VAMNE Magnetic Universal Total RNA Kit

ROA3303

Vazyme

Instruction for Use

Version 24.1

Vazyme

Contents

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

01/Product Description

This kit can quickly separate and purify high-quality total RNA from cell and various tissue samples, achieving high-throughput processing of parallel samples. The kit uses unique embedded superparamagnetic silicon-based magnetic beads. In a unique buffer system, nucleic acids are adsorbed by hydrogen bonding and electrostatic adsorption, without adsorbing proteins and other impurities. The nucleic acid-bound magnetic beads are washed to remove residual proteins and ionic salts. When using a low-salt buffer, the magnetic beads release nucleic acids, achieving the purpose of rapid separation and purification of nucleic acids. The entire operation process is simple, fast, safe, and efficient. The obtained nucleic acids can be directly used for downstream experiments such as reverse transcription, PCR, fluorescence quantitative PCR, RT-PCR, RT-qPCR, next-generation sequencing, and biochip analysis.

02/Components

Components	ROA3303-01 (1 × 96 T)
Binding (Plate 1)	1
Beads (Plate 2)	1
Wash 1 (Plate 3)	1
Wash 2 (Plate 4)	1
Wash 3 (Plate 5)	1
Elution (Plate 6)	1
Elution Buffer	1 ml

03/Storage

Store at 15 ~ 25°C and ship at room temperature.

04/Applications

1. Applicable sample types: cells, animal tissues, simple plant tissues, etc.
2. Sample input: ≤1 × 10^7 Cells; 20 - 50 mg animal tissue/simple plant tissue.

05/Applicable Instruments

It is applicable for fully automatic nucleic acid extraction instrument (Vazyme #VNP-96P) and other similar types of instruments.

▲ If using other brands/models of automated instruments, the matching consumables and procedures need to be adjusted.

06/Notes

1. This kit does not provide Trizol lysis buffer separately. It needs to be purchased separately. We recommend using VeZol Reagent (Vazyme #R411) or FreeZol Reagent (Vazyme #R711) as a combination.
2. Before conducting the experiment, please prepare Trizol reagent and chloroform (or chloroform substitute) (chloroform substitute can be purchased from Vazyme technical support or 1-bromo-3-chloropropane can be purchased by yourself), low-temperature high-speed centrifuge, and RNase-free lab consumables.
3. The key to RNA extraction is to prevent RNase contamination. RNase is widely present in the environment and is extremely stable. Even a small amount of RNase can quickly degrade RNA. Therefore, please take protective measures according to the routine RNA extraction procedure, including wearing masks and disposable sterilized gloves, operating in a separate clean area, and using RNase-free laboratory equipment.
4. Before and after using, the automatic nucleic acid extraction instrument (Vazyme #VNP-96P) and other similar instruments need to be disinfected with UV light for 30 min.
5. After the extraction is completed, there may be trace amounts of magnetic beads remaining in the elution solution. When aspirating the elution solution, try to avoid pipetting the magnetic beads. If the magnetic beads are pipetted out, a magnetic rack can be used for secondary magnetic separation.
6. Reagents with different batch numbers should not be mixed unless otherwise specified, and ensure that the reagent kit is used within its expiration date.

07/Experiment Process

07-1/Sample pre-processing

◇ Cell

Add an appropriate volume of lysis buffer (1 ml VeZol Reagent, Vazyme #R411 or 500 µl FreeZol Reagent, Vazyme #R711) to cells. Vortex or pipette it up and down to fully lyse and let it stand at room temperature for 5 min.

◇ Animal/plant tissue

a. Freeze the fresh tissue with liquid nitrogen, quickly transfer it to a pre-cooled mortar with liquid nitrogen, grind with a pestle, continuously adding liquid nitrogen during the process, until it is ground into a powder form (without obvious visible particles).

b. Transfer the powdered sample to a centrifuge tube, add an appropriate volume of lysis buffer (1 ml VeZol Reagent, Vazyme #R411 or 500 µl FreeZol Reagent, Vazyme #R711). Vortex or pipette it up and down to fully lyse and let it stand at room temperature for 5 min.

07-2/Total RNA Extraction

◇ Extraction with chloroform (VeZol Reagent, Vazyme #R411)

a. Add 1/5 volume of chloroform (or chloroform substitute) to the above lysate. Shake vigorously for 15 sec to obtain an emulsion, and incubate at room temperature for 5 min.

b. Centrifuge at 11,200 rpm (12,000 × g) and 4°C for 15 min.

▲ Centrifuge at a low temperature to avoid increased genomic DNA contamination.

c. Carefully take out the centrifuge tube. The mixture separates into three layers: an upper aqueous phase (containing RNA), an interphase, and a lower red organic phase. Carefully transfer the upper aqueous phase (pipetting 450 µl) to **Binding Plate (Plate 1)**.

▲ Due to the combination with automation, only 450 µl of supernatant is taken.

◇ Extraction without chloroform (FreeZol Reagent, Vazyme #R711)

a. Add Dilution Buffer to the above lysate. For animal tissues, add 100 µl Dilution Buffer per 500 µl FreeZol Reagent. For cells, add 150 µl Dilution Buffer per 500 µl FreeZol Reagent. Close lid and votex to fully mix, and incubate at room temperature for 5 min.

▲ When vortexing, pay attention to make the solution fully mixed into a homogeneous emulsion. Incomplete mixing affects the efficiency of RNA extraction and impurity removal.

b. Centrifuge at 11,200 rpm (12,000 × g) for 15 min at room temperature.

c. Carefully take out the centrifuge tube.The mixture separates into two layers: an upper aqueous phase (containing RNA), and a lower organic phase (including proetin, DNA, polysaccharides and other impurities). Carefully transfer the upper aqueous phase (pipetting 450 µl) to **Binding Plate (Plate 1)**.

▲ Due to the combination with automation, only 450 µl of supernatant is taken.

▲ The supernatant may be slightly turbid or colored for some samples, which will not affect the product yield or purity. It is safe to proceed to the following steps.

07-3/Preparation of Prepackaged reagents

Take out the prepackaged reagents from the kit, invert and mix several times to resuspend the magnetic beads. Gently shake the plate to make the reagents and magnetic beads sink to the bottom of the well. And be carefully tear off the aluminum foil sealing film.

▲ Avoid vibration when tearing off the the sealing foil to prevent liquid from spilling.

07-4/Experiment Process

1. Add 450 µl of the above sample processed supernatant to **Binding Plate (Plate 1)**.
2. Place the 96 well deep plate into the nucleic acid extraction instrument, with the notch of the deep plate facing the upper right corner, and put the magnetic rod sleeves into **Beads Plate (Plate 2)**. Confirm that both the 96 well deep plate and the magnetic rod sleeves are properly installed.
3. Follow the following program to edit (or select the corresponding pre-imported program) for automated extraction:

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	Beads	1	90	0	700	8	/	10	80	0	5
2	1	Binding	10	60	0	900	10	/	10	80	0	5
3	3	Wash 1	1	60	0	700	10	/	10	80	0	5
4	4	Wash 2	1	60	0	700	10	/	10	80	0	5
5	5	Wash 3	1	60	5	700	10	/	10	80	0	5
6	6	Elution	5	30	0	70	10	75	10	80	0	5
7	5	Beads	1	0	0	700	8	/	10	80	0	5
Other settings (in the Option menu): Heating settings (heating and action start at the same time); Adsorption settings (three-stage adsorption)												

4. At the end of the automated procedure, transfer the eluent in **Elution Plate (Plate 6)** for direct use in downstream experiments or store at -30 ~ -15°C for short-term storage, -85 ~ -65°C for long-term storage.

Vazyme Biotech Co.,Ltd.
www.vazyme.com
400-600-9335 (China) +86 400-168-5000 (Global)
support@vazyme.com