VAMNE Magnetic Stool/Soil DNA Extraction Kit (Prepackaged)

DMA5103

Vazyme

Instruction for Use

Version 25.1

Contents

01/Product Description 02
02/Components 02
03/Storage 02
04/Applications 02
05/Applicable Instruments 02
06/Self-prepared Materials 03
07/Notes 03
08/Experiment Process 03
08-1/Sample pretreatment 03
08-2/Automated extraction steps 04

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, and 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 under high-salt buffer conditions. After rinse to remove excess humic acid, proteins, salts, and other impurities, the magnetic beads release nucleic acids under low-salt elution buffer conditions, 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	DMA5103-01 (1 × 96 T)
	Lysis Tube	2 × 48
	Lysis Buffer	96 ml
■	RNase A	500 μl
	PI Buffer	30 ml
	Binding Plate	1
	Beads Plate	1
	W1 Plate	1
	W2 Plate	1
	W3 Plate	1
	Elution Plate	1

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, and water filter membranes, etc.

05/Applicable Instruments

It is applicable for fully automatic nucleic acid extraction instruments (Vazyme #VNP-96P, Thermo #KingFisher Flex) and similar types of instruments.

06/Self-prepared Materials

High-speed centrifuge, grinder or vortex mixer, fully automatic nucleic acid extraction instrument.

07/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. Solution PI Buffer, RNase A should be stored at 2 ~ 8°C upon receipt, and taken out from 2 ~ 8°C before use to achieve their optimal performance.
6. For complex samples with low nucleic acid content (such as mineral soil, sandy soil, coal soil, etc.), contact the relevant salesperson, place an order to obtain Lysis Buffer Pro (Vazyme #DMA5101-C1).
   ▲ 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 before use.

08/Experiment Process

08-1/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, mash, fermentation products, etc.): Add 100 - 250 mg 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 broth, 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 sample 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.

08-2/Automated extraction steps

1. Take out the pre-packaged reagents from the kit, mix by inversion several times to resuspend the magnetic beads, gently flick the plate to concentrate the reagents and magnetic beads at the bottom of the wells. Before use, confirm the well orientation and carefully remove the aluminum parafilm. Avoid vibration when tearing off the sealing foil to prevent liquid from spilling.

2. Carefully transfer the supernatant from **08-1/Sample pretreatment** into the Binding Plate, place the Binding Plate, Beads Plate, W1 Plate, W2 Plate, W3 Plate, and Elution plate in the nucleic acid extraction instrument according to the corresponding order of each plate. Install magnetic rod sleeves and confirm proper installation. Run the the DMA5103 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 Plate. 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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