

# VAMNE Magnetic Pathogen DNA/RNA Kit (Prepackaged)

RM602

Version 23.1



## Product Description

This kit is intended for isolation and purification of DNA and RNA from biological fluid samples (alveolar lavage fluid, sputum, cerebrospinal fluid, swab eluate, serum, plasma, etc.). It combines chemical and mechanical lysis methods and can efficiently lyse bacterial and fungal cells with thick cell walls. The kit uses high-affinity silicon-based magnetic beads, which adsorb nucleic acids in a high-salt buffer through hydrogen bonds and electrostatic forces. Unwanted proteins and salt ions are then rinsed away. Nucleic acids will be released in a low-salt eluent or Nuclease-free ddH<sub>2</sub>O, enabling fast isolation and purification of nucleic acids. The kit is compatible with an automatic nucleic acid extraction instrument (Vazyme #VNP-32P) that leverages magnetic bead-based adsorption. Specially designed magnetic bars are used to adsorb, transfer, and release magnetic beads, allowing for automatic nucleic acid extraction and purification via the transfer of magnetic beads and nucleic acids. The DNA and RNA isolated with this kit are suitable for various downstream applications, including PCR, real-time PCR, metagenomic library preparation, and DNA/RNA library preparation.

## Components

| Components                       | RM602-01<br>(64 T) |
|----------------------------------|--------------------|
| Reagents (Prepackaged for RM602) | 4 × 16 T           |
| Lysis Buffer 3                   | 12.8 ml            |
| Lysis Tube 2                     | 64                 |
| Reagent DX                       | 500 µl             |
| PBS                              | 25.6 ml            |
| Proteinase K                     | 3 × 1 ml           |

## Storage

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

## Applications

Biological fluid samples: fresh or frozen alveolar lavage fluid, sputum, cerebrospinal fluid, synovial fluid, pleural and peritoneal effusion, the eye-intraocular fluid ( $\leq 1 \times 10^7$  cells).

Swab samples: fresh throat, nasal, and oral swab eluates ( $\leq 1 \times 10^7$  cells).

Serum/Plasma samples: fresh or frozen serum and plasma ( $\leq 400$  µl).

## Applicable Instruments

Vazyme #VNP-32P and other similar instruments (heating in Columns 1, 6, 7, 12).

## Self-prepared Materials

High speed centrifuge, vortex mixer, or homogenizer.

## Notes

For research use only. Not for use in diagnostic procedures.

1. Pathogen samples should be processed in a biosafety cabinet.
2. Disinfect the automatic nucleic acid extraction instrument with UV irradiation for at least 30 min before use (for nucleic acid extraction from pathogens, UV irradiation time may be extended to 12 h). After the experiment, wipe the inside of the extraction instrument with 75% ethanol and disinfect it with UV irradiation for 30 min.
3. Before your first use, add 30 µl of Reagent DX to Lysis Buffer 3.

## Experiment Process

### 1. Preparation of Prepackaged Reagent

Take out the prepackaged reagent from the kit, invert it several times to resuspend the magnetic beads, and gently shake the plate so that both the reagent and the magnetic beads are concentrated at the well bottom. Confirm the plate orientation and carefully tear off the sealing aluminum foil before use.

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



## 2. Nucleic Acid Extraction

### 2.1. Pathogen nucleic acid extraction

#### Sample pretreatment:

- Sputum samples: Add 5 volumes of N-acetylcysteine solution (10 g/L) (not provided) to an appropriate amount of sputum and vortex for 30 - 60 min to liquefy. Centrifuge at 7,500 rpm (5,400 × g) for 10 min and discard the supernatant. Resuspend the precipitate in 200 µl of PBS and transfer it to a Lysis Tube 2.
  - ▲ Add to 200 µl with PBS if the sample volume is less than 200 µl.
  - ▲ If the sample volume is more than 200 µl, transfer the sample to a 1.5 ml Nuclease-free centrifuge tube. Centrifuge at 12,000 rpm (13,800 × g) for 5 min and discard the supernatant. Resuspend the precipitate in 200 µl of PBS and transfer it to a Lysis Tube 2.
- Swab and other biological fluid samples: Add 200 µl of sample to a Lysis Tube 2.
  - ▲ Add to 200 µl with PBS if the sample volume is less than 200 µl.
  - ▲ If the sample volume is more than 200 µl, transfer the sample to a 1.5 ml Nuclease-free centrifuge tube. Centrifuge at 12,000 rpm (13,800 × g) for 5 min and discard the supernatant. Resuspend the precipitate in 200 µl of PBS and transfer it to a Lysis Tube 2.

#### Sample extraction:

Add 40 µl of Proteinase K and then 200 µl of Lysis Buffer 3 to the above Lysis Tube 2 containing the sample. Vortex the Lysis Tube 2 on a vortex mixer at maximum speed for 10 min. After vortexing, centrifuge at 12,000 rpm (13,800 × g) for 3 min. Transfer 330 µl of the supernatant to the wells in Columns 1 and 7 of a 96 deep well plate, and proceed to Step 3.

- ▲ If there is still foam after centrifugation at 12,000 rpm (13,800 × g) for 3 min, increase the centrifugation time to remove the foam.

### 2.2. Cell-free nucleic acid extraction from plasma/serum samples

Add 400 µl of the serum/plasma sample and then 40 µl of Proteinase K to the wells in Columns 1 and 7 of a 96 deep well plate, and proceed to Step 3.

- ▲ Add to 400 µl with PBS if the sample volume is less than 400 µl.

## 3. Steps for Automated Extraction

3.1 Place the 96 deep well plate into the nucleic acid extraction instrument in the correct orientation (with the notch facing the upper left). Load the magnetic bar sleeves, and ensure it fully envelops the magnetic bars.

3.2 Set the program as follows (or select the corresponding preset) for automated extraction.

3.2.1 RM602 program (suitable for nucleic acid extraction from all samples types):

| Step   | Slot Position | Name                  | Mixing Time (min) | Adsorption Time (s) | Wait Time (min) | Volume (µl) | Mixing Speed | Temperature (°C) | Mixing Position | Mixing Amplitude | Adsorption Speed | Adsorption Position |
|--|---------------|-----------------------|-------------------|---------------------|-----------------|-------------|--------------|------------------|-----------------|------------------|------------------|---------------------|
| 1  | 2             | Moving magnetic beads | 1                 | 60                  | 0               | 700         | 8            | -                | 10%             | 80%              | 0%               | 5                   |
| 2  | 1             | Lysis                 | 10                | 60                  | 0               | 990         | 10           | 70               | 5%              | 100%             | 0%               | 5                   |
| 3  | 3             | Rinse 1               | 3                 | 60                  | 0               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 4  | 4             | Rinse 2               | 1                 | 60                  | 0               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 5  | 5             | Rinse 2               | 1                 | 60                  | 1               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 6  | 6             | Elution               | 10                | 90                  | 0               | 80          | 10           | 42               | 10%             | 100%             | 0%               | 10                  |
| 7  | 2             | Moving magnetic beads | 0.1               | 0                   | 0               | 700         | 8            | -                | 10%             | 80%              | 0%               | 5                   |
| Other settings (in the Option menu): heating settings (heating and action start at the same time);<br>adsorption settings (three-stage adsorption) |               |                       |                   |                     |                 |             |              |                  |                 |                  |                  |                     |

3.2.2 RM602 Fast mode (only suitable for cell-free nucleic acid rapid extraction from plasma/serum samples):

| Step   | Slot Position | Name                  | Mixing Time (min) | Adsorption Time (s) | Wait Time (min) | Volume (µl) | Mixing Speed | Temperature (°C) | Mixing Position | Mixing Amplitude | Adsorption Speed | Adsorption Position |
|--|---------------|-----------------------|-------------------|---------------------|-----------------|-------------|--------------|------------------|-----------------|------------------|------------------|---------------------|
| 1  | 2             | Moving magnetic beads | 1                 | 60                  | 0               | 700         | 8            | -                | 10%             | 80%              | 0%               | 5                   |
| 2  | 1             | Lysis                 | 5                 | 60                  | 0               | 990         | 10           | 37               | 5%              | 100%             | 0%               | 5                   |
| 3  | 3             | Rinse 1               | 3                 | 60                  | 0               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 4  | 4             | Rinse 2               | 1                 | 60                  | 0               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 5  | 5             | Rinse 2               | 1                 | 60                  | 1               | 700         | 10           | -                | 10%             | 100%             | 0%               | 10                  |
| 6  | 6             | Elution               | 10                | 90                  | 0               | 80          | 10           | 42               | 10%             | 100%             | 0%               | 10                  |
| 7  | 2             | Moving magnetic beads | 0.1               | 0                   | 0               | 700         | 8            | -                | 10%             | 80%              | 0%               | 5                   |
| Other settings (in the Option menu): heating settings (heating and action start at the same time);<br>adsorption settings (three-stage adsorption) |               |                       |                   |                     |                 |             |              |                  |                 |                  |                  |                     |

3.3 At the end of the automated procedure, transfer the eluent in Columns 6 and 12 to clean Nuclease-free centrifuge tubes for use directly in downstream experiments or storage at -20°C.

