VAMNE Magnetic Pathogen DNA/RNA Kit

RM601

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

This kit is intended for isolation and purification of DNA and RNA from biological fluid samples (alveolar lavage fluid, blood, sputum, cerebrospinal fluid, swab eluate, etc.) and microbial cultures. 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_2O, enabling fast isolation and purification of nucleic acids. The extraction process with this kit is safe, fast, and simple as toxic reagents such as phenol and chloroform are not required. While ensuring efficiency in nucleic acid extraction, the kit also minimizes proteins, inorganic salts, and other impurities. The isolated DNA and RNA are suitable for various downstream applications, including PCR, real-time PCR, metagenomic library preparation, and DNA/RNA library preparation.

02/Components

Components	RM601-01 (50 rxns)
Lysis Buffer 2	25 ml
Binding Buffer 2	10 ml
Proteinase K	2 × 1 ml
Magnetic Beads	1 ml
Wash Buffer A	51 ml
Wash Buffer B	24 ml
Nuclease-free ddH_2O	5 × 1 ml
PBS	20 ml
Reagent DX	500 µl
Lysis Tube 2	50
2 ml Nuclease-free Tube	50
1.5 ml Nuclease-free Tube	2 × 50

03/Storage

Store Proteinase K at 2 ~ 8°C and adjust the shipping method according to the destination. Store the other components at 15 ~ 25°C and transport at room temperature.

04/Applications

Biological fluid samples

Blood samples: fresh or frozen anticoagulated whole blood (≤400 µl).

Other biological fluid samples: 0.4 - 3 ml of fresh or frozen alveolar lavage fluid, sputum, cerebrospinal fluid, synovial fluid, pleural and peritoneal effusion (≤5 × 10^6 cells).

Swab samples: 0.4 - 3 ml of eluate from nasal, pharyngeal, oral swabs, etc. (≤5 × 10^6 cells).

Microbial cultures

Bacteria (≤1 × 10^9 cells) and fungi (≤1 × 10^9 cells).

05/Self-prepared Materials

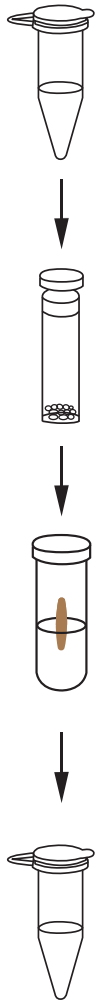
Absolute ethanol, isopropanol, nuclease-free pipette tips, high speed centrifuge, thermostatic water bath, magnetic rack, vortex mixer or homogenizer.

06/Notes

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

1. When using the kit, wear a lab coat, disposable latex gloves, and a disposable mask.
2. Pathogen samples should be processed in a biosafety cabinet.
3. Use Nuclease-free pipette tips and Nuclease-free centrifuge tubes to avoid contamination by exogenous microorganisms and nucleic acids.
4. Before your first use, add 60 μl of Reagent DX to Lysis Buffer 2, 69 ml of absolute ethanol to Wash Buffer A, and 96 ml of absolute ethanol to Wash Buffer B according to the labels, and mark the bottle bodies and caps.
5. Check for precipitates in Lysis Buffer 2 and Binding Buffer 2 before use. Redissolve any precipitate in a 37°C water bath. Mix well before use.
6. Do not frozen Magnetic Beads.

07/Mechanism & Workflow



Sample pretreatment:

1. Sample type

◇ Biological fluid samples

Input 400 µl of the biological fluid sample (anticoagulated whole blood, sputum, swab eluate, etc.).

◇ Microbial cultures

2. Refer to **08-1/Sample Pretreatment** for the specific pretreatment methods for different types of samples.

Sample lysis:

◇ Biological fluid samples

Add 40 µl of Proteinase K, **200 µl of Lysis Buffer 2**, then 200 µl of Binding Buffer 2.

◇ Microbial cultures

Add 40 µl of Proteinase K, **500 µl of Lysis Buffer 2**, then 200 µl of Binding Buffer 2.

Nucleic acid adsorption:

◇ Biological fluid samples

Add **350 µl of isopropanol** and 20 µl of Magnetic Beads.

◇ Microbial cultures

Add **250 µl of isopropanol** and 20 µl of Magnetic Beads.

Removal of proteins: Rinse with Wash Buffer A twice.

Removal of salt ions: Rinse with Wash Buffer B twice.

Removal of ethanol: Discard all supernatant and leave for 1 min uncapped.

Elution of nucleic acids:

Add 100 µl of Nuclease-free ddH_2O to elute the nucleic acids.

08/Experiment Process

1. Before your first use, add 60 µl of Reagent DX to Lysis Buffer 2, 69 ml of absolute ethanol to Wash Buffer A, and 96 ml of absolute ethanol to Wash Buffer B according to the labels, and mark the bottle bodies and caps.
2. Check for precipitates in Lysis Buffer 2 and Binding Buffer 2 before use. Redissolve any precipitate in a 37°C water bath. Mix well before use.

08-1/Sample Pretreatment

1. Biological fluid samples

◇ **Anticoagulated whole blood samples**

Add 400 µl of the anticoagulated whole blood sample to a Lysis Tube 2.

If the sample volume is less than 400 µl, transfer the anticoagulated whole blood sample to a Lysis Tube 2, make up the volume to 400 µl with PBS, then proceed to 08-2/DNA/RNA Co-extraction from Biological Fluid Samples.

◇ **Sputum samples**

Add an appropriate amount of liquefied sputum sample (Refer to 09/FAQ & Troubleshooting for the liquefaction methods) to a 1.5 ml Nuclease-free Tube. Centrifuge at 12,000 rpm (13,800 × g) for 10 min and discard the supernatant. Resuspend the precipitate in 400 µl of PBS and transfer it to a Lysis Tube 2. Then proceed to 08-2/DNA/RNA Co-extraction from Biological Fluid Samples.

◇ **Swab eluate and other biological fluid samples:**

Add 400 µl of swab eluate or other biological fluid to a Lysis Tube 2.

If the sample volume is more than 400 µl, transfer the sample to a 1.5 ml Nuclease-free Tube. Centrifuge at 12,000 rpm (13,800 × g) for 5 min and discard the supernatant. Resuspend the precipitate in 400 µl of PBS and transfer it to a Lysis Tube 2. Then proceed to 08-2/DNA/RNA Co-extraction from Biological Fluid Samples.

If the sample volume is less than 400 µl, transfer the sample to a Lysis Tube 2, make up the volume to 400 µl with PBS, and then proceed to 08-2/DNA/RNA Co-extraction from Biological Fluid Samples.

2. Microbial cultures

Add a microbial culture sample to a 1.5 ml Nuclease-free Tube. Centrifuge at 13,000 rpm (16,200 × g) for 5 min and discard the supernatant. Resuspend the precipitate in 100 µl of PBS and transfer it to a Lysis Tube 2. Then, proceed to 08-3/DNA/RNA Co-extraction from Microbial Cultures.

08-2/DNA/RNA Co-extraction from Biological Fluid Samples

1. Add 40 µl of Proteinase K, 200 µl of Lysis Buffer 2, then 200 µl of Binding Buffer 2 to the Lysis Tube 2, and vortex to mix well.

   ▲ Lysis Buffer 2 and Proteinase K can be mixed in advance according to the sample number. Binding Buffer 2 needs to be added separately and last.

2. Lyse the cells using one of the following recommended methods:

   a. Vortex the Lysis Tube 2 on a vortex mixer at maximum speed for 10 min.

   b. Place the Lysis Tube 2 in a homogenizer and disrupt cells by running an appropriate program. For example, use FastPrep-24 from MP Biomedicals with the following settings: 6.5 m/sec, on 90 sec, off 3 min, 2 cycles.

3. Incubate the Lysis Tube 2 in a 56°C water bath for 10 min, centrifuge at 12,000 rpm (13,800 × g) for 3 min to remove the foam, and then transfer 600 µl of the supernatant into a new 2 ml Nuclease-free Tube.
   ▲ Do not pipette glass beads when transferring the supernatant. You can slightly tilt the Lysis Tube 2, place the pipette tip closely against the tube wall, and slowly move it down with the falling liquid surface to collect the supernatant.
   ▲ The solution may become turbid when transferring the supernatant. Transfer the supernatant cautiously.
4. Add 350 µl of isopropanol to the centrifuge tube of Step 3, vortex to mix well, and collect the liquid on the tube cap by instantaneous centrifugation for 1 - 3 sec.
5. Add 20 µl of Magnetic Beads to the centrifuge tube of Step 4. Vortex the tube to mix well in a vortex mixer at 1,500 rpm for 3 min and let it stand for 1 min. Then instantaneously centrifuge the tube for 1 - 3 sec, and leave it on a magnetic rack for 1 min.
   ▲ Mix the magnetic beads well before use.
   ▲ Do not centrifuge for too long. Otherwise, Magnetic Beads will adhere to the tube wall.
6. Discard the supernatant and take the centrifuge tube off the magnetic rack. Add 900 µl of Wash Buffer A (check whether absolute ethanol has been added before use) along the tube wall. Vortex to mix well for 1 min and leave to stand for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 1 min.
7. Repeat Step 6 once.
8. Discard the supernatant and take the centrifuge tube off the magnetic rack. Add 900 µl of Wash Buffer B (check whether absolute ethanol has been added before use) along the tube wall. Vortex to mix well for 1 min and leave stand for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 1 min.
9. Repeat Step 8 once.
10. Discard the supernatant as completely as possible, and air dry the tube content for 1 min. Add 100 µl of Nuclease-free ddH_2O, and vortex to mix well for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 3 min.
    ▲ After discarding all the supernatant, immediately add 100 µl of Nuclease-free ddH_2O for product elution. Do not air dry for too long, or the elution efficiency will decrease.
11. Pipette 85 µl of the eluate to a new 1.5 ml Nuclease-free Tube for direct use in downstream experiments or storage at -80°C for a long time.

08-3/DNA/RNA Co-extraction from Microbial cultures

1. Add 40 µl of Proteinase K, 500 µl of Lysis Buffer 2, then 200 µl of Binding Buffer 2 to the Lysis Tube 2, and vortex to mix well.
   ▲ Lysis Buffer 2 and Proteinase K can be mixed in advance according to the sample number. Binding Buffer 2 needs to be added separately and last.

2. Lyse the cells using one of the following recommended methods:
   a. Vortex the Lysis Tube 2 on a vortex mixer at maximum speed for 10 min.
   b. Place the Lysis Tube 2 in a homogenizer and disrupt cells by running an appropriate program. For example, use FastPrep-24 from MP Biomedicals with the following settings: 6.5 m/sec, on 90 sec, off 3 min, 2 cycles.
3. Incubate the Lysis Tube 2 in a 56°C water bath for 10 min, centrifuge at 12,000 rpm (13,800 × g) for 3 min to remove the foam, and then transfer 600 µl of the supernatant into a new 2 ml Nuclease-free Tube.
   ▲ Do not pipette glass beads when transferring the supernatant. You can slightly tilt the Lysis Tube 2, place the pipette tip closely against the tube wall, and slowly move it down with the falling liquid surface to collect the supernatant.
   ▲ The solution may become turbid when transferring the supernatant. Transfer the supernatant cautiously.
4. Add 250 µl of isopropanol to the centrifuge tube of Step 3, vortex to mix well, and collect the liquid on the tube cap by instantaneous centrifugation for 1 - 3 sec.
5. Add 20 µl of Magnetic Beads to the centrifuge tube of Step 4. Vortex the tube to mix well in a vortex mixer at 1,500 rpm for 3 min and let it stand for 1 min. Then instantaneously centrifuge the tube for 1 - 3 sec, and leave it on a magnetic rack for 1 min.
   ▲ Mix the magnetic beads well before use.
   ▲ Do not centrifuge for too long. Otherwise, Magnetic Beads will adhere to the tube wall.
6. Discard the supernatant and take the centrifuge tube off the magnetic rack. Add 900 µl of Wash Buffer A (check whether absolute ethanol has been added before use) along the tube wall. Vortex to mix well for 1 min and leave to stand for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 1 min.
7. Repeat Step 6 once.
8. Discard the supernatant and take the centrifuge tube off the magnetic rack. Add 900 µl of Wash Buffer B (check whether absolute ethanol has been added before use) along the tube wall. Vortex to mix well for 1 min and leave stand for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 1 min.
9. Repeat Step 8 once.
10. Discard the supernatant as completely as possible, and air dry the tube content for 1 min. Add 100 µl of Nuclease-free ddH_2O, and vortex to mix well for 1 min. Centrifuge instantaneously for 1 - 3 sec and leave the tube on the magnetic rack for 3 min.
    ▲ After discarding all the supernatant, immediately add 100 µl of Nuclease-free ddH_2O for product elution. Do not air dry for too long, or the elution efficiency will decrease.
11. Pipette 85 µl of the eluate to a new 1.5 ml Nuclease-free Tube for direct use in downstream experiments or storage at -80°C for a long time.

09/FAQ & Troubleshooting

Question	Reasons	Solutions
Inaccurate loading of sputum samples	Viscous sputum samples are difficult to be loaded accurately	1. If the sample is white foamy thin sputum, take an appropriate volume of it, add five volumes of N-acetylcysteine solution (10 g/L), shake to mix well and liquefy for 30 - 60 min before extraction. 2. If the sample is white or yellow-white viscous sputum, yellow sputum with blood streaks, or bloody sputum, take an appropriate volume of it, add 10 volumes of N-acetylcysteine solution (10 g/L), and shake to liquefy for 30 - 60 min before extraction.
Low detection rate in viral samples	Loss of lysed viral material during supernatant transfer	If virus detection is your main concern, skip the lysis step in Lysis Tube 2 and operate as follows: 1.Add 400 µl of the sample, 40 µl of Proteinase K, 200 µl of Lysis Buffer 2, then 200 µl of Binding Buffer 2 to a 2 ml Nuclease-free Tube, and vortex to mix well. 2. Lyse the virus using one of the methods described in 08-2/ DNA/RNA Co-extraction from Biological Fluid Samples/Step 2. 3. Incubate the 2 ml Nuclease-free Tube in a 56°C water bath for 10 min, centrifuge instantaneously, and add 490 µl of isopropanol to the centrifuge tube. 4. Perform Steps 5 - 11 in 08-2/DNA/RNA Co-extraction from Biological Fluid Samples.
Low nucleic acid yield	Incomplete cell disruption	Extend the mechanical lysis time appropriately for complete cell disruption.
	Low elution efficiency	Pre-heat Nuclease-free ddH_2O to 56°C before elution.
	Wash Buffer A/Wash Buffer B not supplemented with absolute ethanol	1. Add 1.35 volumes of absolute ethanol to the remaining Wash Buffer A. 2. Add 4 volumes of absolute ethanol to the remaining Wash Buffer B.
Low nucleic acid purity	Protein contamination	Perform an additional rinse with Wash Buffer A.
	Ion contamination	Perform an additional rinse with Wash Buffer B.

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