

**VAMNE Magnetic Cell/Tissue
Total RNA Kit**

RMA3301



Instruction for Use

Version 25.1

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

01/Product Description

VAMNE Magnetic Cell/Tissue Total RNA Kit is designed for the extraction of high-purity RNA from cells and animal tissues, achieving high-throughput processing of samples. The kit is based on superparamagnetic particle purification technology with an optimized reagent system, which can effectively remove various impurities and specifically bind nucleic acids. DNase I can efficiently eliminate DNA. The fully automated workflow is simple, rapid, and safe. The obtained RNA can be directly used for downstream molecular biology applications such as RT-PCR, qRT-PCR, Northern blot, Dot blot, *in vitro* translation, and Next-generation sequencing.

02/Components

| | Components | RMA3301-01 (96 rxns) |
|-------|----------------|-------------------------|
| BOX 1 | Binding Buffer | 23 ml |
| | Beads | 24 ml |
| | Wash Buffer 1 | 70 ml |
| | Wash Buffer 2 | 70 ml |
| | Wash Buffer 3 | 70 ml |
| | Elution Buffer | 10 ml |
| | Lysis Buffer | 50 ml |
| | RDD Buffer | 3 × 1.8 ml |
| BOX 2 | Proteinase K | 3 ml |
| | DNase I | 300 µl |
| | Buffer TP | 2 × 1 ml |

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 automated nucleic acids extraction instruments (Vazyme #VNP-32P/VNP-96P) and equivalent instruments.

05/Applications

≤30 mg animal tissue.

≤5 × 10⁶ cultured cells.

06/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 automated 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 absorption 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 only reagents and does not include consumables. Please contact sales representatives to purchase separately.

07/Experiment Process

1. Reagents Preparation

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

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

Match with Vazyme #VNP-32P

| Well Position | Reagent | Volume per Well |
|----------------|----------------|-----------------|
| Columns 1 & 7 | Binding Buffer | 230 μ l |
| Columns 2 & 8 | Beads | 250 μ l |
| Columns 3 & 9 | Wash Buffer 1 | 700 μ l |
| Columns 4 & 10 | Wash Buffer 2 | 700 μ l |
| Columns 5 & 11 | Wash Buffer 3 | 700 μ l |
| Columns 6 & 12 | Elution Buffer | 70 μ l |

Match with Vazyme #VNP-96P

| Plate ID | Plate Position | Reagent | Volume per Well |
|---------------|----------------|----------------|-----------------|
| Binding Plate | 1 | Binding Buffer | 230 μ l |
| Beads Plate | 2 | Beads | 250 μ l |
| Wash Plate 1 | 3 | Wash Buffer 1 | 700 μ l |
| Wash Plate 2 | 4 | Wash Buffer 2 | 700 μ l |
| Wash Plate 3 | 5 | Wash Buffer 3 | 700 μ l |
| Elution Plate | 6 | Elution Buffer | 70 μ l |

2. Sample Pretreatment

Sample Pre-processing Solution preparation (520 μ l): Mix 470 μ l of Lysis Buffer, 30 μ l of Proteinase K, and 20 μ l of Buffer TP thoroughly.

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

a. Animal tissue: Weigh ≤ 30 mg of animal tissue powder pre-ground in liquid nitrogen into a 1.5 ml microcentrifuge tube. Add 520 μ l of Pre-processing Solution, and immediately mix thoroughly by pipetting or vortexing until no obvious clumps remain. Alternatively, weigh ≤ 30 mg of animal tissue, add 520 μ l of Pre-processing Solution, and homogenize immediately until no obvious clumps remain. Transfer all of the liquid to the well/plate containing Binding Buffer.

▲ After processing muscle, skin, bone or tail samples from rats or mice, incubate at room temperature for 5 min. Centrifuge at 12,000 rpm ($13,400 \times g$) for 1 min, and carefully transfer 500 μ l of the supernatant to the well/plate containing Binding Buffer.

▲ For animal tissue with high endogenous RNase content (e.g., spleen, pancreas), the recommended input amount is ≤ 10 mg.

▲ Thymus samples contain high levels of genomic DNA, and the input amount should be ≤ 10 mg.

b. Cell culture: Take $\leq 5 \times 10^6$ cells to a 1.5 ml centrifuge tube, add 520 μ l Pre-processing Solution, immediately pipette or vortex until no cell clumps, transfer all the lysate to the well/plate containing Binding Buffer.

3. Aliquoting DNase I

a. Prepare DNase I Working Solution: Mix 47 μ l of RDD Buffer with 3 μ l of DNase I thoroughly.

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

b. Add prepared DNase I Working Solution to well/plate containing Beads, dispensing 50 μ l into per well.

▲ Make sure DNase I is added to the liquid, adding to the well wall will reduce the efficiency of genome elimination.

4. Operation on the automatic instrument

a. Vazyme #VNP-32P: Place the 96-deep-well plate into the nucleic acid 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 nucleic acid extraction instrument with the notch toward the inner **right** front of the instrument. Place the magnetic rod sleeves into the Beads Plate and ensure they are properly seated.

b. Edit the program as below (or select the corresponding pre-imported program) to perform automated extraction.

Compatible with the Vazyme #VNP-32P (RMA3302) program.

| Step | Well Position | Name | Mixing Time (min) | Absorption Time (sec) | Waiting Time (min) | Volume (μl) | Mixing Speed | Temperature (°C) | Mixing Position | Mixing Amplitude | Absorption Position | Absorption Speed |
|------|---------------|---------|-------------------|-----------------------|--------------------|-------------|--------------|------------------|-----------------|------------------|---------------------|------------------|
| 1 | 1 | Lysis | 2 | 0 | 0 | 750 | 8 | 58 | 10% | 80% | 0 | 10 |
| 2 | 2 | Beads | 0.5 | 30 | 0 | 300 | 8 | - | 10% | 80% | 0 | 10 |
| 3 | 1 | Lysis | 5 | 60 | 0 | 750 | 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 | 80 | 0 | 70 | 8 | 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); Absorption setting (Absorption type: **Reciprocate absorption**);
Drying setting (Drying position: Above the kit; Drying fan: OFF)

Compatible with the Vazyme #VNP-96P (RMA3303) program.

| Step | Plate Position | Name | Mixing Time (min) | Absorption Time (sec) | Waiting Time (min) | Volume (μl) | Mixing Speed | Temperature (°C) | Mixing Position | Mixing Amplitude | Absorption Position | Absorption Speed |
|------|----------------|---------|-------------------|-----------------------|--------------------|-------------|--------------|------------------|-----------------|------------------|---------------------|------------------|
| 1 | 2 | Beads | 0.1 | 0 | 0 | 300 | 8 | - | 10% | 80% | 0 | 10 |
| 2 | 1 | Lysis | 2 | 0 | 0 | 750 | 8 | 58 | 10% | 80% | 0 | 10 |
| 3 | 2 | Beads | 0.5 | 30 | 0 | 300 | 8 | - | 10% | 80% | 0 | 10 |
| 4 | 1 | Lysis | 5 | 60 | 0 | 750 | 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 | 80 | 0 | 70 | 8 | 65 | 10% | 80% | 0 | 10 |
| 11 | 2 | Beads | 0 | 0 | 0 | 300 | 8 | - | 10% | 80% | 0 | 10 |

Other settings (in the Option menu):

Temperature control (TC) setting (TC Type: Start with TC); Absorption setting (Absorption type: **Reciprocate absorption**);
Drying setting (Drying position: Above the kit; Drying fan: OFF)

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



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