VAMNE Magnetic Stool/Soil DNA Extraction Kit

DMA5101

Vazyme

Instruction for Use

Version 25.1

Vazyme

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

This kit is applicable for extracting DNA from various samples including soil, stool, intestinal contents, fermentation products, water filter membranes, etc. The kit combines chemical and mechanical methods, which can efficiently lyse bacteria and fungi with thick cell walls. The kit applies a unique humic acid removal technology to effectively remove humic acid and other inhibitors from the sample. The high-affinity magnetic beads used in this kit can adsorb nucleic acids through hydrogen bonds and electrostatic interactions in high-salt buffer solutions. After rinse to remove excess humic acid, proteins, salts, and other impurities, the magnetic beads release nucleic acids under the action of low-salt elution solution, thus achieving the purpose of rapid separation and purification of nucleic acids. This kit can quickly extract high-yield, high-purity DNA, and the purified DNA can be directly used for PCR, qPCR, next-generation sequencing, and other experiments.

02/Components

	Components	DMA5101-01 (96 rxns)
	Lysis Tube	2 × 48
	Lysis Buffer	96 ml
■	RNase A	500 μl
	PI Buffer	30 ml
	Binding Buffer	48 ml
	Beads	2 ml
	Buffer W1	68 ml
	Buffer W2	33 ml
	Buffer W3	15 ml
	Elution Buffer	7 ml

03/Storage

BOX 1: RNase A, PI Buffer. Store at 2 ~ 8°C and ship on ice.

BOX 2: Other Components. Store at 15 ~ 25°C and ship at room temperature.

04/Applications

It is applicable for soil, stool, intestinal contents, fermentation products, water filter membranes, etc.

05/Self-prepared Materials

Manual extraction: high-speed centrifuge, homogenizer or vortex mixer, magnetic rack, thermostatic water bath;

Automated extraction: high-speed centrifuge, homogenizer or vortex mixer, fully automatic nucleic acid extraction instrument, 96 well deep plate, magnetic rod sleeves.

06/Notes

1. Before use, please read the instructions for use in detail and strictly follow the instructions.
2. The vessels, pipettes, etc., used in this kit are all dedicated. The centrifuge tubes, pipette tips, and other disposable consumables used should be DNase and RNase-free. Sample processing should be carried out in a laminar flow cabinet or biosafety cabinet. Operators should wear powder-free gloves, masks, etc.
3. Before and after using the fully automatic nucleic acid extraction instrument, it is necessary to perform ultraviolet disinfection on the instrument for 30 min.
4. After extraction, if there are trace amounts of magnetic beads remaining in the elution solution, care should be taken to avoid aspirating the beads when taking the elution. If beads are aspirated, a magnetic rack can be used for secondary magnetic attraction.
5. Beads, PI Buffer, RNase A should be stored at 2 ~ 8°C after receipt. Remove from 2 ~ 8°C before use and equilibrate to room temperature for optimal use effect.
6. For complex samples with low nucleic acid content (such as mineral soil, sandy soil, coal soil, etc.), it's recommended to use Lysis buffer Pro (Vazyme #DMA5101-C1) which is not provided in this kit. Please contact our sales agent for Lysis buffer Pro.
   ▲ The reagent is prone to precipitation at low temperatures. If precipitate forms, it can be heated in a 37°C water bath to dissolve. Use after mixing evenly.
7. Before use, please prepare the Lysis Buffer/RNase A mixture: add 5 μl RNase A per reaction, mix well and then use. And it is recommended to be prepared freshly.
8. **Before using the kit for the first time, add the labeled volume of absolute ethanol to Buffer W2 and Buffer W3, respectively (add 42 ml of absolute ethanol to Buffer W2, add 60 ml of absolute ethanol to Buffer W3), mix thoroughly and mark on the label.**

07/Experiment Process

07-1/Sample pretreatment

1. Add the sample to the Lysis Tube, then add the Lysis Buffer/RNase A mixture.
   ▲ Lysis Buffer/RNase A mixture can be prepared in advance: add 5 μl RNase A per reaction, mix well and use.

   a. Soil sample: Add 100 - 250 mg soil sample, then add 700 μl Lysis Buffer/RNase A

mixture.

b. Stool sample: Add 50 - 150 mg stool sample, then add 700 µl Lysis Buffer/RNase A mixture.

c. Filter membrane sample: Add the cut filter membrane (approximately one piece of 47 mm diameter filter membrane), then add 700 µl Lysis Buffer/RNase A mixture. If the filter membrane has strong water absorption, the amount of Lysis Buffer/RNase A can be increased to 1 ml, or the amount of filter membrane can be appropriately reduced.

d. Other solid samples (intestinal contents, fermented mash, fermentation products, etc.): Add 100 - 250 mg of sample, then add 700 µl Lysis Buffer/RNase A mixture.

▲ For dry samples with highly water-absorbent, the amount of Lysis Buffer/RNase A can be increased, but it should not exceed 1 ml.

e. Liquid samples (loose stool, fermentation liquid, sludge, etc.): Add 200 µl of liquid sample, then add 500 µl of Lysis Buffer/RNase A mixture.

f. Microbial cultures (bacteria, fungi, yeast) : Add an approximate amount of to a Lysis Tube. For the liquid cultures, 10^9 input is recommended. For mycelium, 20 - 100 mg input is recommended. Then add 300 µl Lysis Buffer/RNase A mixture.

2. Vortex or homogenize the mixture, recommended vortex conditions: maximum speed for 5 min; homogenization conditions: 60 Hz for 2 min.

▲ If higher requirements for DNA integrity are needed, mild grinding conditions can be adopted, but grinding intensity reduction may affect nucleic acid yield. It is recommended to vortex at medium speed for 5 min or homogenize at 25 Hz for 5 min.

3. Centrifuge at 12,500 rpm (15,000 × g) for 2 min. Transfer approximately 500 µl supernatant (if less than 500 µl, aspirate completely) to a 1.5 ml centrifuge tube (self-prepared).

4. Add 300 µl PI Buffer. Vortex mix for 5 - 10 sec or mix by inversion 20 times. Centrifuge at 12,500 rpm (15,000 × g) for 2 min. Transfer 500 µl supernatant (if less than 500 µl, aspirate all) for the next step. For microbial cultures, 300 µl PI Buffer is omitted.

▲ For samples with low humic acid content (such as filter membranes, water bodies, etc.), the amount of PI Buffer can be reduced to 100 µl, or PI Buffer can be omitted.

07-2/Manual extraction steps

1. Carefully transfer supernatant from the **07-1/Sample pretreatment** to a centrifuge tube, add 500 µl Binding Buffer and 20 µl Beads. Vortex and mix at maximum speed for 4 min. Briefly centrifuge, place the tube on a magnetic rack and let it stand for 1 min. When the magnetic beads are completely adsorbed, carefully discard the supernatant.

2. Add 700 µl Buffer W1 to the centrifuge tube. Vortex at maximum speed for 1 min. Place the centrifuge tube on the magnetic rack and let it stand for 1 min. Wait until the magnetic beads are completely adsorbed. Carefully discard the supernatant.

3. Add 700 μl Buffer W2 to the centrifuge tube (check if absolute ethanol has been added before use). Vortex at maximum speed for 1 min. Place the centrifuge tubes on a magnetic rack and let it stand for 1 min. Wait until the magnetic beads are completely adsorbed. Carefully discard the supernatant.

4. Add 700 μl Buffer W3 to the centrifuge tube (check if absolute ethanol has been added before use). Vortex at maximum speed for 1 min. Place the centrifuge tubes on a magnetic rack and let it stand for 1 min. Wait until the magnetic beads are completely adsorbed. Carefully discard the supernatant.

   ▲ The residual liquid can be completely aspirated with a 10 μl pipette tip.

   ▲ If higher purity is required, an additional 80% absolute ethanol rinse (refer to step 4 for rinse method) can be added to increase the 260/230 ratio.

5. Keep the centrifuge tubes on the magnetic rack. Open the tube caps, and air dry at room temperature for 2 - 5 min.

   ▲ Ethanol residue may interfere with subsequent enzymatic reactions. Ensure that the absolute ethonal evaporates completely, but avoid excessive drying of magnetic beadsto prevent affecting elution.

6. Add 70 μl Elution Buffer to the centrifuge tube, use a pipette to pipette up and down several times to mix beads thoroughly and transfer the mixture to a new centrifuge tube. Vortex the new tube at maximum speed for 1 min. Then place it in a 65°C water bath for 3 - 5 min, and during this time mix by inversion 3 - 5 times.

   ▲ During the manual extraction process, the wash buffer may be left in the centrifuge tube and affect the 260/230 ratio of the DNA product. To avoid a low 260/230 ratio, it's necessary to replace a new tube before incubation.

7. Place the centrifuge tube on a magnetic rack and let it stand for 1 min, until the magnetic beads are completely adsorbed. Then transfer the elution product to a clean Nuclease-free centrifuge tube. If not used immediately, store the elution product at -20°C.

07-3/Automated extraction steps

1. Add the following reagents to the 96 well deep plate according to the table below.

Position	Order	Reagent	Volume/Well
1/7	1	Binding Buffer	500 μl
2/8	2	Beads	20 μl Beads + 680 μl ddH_2O
3/9	3	Buffer W1	700 μl
4/10	4	Buffer W2	700 μl
5/11	5	Buffer W3	700 μl
6/12	6	Elution Buffer	70 μl

2. Carefully transfer the supernatant from **07-1/Sample pretreatment** into the Binding Buffer well of the 96 well deep plate. Place the 96 well deep plate in the correct order into the nucleic acid extraction instrument. Install magnetic rod sleeves and confirm proper installation, run the DMA5101 program for automated extraction.

3. Automated extraction procedure

Step	Plate Position	Name	Mixing Time (min)	Adsorption Time (sec)	Waiting Time (min)	Volume (μl)	Mixing Speed	Temperature (°C)	Mixing Position	Mixing Amplitude	Adsorption Position	Adsorption Speed
1	2	Movebeads	0.5	30	0	700	10	-	10%	80%	0%	10
2	1	Binding	4	45	0	1,000	10	-	10%	80%	0%	10
3	3	W1	1	30	0	700	8	-	10%	80%	0%	10
4	4	W2	1	30	0	700	8	-	10%	80%	0%	10
5	5	W3	1	30	2	700	8	-	10%	80%	0%	10
6	6	Elution	3	30	0	70	10	65	5%	80%	0%	10
7	2	Movebeads	0.3	0	0	700	10	-	10%	80%	0%	10
Other settings (in the Option menu): Heating settings (heating and action start at the same time) Adsorption settings (three-stage adsorption)												

4. After the automated procedure ends, the purified nucleic acid product will be in the Elution Buffer wells. Transfer the elution product to a clean, nuclease-free centrifuge tube. If not used immediately, store the elution product at -20°C.

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