7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
Email: info@abpbio.com

EasyMag Viral RNA Mini Kit

Catalog Number: D144-1, D144-2

Table 1. Kit Components and Storage

Kit Component	D144-1 (50 preps)	D144-2 (200 preps)	Storage	Stability
Buffer AVL	30 mL	120 mL	RT	The product is stable for one year when stored as directed.
Buffer AW1*	15 mL	53 mL	RT	
Buffer AW2*	10 mL	25 mL	RT	
RNase-Free ddH_2O	10 mL	30 mL	RT	
Carrier RNA (1 µg/µL)	250 µL	1000 µL	-20°C	
MagBinding Beads	2.5 mL	10 mL	4°C	

***** Prior to use, add absolute ethanol to **Buffer AW1** and **Buffer AW2** according to the bottle label.

Product Description

EasyMag Viral RNA Mini Kit is designed for the fastest and easiest way to purify viral RNA from serum, plasma, swab, urine, cell culture media, and other cell-free body fluids. The magnetic beads technology enables purification of high-quality RNA that is free of proteins, nucleases, and other impurities. In addition to easily being adapted with automated systems, this procedure can be also be scaled up or down depending on the amount of starting sample. The sample is first lysed under highly denaturing conditions to inactivate RNase and to ensure isolation of intact viral RNA. Buffering conditions are then adjusted to provide optimum binding of the RNA to the magnetic beads. The RNA binds to the magnetic beads, and contaminants are efficiently washed away using washing buffers. High-quality RNA is then eluted from magnetic beads for direct use or safe storage. The purified RNA is ready for downstream applications such as Next-Gen sequencing, and RT-qPCR detection.

Features

- High sensitivity: the kit can be used to isolate viral RNA as little as 10 copies of virus.
- Fast: the purification process takes only 20 minutes.
- Good stability: the optimized buffer system provides consistent results.
- Universal: the kit is compatible with a wide variety of viruses.

Sample Preparation

This protocol is for purification of viral RNA from 140 µL plasma, serum, urine, swab, cell culture media, or other cell-free body fluids.

Larger starting volumes, up to 560 µL, can be processed by increasing the initial volumes proportionally, as described below in the protocol.

Some samples with very low viral titers should be concentrated before the purification procedure. In these cases, concentrating samples of up to 3.5 mL to a final volume of 140 µL is recommended. Using centrifugal microconcentrators, such as Centricon® 100 (Amicon: 2 ml, cat. no. 4211), Microsep 100 (Filtron: 3.5 ml, cat. no. OD100C40), Ultrafree® CL (Millipore: 2 ml, cat. no. UFC4 THK 25) is recommended.

Purification Protocol

This protocol is described for purification of viral RNA from 140 µL plasma, serum, urine, swab, cell culture media, or other cell-free body fluids. Larger starting volumes can be processed by increasing the initial volumes proportionally, as described below in the protocol.

1. Add 560 µL Buffer AVL and 5 µL Carrier RNA to a 1.5 mL microcentrifuge tube.
   Note: If the sample volume is larger than 140 µL, increase the amount of Buffer AVL and carrier RNA proportionally (e.g., a 280 µL sample will require 1120 µL Buffer AVL and 10 µL carrier RNA) and use a larger tube.
2. Add 140 µL plasma, serum, urine, swab storage media, cell culture supernatant or cell-free body fluid to the Buffer AVL-carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 second.
   Note: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer AVL to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.
3. Incubate at room temperature for 10 min.

   Note: Viral particle lysis is complete after lysis for 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA.
4. Briefly centrifuge the tube to remove drops from the inside of the lid.
5. Add 350 µL isopropanol and 50 µL MagBinding Beads to the sample, and vortex for 15 second. Incubate at room temperature for 5 min.
   Note: To ensure efficient binding, it is essential that the sample is mixed thoroughly with the isopropanol and MagBinding Beads to yield a homogeneous solution. If the sample volume is greater than 140 µL, increase the amount of isopropanol and MagBinding Beads proportionally (e.g., a 280 µL sample will require 700 µL isopropanol and 100 µL MagBinding Beads).
6. Place the tube to a magnetic stand for an additional 2 minutes or until beads pellet and supernatant is cleared. With the tube on the magnetic stand remove the supernatant and discard.
7. Remove the tube from the magnetic stand. Add 500 µL of Buffer AW1, and resuspend the beads by pipetting up and down or vortexing the tube at 1,500 rpm for 30 seconds. Replace the tube on the magnetic stand for 2 minutes or until beads pellet. Remove and discard the supernatant.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
8. Remove the tube from the magnetic stand. Add 500 µL of Buffer AW2, and resuspend the beads by pipetting up and down or vortexing the tube at 1,500 rpm for 30 seconds. Replace the tube on the magnetic stand for 2 minutes or until beads pellet. Remove and discard the supernatant.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
9. Leave the tube on the magnetic stand, and remove any residue liquid with a pipettor. Air dry the beads at room temperature for 5-10 min.

   Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
10. Add 60 µL RNase-Free ddH_2O, and resuspend the beads by pipetting up and down. Incubate at 55ºC for 3 min.
11. Replace the tube on the magnetic stand for 2 minutes or until beads pellet. Transfer the cleared supernatant to a clean tube.
12. Store the viral RNA at -20-80ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• Sample frozen and thawed more than once: Repeated freezing and thawing should be avoided. Always use fresh samples or samples thawed only once. • Low concentration of virus in the sample: Concentrate the sample volume to 140 µl using a microconcentrator. Repeat the RNA purification procedure. • No isopropanol added to the lysate: Repeat the purification procedure with a new sample. • Improper washing: Buffer AW1, buffer AW2 must be diluted with absolute ethanol before use. • RNA degraded: Often RNA is degraded by RNases in the starting material (plasma, serum, body fluids). Ensure that the samples are processed quickly. If necessary, add RNase inhibitor to the sample. Check for RNase contamination of buffers and water, and ensure that no RNase is introduced during the procedure.
Poor performance in downstream applications	• Salt contamination: Repeat Buffer AW2 wash twice. • Ethanol contamination: Dry the magnetic beads completely before elution. • Reduced sensitivity: Determine the maximum volume of eluate suitable for your RT-PCR. Reduce the volume of elute added to the RT-PCR.