 mg. For various wet stool samples, it is recommended to use between 0.1 g and 0.2 g.

Q2: What should be done if DNA does not amplify?

A: An excessive amount of DNA can inhibit the PCR reaction. It is advisable to perform a gradient dilution of the template DNA to determine the optimum concentration.

Q3: What alternative lysis methods can be used?

A: If cells are challenging to lyse, vortexing the sample for 10 minutes after adding SP Buffer and Lysis S Buffer may help.

Q4: How can DNA be concentrated?

A: The final volume of the eluted DNA should be 100 µL. To concentrate the DNA, add 4 µL of 5M NaCl and vortex 5 times to mix. Next, add 200 µL of 100% cold ethanol and invert the mixture 5 times to ensure good mixing. Centrifuge at 12,000 x g for 5 minutes at room temperature. Carefully decant all the liquid and remove any residual ethanol using a speed vacuum, desiccator, or by air drying. Finally, resuspend the precipitated DNA in sterile water or sterile 10 mM Tris buffer.

Q5: How can complete genomic DNA be ensured?

A: Intense shock force and centrifugal force can lead to shearing of genomic DNA. To obtain complete genomic DNA, consider reducing the amplitude of the grinding machine and lowering the centrifugal force from 14,000 g to 10,000 g.