Magnetic Beads Virus DNA/RNA Extraction Kit II

For research use only

Sample : up to 200 µl plasma, serum, body fluid, and supernatant of viral infected cell cultures nasopharyngeal and oropharyngeal swabs
Format : magnetic beads
Sensitivity : as low as 10E1 copy number of virus
Operation method : magnetic bead separation instruments/ manual
Operation time : 60 minutes
Elution volume : 30 µl – 100 µl

CERTIFICATE NO. QAIC/TW/50077

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Introduction

The Magnetic Beads Virus DNA/RNA Extraction Kit II was designed for high-throughput purification of high-quality of viral DNA and viral RNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. Viral DNA/RNA is bound to the surface of the magnetic beads and released using a proprietary buffer system. The Magnetic Beads Viral DNA/RNA Kit can be easily adapted to automated magnetic bead separation instruments and workstations. The purified viral DNA/RNA can be used directly in qPCR and qRT-PCR assays.

Quality Control

The quality of Magnetic Beads Virus DNA/RNA Extraction Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system by isolating viral DNA/RNA from a 200 µl plasma sample.

Kit Contents

Component	MV004	MV048	MV096	MV480
MV1 Buffer	2 ml	30 ml	60 ml	130 ml x1 80 ml x1
W1 Buffer*	2 ml	50 ml	80 ml	130 ml x2 80 ml x1
Wash Buffer*[1] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml x2 (200 ml) 25 ml x1 (100 ml)
RNase-free Water	2 ml	15 ml	15 ml	60 ml
MV Magnetic Beads	50 µl	500 µl	1 ml	5 ml
Carrier RNA[2] (Add RNase-free water)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)	1 mg (1 ml)
96 Deep Well Plate	-	1 pc	1 pc	5 pcs
Adhesive Film	-	1 pc	1 pc	5 pcs

[1] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]Carrier RNA is shipped at room temperature and should be stored at -20ºC once received the kit for extended periods. Add 1 ml of RNase-free Water to Carrier RNA then vortex to ensure Carrier RNA is completely dissolved to obtain a working solution of 1 µg/µl. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes. The Carrier RNA and RNase-free Water solution should be stored at -20ºC. Do not freeze and thaw Carrier RNA solution more than 3 times.

Caution

MV1 Buffer contain chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free).

Additional Requirements:

For manual procedure: Orbital shaker for 96 well plate (ex. Eppendorf MixMate), magnetic separator for 96 well plate, absolute ethanol, isopropanol.

For automatic procedure: MagMAX™ Express-96 Deep Well Magnetic Particle Processor, additional 96 deep well plates, absolute ethanol, isopropanol.

Important before use

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle.
2. Add 1 ml of RNase-free Water to Carrier RNA then vortex to ensure Carrier RNA is completely dissolved to obtain a working solution of 1 µg/µl. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes and store at -20ºC. Do not freeze and thaw Carrier RNA solution more than 3 times.
3. Vortex MV magnetic beads to ensure they are in suspension prior to initial use.
4. Determine the maximum plate shaker setting: Add 1 ml of water into each well of a 96 Deep Well Plate, determine the maximum shaking speed with your orbital shaker without spilling sample. Use this speed for all of the shaking incubations in the protocol.

Magnetic Beads Virus DNA/RNA Extraction Kit Manual Protocol

Step 1 Sample preparation	**For cell-free samples (serum, plasma, body fluids)** • For 96 samples: add **40 ml of MV1 Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 10 seconds. • Add **400 µl of MV1 Buffer containing Carrier RNA** into each well of a 96 Deep Well Plate using a multichannel pipette. • Transfer **200 µl sample** into each well of the 96 Deep Well Plate. Note: If the prepared sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. Careful adding sample into each well to prevent cross contamination is obligatory. • Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes. **For nasopharyngeal and oropharyngeal swabs preserving in the transport medium** • For 96 samples: add **40 ml of MV1 Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 10 seconds. • Add **400 µl of MV1 Buffer containing Carrier RNA** into each well of a 96 Deep Well Plate using a multichannel pipette. • Vortex the preservation tubes containing swabs for 1 minute. • Transfer **200 µl of medium such as VTM, UTM and PBS** into each well of the 96 Deep Well Plate. • Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes. **For samples preserving in Geneaid SYNCstore™ STM** • Add **1 µl of Carrier RNA** into each tube of SYNCstore™ STM and vortex briefly. • Transfer **600 µl of medium** into each well of a clean 96 Deep Well Plate. Note: Careful adding sample into each well to prevent cross contamination is obligatory.
Step 2 Viral Nucleic Acid Binding	• For 96 samples: add **40 ml of isopropanol** and **1 ml of MV Magnetic Beads (vortex magnetic beads to ensure they are in suspension)** into a clean 50 ml tube, mix by vortex for 30 seconds. • Add **400 µl of isopropanol containing MV Magnetic Beads** into each well of the 96 Deep Well Plate using a multichannel pipette. • Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes. • Transfer the 96 Deep Well Plate to a magnetic separator to capture the **MV Magnetic Beads**. Leave the plate on the magnetic separator for at least 3 minutes. • Carefully aspirate and discard the supernatant without disturbing the beads using a multichannel pipette. • Remove the 96 Deep Well Plate from the magnetic separator.

Step 3 Wash	• Add **400 µl of W1 Buffer** into each well of the 96 Deep Well Plate using a multichannel pipette and shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes. • Transfer the 96 Deep Well Plate to a magnetic separator to capture the **MV Magnetic Beads**. Leave the plate on the magnetic separator for at least 1 minute. • Carefully aspirate and discard the supernatant without disturbing the beads using a multichannel pipette and remove the 96 Deep Well Plate from the magnetic separator. • Add **600 µl of Wash Buffer (make sure ethanol was added)** into each well of the 96 Deep Well Plate using a multichannel pipette and shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes. • Transfer the 96 Deep Well Plate to a magnetic separator to capture the **MV Magnetic Beads**. Leave the plate on the magnetic separator for at least 1 minute. • Carefully aspirate and discard the supernatant without disturbing the beads using a multichannel pipette and remove the 96 Deep Well Plate from the magnetic separator. • Repeat to wash the **MV Magnetic Beads** with **600 µl of Wash Buffer.** Shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes. • Transfer the 96 Deep Well Plate to a magnetic separator for 1 minute to capture the **MV Magnetic Beads**. • Carefully aspirate and discard the supernatant using a multichannel pipette without disturbing the beads. • Shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes to dry the **MV Magnetic Beads**. Note: DO NOT over dry the beads. Over dry the beads could result in low DNA/RNA yield.
Step 4 Elution	• Add **30 µl – 100 µl of RNase-free water** into each well of the 96 Deep Well Plate using a multichannel pipette and shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes. • Transfer the 96 Deep Well Plate to a magnetic separator to capture the **MV Magnetic Beads**. Leave the plate on the magnetic separator for at least 1 minute. • Transfer the supernatant containing the purified Viral DNA/RNA into each well of a RNase-free 0.35 ml 96 well plate (not provided), seal the Plate with an **Adhesive Film** and store at -70 ºC.

Magnetic Beads Virus DNA/RNA Extraction Kit Automatic Protocol

For using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor

Step 1 Buffer Preparation	• Add the reagents to the appropriate plates using a multichannel pipette. 1. Add **300 µl of W1 Buffer** per well into two Deep Well Plates. 2. Add **450 µl of Wash Buffer** per well into two Deep Well Plates. 3. Add **90 µl of RNase-free Buffer** per well into one Standard Plate.

Step 2
Sample Preparation

For cell-free samples (serum, plasma, body fluids)

- Add **40 ml of MV1 Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 30 seconds.
- Add **400 µl of MV1 Buffer containing Carrier RNA** into each well of a 96 Deep Well Plate using a multichannel pipette.
- Transfer **200 µl sample** into each well of the 96 Deep Well Plate.

Note: Careful adding sample into each well to prevent cross contamination is obligatory.

- Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes.
- During incubation, add **35 ml of isopropanol** and **1 ml of MV Magnetic Beads (vortex magnetic beads to ensure they are in suspension)** into a clean 50 ml tube, mix by vortex for 30 seconds.
- Add **350 µl of isopropanol containing MV Magnetic Beads** into each well of the 96 Deep Well Plate using a multichannel pipette.

For nasopharyngeal and oropharyngeal swabs preserving in the transport medium

- Add **40 ml of MV1 Buffer** and **100 µl of Carrier RNA** into a clean 50 ml tube, mix by vortex for 30 seconds.
- Add **400 µl of MV1 Buffer containing Carrier RNA** into each well of a 96 Deep Well Plate using a multichannel pipette.
- Vortex the preservation tubes containing swabs for 1 minute.
- Transfer **200 µl of medium such as VTM, UTM and PBS** into each well of the 96 Deep Well Plate.

Note: Careful adding sample into each well to prevent cross contamination is obligatory.

- Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes.
- During incubation, add **35 ml of isopropanol** and **1 ml of MV Magnetic Beads (vortex magnetic beads to ensure they are in suspension)** into a clean 50 ml tube, mix by vortex for 30 seconds.
- Add **350 µl of isopropanol containing MV Magnetic Beads** into each well of the 96 Deep Well Plate using a multichannel pipette.

For samples preserving in Geneaid SYNCstore™ STM

- Add **1 µl of Carrier RNA** into each tube of SYNCstore™ STM and vortex briefly.
- Transfer **600 µl of medium** into each well of a clean 96 Deep Well Plate.
- Add **35 ml of isopropanol** and **1 ml of MV Magnetic Beads (vortex magnetic beads to ensure they are in suspension)** into a clean 50 ml tube, mix by vortex for 30 seconds.
- Add **350 µl of isopropanol containing MV Magnetic Beads** into each well of the 96 Deep Well Plate using a multichannel pipette.

Step 3
Instrument setup

- Load all plates onto the instrument following the table below:

<table>
<tr><th colspan="2">Plate position</th><th colspan="2">reagent</th><th>Plate type</th><th>Volume</th></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Sample plate</td><td>• MV1 Buffer
• Sample</td><td>• SYNCstore™ STM Medium</td><td rowspan="2">Deep well plate</td><td rowspan="2">950 µl</td></tr>
<tr><td colspan="2">Isopropanol
MV Magnetic beads</td></tr>
<tr><td>2</td><td>1st Wash plate</td><td colspan="2">W1 Buffer</td><td>Deep well plate</td><td>300 µl</td></tr>
<tr><td>3</td><td>2nd Wash plate</td><td colspan="2">W1 Buffer</td><td>Deep well plate</td><td>300 µl</td></tr>
<tr><td>4</td><td>3rd Wash plate</td><td colspan="2">Wash Buffer</td><td>Deep well plate</td><td>450 µl</td></tr>
<tr><td>5</td><td>4th Wash plate</td><td colspan="2">Wash Buffer</td><td>Deep well plate</td><td>450 µl</td></tr>
<tr><td>6</td><td>Elution plate</td><td colspan="2">RNase-free water</td><td>Standard plate</td><td>90 µl</td></tr>
<tr><td>7</td><td>Tip comb plate</td><td colspan="4">Tip Comb</td></tr>
</table>

- Select the 4462359_DW_HV protocol on the instrument and start to run the protocol.
- After program finish, seal the Elution plate with an **Adhesive Film** and store at -70 ºC.