Magnetic Beads Virus DNA/RNA Extraction Kit

For research use only

Catalogue Numbers	Quantity
MV048	48 rxns
MV096	96 rxns

CERTIFICATE NO. QAIC/TW/50077

ISO 9001:2008 QMS

Introduction

The Magnetic Beads Virus DNA/RNA Extraction Kit was designed specifically for efficient purification 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 DNA/RNA can be used in qPCR and qRT-PCR assays.

Quality Control

The 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 serum sample.

Advantages

- High Sensitivity: virus RNA/DNA can be successfully extracted and detected from as low as 10E1 copy number
- Easily adapted to automated magnetic bead separation instruments and workstations
- Sample: up to 200 µl of virus samples (plasma, serum, body fluid or the supernatant of viral infected cell cultures)
- Operation time: within 30 minutes (manual)
- Storage: dry at room temperature (15-25ºC)

Caution

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) and used only for RNA procedures.

Components and Storage

Item	Volume	Product	Shipping	Storage
MV1 Buffer	2 ml	MV004	room temperature	dry at room temperature (15-25ºC)
	30 ml	MV048		
	60 ml	MV096		
MV2 Buffer[1](Add Ethanol)	1.5 ml (3 ml)	MV004	room temperature	dry at room temperature (15-25ºC)
	14 ml (20 ml)	MV048		
	28 ml (40 ml)	MV096		
MV3 Buffer	2 ml	MV004	room temperature	dry at room temperature (15-25ºC)
	30 ml	MV048		
	50 ml	MV096		
MV4 Buffer[2] (Add Ethanol)	1 ml (4 ml)	MV004	room temperature	dry at room temperature (15-25ºC)
	12.5 ml (50 ml)	MV048		
	25 ml (100 ml)	MV096		
RNase-free water	2 ml	MV004	room temperature	dry at room temperature (15-25ºC)
	15 ml	MV048		
	15 ml	MV096		
MV Magnetic Beads	220 µl	MV004	room temperature	dry at room temperature (15-25ºC)
	2.5 ml	MV048		
	5 ml	MV096		
Carrier RNA[3] (Add RNase-free Water)	1 mg (1 ml)	MV004	room temperature	dry at room temperature (15-25ºC)
	1 mg (1 ml)	MV048		
	1 mg (1 ml)	MV096		

[1-2]Add absolute ethanol (see the bottle label for volume) to MV2 and MV4 Buffers 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.

[3]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.

Magnetic Beads Virus DNA/RNA Kit Protocol Procedure

IMPORTANT BEFORE USE:
1. Vortex magbeads to ensure they are in suspension prior to initial use.
2. Add absolute ethanol (see the bottle label for volume) to the MV2 and MV4 Buffers and 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.
3. 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. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. 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.
Additional requirements: absolute ethanol, microcentrifuge tubes (DNase and RNase-free), Phosphate-Buffered Saline (PBS), magnetic separator

1. **Transfer a 200 µl sample** (e.g. serum, plasma, body fluids or the supernatant of a viral infected cell culture) to a 1.5 ml RNase-free microcentrifuge tube. NOTE: If the prepared sample is less than 200 µl, adjust the sample volume to 200 µl with PBS. For RNA virus, adding 1 µl of Carrier RNA solution to the sample before extraction is recommended.

2. **Add 400 µl of MV1 Buffer** to the sample then mix by vortex. Incubate at room temperature for 10 minutes.

3. **Add 450 µl of MV2 Buffer (make sure ethanol was added)** to the sample lysate then mix by vortex. Vortex MV Magnetic Beads for 10 seconds to ensure the beads are kept in suspension before use. **Add 50 µl of MV Magnetic beads** to the sample lysate then gently shake the tube for 5 minutes to mix. Be sure the MV Magnetic Beads are dispersed completely in the sample mixture. Place the tube in a magnetic separator for 30 seconds or until MV Magnetic Beads have pelleted then remove and discard the supernatant.

4. **Add 400 µl of MV3 Buffer** then gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until MV Magnetic Beads have pelleted then remove and discard the cleared supernatant.

5. **Add 600 µl of MV4 Buffer (make sure ethanol was added)** then gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until MV Magnetic beads have pelleted then remove and discard the supernatant. **Once again, add 600 µl of MV4 Buffer (make sure ethanol was added)** then gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until MV Magnetic Beads have pelleted then remove and discard the supernatant.

6. Incubate the tube on a 40ºC hot plate for 3 minutes to dry the MV Magnetic Beads. **Add 50–100 µl of RNase-free Water** then mix the sample by pipetting and incubate the sample at room temperature for 3 minutes. During incubation, keep the MV Magnetic Beads in suspension by mixing. Place the tube in a magnetic separator for 30 seconds or until MV Magnetic Beads have pelleted. Transfer the supernatant containing the purified Viral DNA/RNA to a RNase-free 1.5 ml microcentrifuge tube.

Magnetic Beads Virus DNA/RNA Extraction Kit Functional Test Data

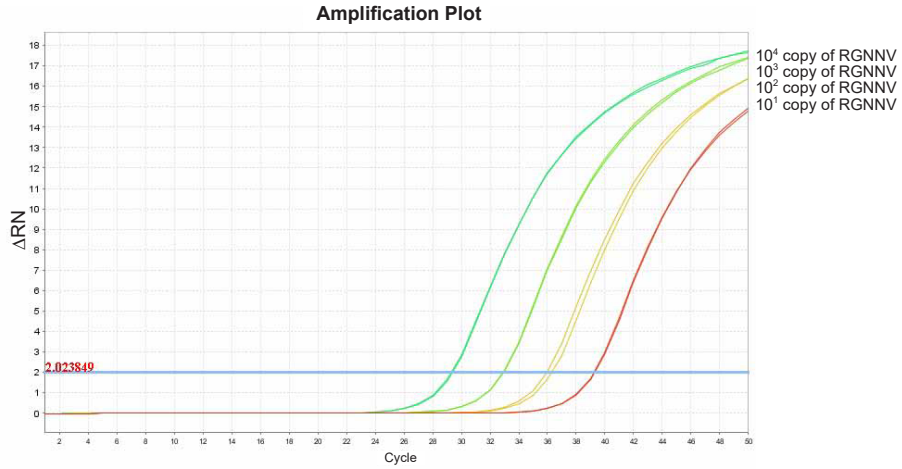

Figure 1. Virus RNA was purified from 10E1-10E4 copy number of Red Spotted Grouper Nervous Necrosis Virus (RGNNV) using the Geneaid Magnetic Beads Virus DNA/RNA Kit (2 replications of each copy number). The purified RNA was eluted with 50 µl RNase-free Water. cDNA synthesis was carried out with a 10 µl aliquot of purified RNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche) in a final volume of 20 µl. A Real-time PCR assay was then performed with 5 µl of synthesized cDNA as template, primers (designed to amplify the T4 region on the RNA2 segment), and Fast SYBR Green PCR Master Mix using the StepOnePlusTM Real-Time PCR system (Applied Biosystems). The results confirmed that virus RNA can be successfully extracted and detected from as low as 10E1 copy number of RGNNV.