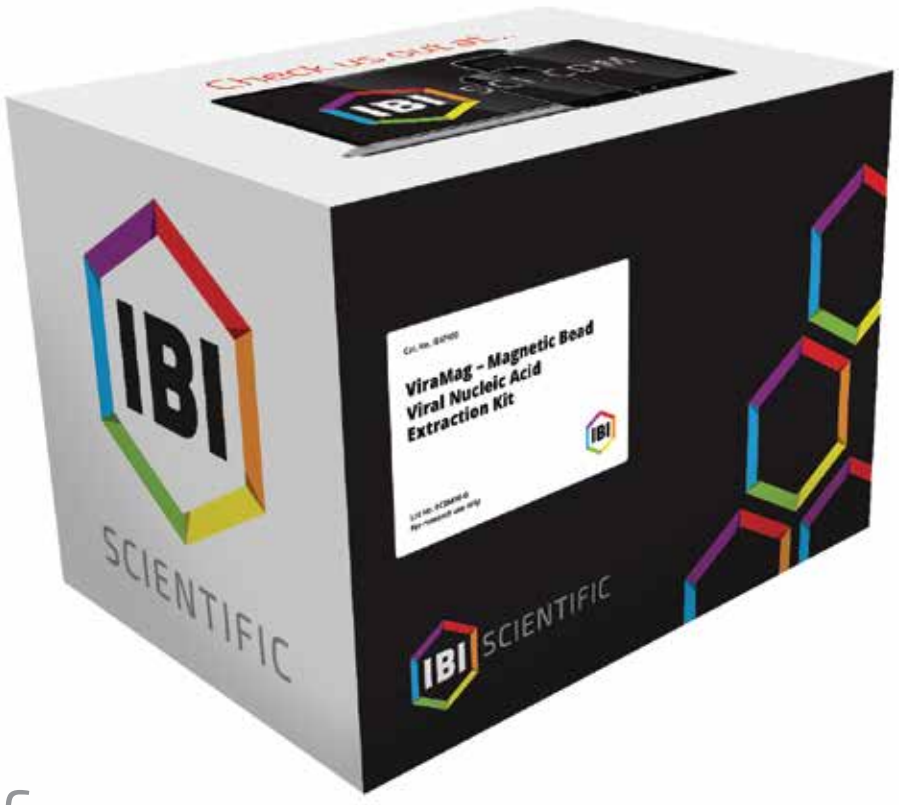
For Research Use Only



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INSTRUCTION MANUAL

ViraMag - Magnetic Bead Virus DNA/RNA Extraction Kit

IB47460
IB47461
IB47462
IB47463

Sample: up to 200 µl plasma, serum, body fluid, and supernatant of viral infected cell cultures

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

Introduction

IBI ViraMag Virus DNA/RNA Extraction Kit 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 lound to the surface of the magnetic beads and released using a proprietary buffer system. The ViraMag 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 DNARNA Extraction Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system by isolating viral DNA/RNAfrom a 200 pl serum sample.

Kit Contents

Component	IB47460	IB47461	IB47462	IB47463
MV1 Buffer	2 ml	30 ml	60 ml	130 ml x2
W1 Buffer*	2ml	30 ml	50 ml	130 mlx2
Wash Buffer*[1] (Add Ethanol)	1ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml x3 (200 ml)
RNase-free Water	2ml	15 ml	15 ml	60 ml
MV Magnetic Beads	50µl	500µl	1ml	5ml
Carrier RNA[2] (Add RNase-free water)	1mg (1ml)	1mg (1 ml)	1mg (1 ml)	1mg (1 ml)
96 Deep Well Plate	-	1pc	1pc	5 pcs
Adhesive Film	-	1pc	1pc	5 pcs

[1] Add absolute ethanol (see the bottle label forvolume) 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] 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 -20°C. 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, magnetic separator for 96 well plate, absolute ethanol, isopropanol. For automatic, procedure MdgMAX™ Express-96 Deep Well Magnetic Particle Processor, additional 96 deep well plates, absolute ethanol, isopropanol.

IMPORTANT BEFORE USE!

- 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.
- 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 1µg/µl. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes and store at -20°C. Do not 'treeze and thaw Carrier RNA solution more than 3 times.

• Vortex MV magnetic beads to ensure they are in suspension prior to initial use.

• Determine the maximum plate shaker setting: Add 1.1 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.

ViraMag Virus DNA/RNA Extraction Kit Manual Protocol

Step 1 Sample Prep.	**For cell-free samples (serum, plasma, body fluids)** • For 96 samples: add 40 ml of MVI Buffer and 100 pl 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. • 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 wellto prevent cross contamrnation is obligatory. • Shake the Deep Well Plate on an orbital shaker at the maximal speed for 10 minutes.
Step 2 Viral Nucleic Acid Binding	• For 96 samples: add 45 ml of isopropanol and 1 ml of MV Magnetic Beads (vortex magbeads to ensure they are in suspension) into a clean 50 ml tube, mix by vortex for 30 seconds. •Add 450 µl of isopropanol containing MV Magnetic Beads into each well of the 96 Deep Well Plate. Note: Careful adding isopropanol 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. • 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 beats. • Remove the 96 Deep Well Plate from the magnetic separator.
Step 3 Wash	• Add 400 µl of Wl Buffer into each well of the 96 Deep Well Plate 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 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 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 and remove the 96 Deep Well Plate from the magnetic separator. • Repeat to wash the MV Magnetic Beads with 600µl 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 th MV Magnetic Beads. • Carefully aspirate and discard the supernatant 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 beads. Over dry beads could result in low DNA/RNA yield.
Step 3 Wash	Add 30µl -100µl of Rnase-free water into each well of the 96 Deep Well Plate and shake the Deep Well Plate on an orbital shaker at the maximal speed for 3 minutes. Transfer the 96 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 purfied 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°C.

Magnetic Beads Virus DNA/RNA Extraction Kit
Automatic Protocol

<table>
<tr><td>Step 1
Buffer
Preparation</td><td>• Add the reagents to the appropriate plates.
1. Add 300 µl of W1 Buffer per well into two standard well plates.
2. Add 450 µl of Wash Buffer per well into two standard well plates.
3. Add 90 µl of RNase-free Buffer per well into one standard well plate.</td></tr>
<tr><td>Step 2
Sample
Preparation</td><td>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.
• Transfer 200 µl sample into each well of the 96 Deep Well Plate.
Note: Careful adding sample into each wellto 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 magbeads 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.</td></tr>
<tr><td>Step 3
Instrument
setup</td><td>• Load all plates onto the instrument the table below:
<table>
<tr><th colspan="2">Plate position</th><th>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 rowspan="2">Deep well plate</td><td rowspan="2">950 µl</td></tr>
<tr><td>Isopropanol
MV Magbead</td></tr>
<tr><td>2</td><td>1st Wash plate</td><td>W1 Buffer</td><td>Deep well plate</td><td>300 µl</td></tr>
<tr><td>3</td><td>2nd Wash plate</td><td>W1 Buffer</td><td>Deep well plate</td><td>300 µl</td></tr>
<tr><td>4</td><td>3rd Wash plate</td><td>Wash Buffer</td><td>Deep well plate</td><td>450 µl</td></tr>
<tr><td>5</td><td>4th Wash plate</td><td>Wash Buffer</td><td>Deep well plate</td><td>450 µl</td></tr>
<tr><td>6</td><td>Elution plate</td><td>RNase-free water</td><td>Standard plate</td><td>90 µl</td></tr>
<tr><td>7</td><td>Tip comb plate</td><td colspan="3">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°C</td></tr>
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