VAMNE Magnetic Universal Plant Total RNA Kit

RMA4101

Vazyme

Instruction for Use

Version 25.1

Contents

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

01/Product Description

VAMNE Magnetic Universal Plant Total RNA Kit is designed for the extraction of high-purity RNA from various plant tissues, achieving high-throughput processing of samples. The kit is based on superparamagnetic particle purification technology with an optimized reagent system, which can specifically adsorp nucleic acids and effectively remove various impurities. DNase I can efficiently eliminate DNA. The fully automated workflow is simple, rapid, safe and efficient. The obtained RNA can be directly used for downstream experiments such as RT-PCR, qRT-PCR, Northern Blot, Dot Blot, *in vitro* translation, and high-throughput sequencing.

02/Components

	Components	RMA4101-01 (96 rxns)
BOX 1	Binding Buffer A	21 ml
	Beads	26 ml
	Wash Buffer 1	34 ml
	Wash Buffer 2	70 ml
	Wash Buffer 3	20 ml
	Elution Buffer	7 ml
	Lysis Buffer PLA	80 ml
	Buffer RDD	5 ml
BOX 2	Proteinase K	2.5 ml
	Buffer TP	3 × 1.2 ml
	DNase I	320 µl

03/Storage

BOX 1: Store at 15 ~ 25°C and ship at room temperature.

BOX 2: Store at -30 ~ -15°C and ship at ≤0°C.

04/Applicable Instruments

For use with the fully automatic nucleic acids extraction instruments (Vazyme #VNP-32P/VNP-96P) and equivalent instruments.

▲ For automated instruments of other brands or models, compatible consumables and operating programs must be adjusted accordingly.

05/Applications

Plant leaves and fungi: 20 - 100 mg;

Polysaccharide-rich tubers, roots and seeds: 20 - 50 mg;

Fruits: 50 - 200 mg.

06/Self-prepared Materials

Isopropanol, anhydrous ethanol and Seed Lysis Buffer (Vazyme #DMA4101-C2) (Optional).

07/Notes

1. The key to successful RNA extraction is to prevent RNase contamination. RNases are ubiquitous and extremely stable in the environment. Even trace amounts of RNase can rapidly degrade RNA. Therefore, please follow standard RNA extraction precautions, including wearing masks and disposable sterilized gloves, operating in a dedicated and clean area, and using RNase-free laboratory consumables.
2. The automate nucleic acid extraction instruments should be disinfected with the UV light for 30 min before and after use.
3. Traces of magnetic beads may remain in the eluate after extraction. Avoid aspirating the magnetic beads. If magnetic beads are accidentally aspirated, repeat magnetic adsorption once more using a magnetic rack.
4. Properly dispose of all samples and reagents, thoroughly wipe down and disinfect all work surfaces with 75% ethanol.
5. This kit contains reagents only and does not include consumables. Please contact sales representatives for purchasing consumables.

08/Experiment Process

08-1/Reagents Preparation

Before use, add 9 ml **isopropanol** to Binding Buffer A, 66 ml **anhydrous ethanol** to Wash Buffer 1, and 80 ml **anhydrous ethanol** to Wash Buffer 3. Mark the bottles accordingly after addition.

Aliquot the reagents into the corresponding wells of the 96-well plate as indicated in the below table.

▲ Vortex the magnetic beads thoroughly before aliquoting. After dispensing into every 3 wells, vortex again before continuing aliquoting.

Compatible with Vazyme #VNP-32P

Well Position	Reagent	Volume per Well
Columns 1 & 7	Binding Buffer A	220 µl
Columns 2 & 8	Beads	255 µl
Columns 3 & 9	Wash Buffer 1	700 µl
Columns 4 & 10	Wash Buffer 2	700 µl
Columns 5 & 11	Wash Buffer 3	700 µl
Columns 6 & 12	Elution Buffer	70 µl

Compatible with Vazyme #VNP-96P

Plate ID	Plate Position	Reagent	Volume per Well
Binding (Plate 1)	1	Binding Buffer A	220 µl
Beads (Plate 2)	2	Beads	255 µl
Wash 1 (Plate 3)	3	Wash Buffer 1	700 µl
Wash 2 (Plate 4)	4	Wash Buffer 2	700 µl
Wash 3 (Plate 5)	5	Wash Buffer 3	700 µl
Elution (Plate 6)	6	Elution Buffer	70 µl

08-2/Sample Pretreatment

1. Sample grinding: Rapidly transfer fresh or frozen plant tissue stored at -85 ~ -65°C into a liquid nitrogen–precooled mortar and grind thoroughly. Continuously add liquid nitrogen to maintain the tissue at a low temperature until it is ground into a fine powder with no obvious particles. Alternatively, rapidly grind the tissue into a powder at low temperature using a fully automated tissue homogenizer. Recommended parameters are provided in the table below.

Sample type	Parameters settings	
Plant leaves, flowers, fruits, fungi, etc.	40 - 60 Hz	45 sec, 1 cycle
Plant polysaccharide-rich tubers, roots, etc.	40 - 60 Hz	45 sec, 2 cycles
Plant seeds such as soybeans, wheat seeds, etc.	60 Hz	45 sec, 3 cycles

▲ The grinding parameters can be adjusted according to the difficulty of disrupting sample. Laboratory-standard grinding parameters may also be used as appropriate.

▲ All grinding accessories should be pre-cooled with liquid nitrogen prior to use, and re-precooled between successive grinding cycles.

2. Sample Pre-processing Solution preparation: Add 30 µl Buffer TP and 20 µl Proteinase K to each 800 µl of Lysis Buffer PLA and mix well. Transfer the ground tissue into a 1.5 ml centrifuge tube. Immediately add 850 µl Pre-processing Solution. Vigorously vortex for 30 - 60 sec to thoroughly mix the sample and Pre-processing Solution until no obvious large tissue clumps remain, then incubate at room temperature for 5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 5 min at room temperature. Transfer 700 µl of supernatant to the Binding Buffer A wells or Binding (Plate 1).

▲ Pre-processing Solution needs to be prepared freshly and used immediately. It is recommended to prepare Pre-processing Solution for N + 1 reactions.

▲ For samples with low RNA content, the input amount can be appropriately increased. Due to the diversity of plant samples, the input amount can be adjusted based on specific experimental requirements and experience.

▲ For certain seed or polysaccharide-rich samples, a transparent gel-like layer may appear between the pellet and the supernatant after centrifugation. Accidentally aspirating this layer can affect extraction efficiency. To ensure optimal results, the volume of the supernatant can be appropriately reduced. For seed samples (e.g., wheat seeds, rice seeds) ≥30 mg, Seed Lysis Buffer (Vazyme #DMA4101-C2) is recommended. Contact sales representatives for acquisition.

08-3/Aliquoting DNase I

1. DNase I Working Solution Preparation: For each reaction, mix 47 µl Buffer RDD with 3 µl DNase I by gentle pipetting.

2. Add prepared DNase I Working Solution to Beads wells, dispensing 50 µl per well.

▲ It is recommended to prepare DNase I Working Solution for N + 2 reactions.

▲ DNase I Working Solution needs to be prepared freshly and used immediately. Make sure to add the DNase I Working Solution directly into the liquid; adding it onto the well wall may reduce the efficiency of genomic DNA removal.

08-4/Operation on the automatic instrument

1. Vazyme #VNP-32P: Place the 96-deep-well plate into the Automatic nucleic acids extraction instrument **with the notch toward the inner left front of the instrument**. Install the magnetic rod sleeves and ensure they are properly seated.
   Vazyme #VNP-96P: Place the 96-deep-well plate into the Automatic nucleic acids extraction instrument **with the notch toward the inner right front of the instrument**. Place the magnetic rod sleeves into the Beads Plate (Plate 2) and ensure they are properly seated.
2. Edit the program as below (or select the corresponding pre-imported program) to perform automated extraction.

The Program for Vazyme #VNP-32P (RMA4102)

Step	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	1	Lysis	5	0	0	900	8	58	10	80	0	10
2	2	Beads	0.5	30	0	300	8	-	10	80	0	10
3	1	Lysis	2	60	0	900	8	58	10	80	0	10
4	3	Wash 1	1	30	0	700	8	-	10	80	0	10
5	2	DNase I	10	30	0	300	8	-	10	80	0	10
6	3	Wash 1	1	30	0	700	8	-	10	80	0	10
7	4	Wash 2	1	30	0	700	8	-	10	80	0	10
8	5	Wash 3	1	30	5	700	8	-	10	80	0	10
9	6	Elution	2.5	60	0	70	10	75	10	80	0	10
10	2	Beads	0.1	0	0	300	8	-	10	80	0	10
Other settings (in the Option menu): Temperature control (TC) setting (TC Type: Start with TC); **Adsorption setting (Adsorption type: three-stage adsorption)**; Drying setting (Drying position: Above the kit; Drying fan: OFF)												

The Program for Vazyme #VNP-96P (RMA4103)

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	0.1	0	0	300	8	-	10	80	0	10
2	1	Lysis	5	0	0	900	8	58	10	80	0	10
3	2	Beads	0.5	30	0	300	8	-	10	80	0	10
4	1	Lysis	2	60	0	900	8	58	10	80	0	10
5	3	Wash 1	1	30	0	700	8	-	10	80	0	10
6	2	DNase I	10	30	0	300	8	-	10	80	0	10
7	3	Wash 1	1	30	0	700	8	-	10	80	0	10
8	4	Wash 2	1	30	0	700	8	-	10	80	0	10
9	5	Wash 3	1	30	5	700	8	-	10	80	0	10
10	6	Elution	2.5	60	0	70	10	75	10	80	0	10
11	2	Beads	0.1	0	0	300	8	-	10	80	0	10
Other settings (in the Option menu): Temperature control (TC) setting (TC Type: Start with TC); **Adsorption setting (Adsorption type: three-stage adsorption)**; Drying setting (Drying position: Above the kit; Drying fan: OFF)												

3. After the automated extraction, promptly transfer the eluates from Elution wells (note the valid wells) into clean RNase-free centrifuge tubes.The extracted product can be stored at -85 ~ -65°C for long-term storage, and -30 ~ -15°C for short-term storage.

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