Viral RNA Isolation Kit
(vRNAEx)
Catalog #MB891
100 Preps

Description

ScienCell's Viral RNA Isolation Kit provides a fast and reliable way to purify viral RNA from a variety of samples such as cell culture media, plasma, serum, saliva, bronchoalveolar lavage, nasal/nasopharyngeal/oropharyngeal swab specimens, and other body fluids. The vRNAEx combines an optimized buffer system with a convenient spin column-based purification which facilitates fast and efficient viral particle lysis and RNA isolation and purification. Recovery rates are high with greater than 80% recovery for RNA longer than 200 nucleotides. The concentrated and purified RNA recovered from the kit is suitable for downstream applications such as RT-PCR detection and next-generation sequencing (NGS).

Kit Components

Cat #	Item	Quantity
MB891a	Buffer VL	35 mL
MB891b	Buffer VW1	24 mL
MB891c	Buffer VW2	17 mL
MB891d	Nuclease-free water	8 mL
MB891e	Carrier RNA (poly-A), lyophilized	1 vial
MB891f	RNA spin columns (in wash tubes)	100 pieces
MB891g	Wash tubes	100 pieces

Additional Materials Required (Not Provided)

Ethanol (96-100%)
1.7 mL (or 1.5 mL) microcentrifuge tubes (DNase/RNase free)
Optional: RNase-Free DNase Set (Qiagen, Cat #79254)

Quality Control

vRNAEx is tested by isolation and purification of RNA from spiked clinical specimens. Yield and quality of extracted RNA is evaluated by RT-qPCR, spectrophotometry, and gel electrophoresis.

Product Use

vRNAEx is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

Ambient temperature. Upon receipt, store Carrier RNA (Cat #MB891e) at -20°C in a manual defrost freezer. Other components of the kit should be stored at room temperature.

Reagent Preparation

1. Buffer VW1 (24 mL, Cat #MB891b) is provided as a concentrate. Prior to use for the first time, add **32 mL** of ethanol (96-100%) to make complete buffer VW1 and mix well. Store closed at room temperature.

2. Buffer VW2 (17 mL, Cat #MB891c) is provided as a concentrate. Prior to use for the first time, add **40 mL** of ethanol (96-100%) to make complete buffer VW2 and mix well. Store closed at room temperature.

3. Carrier RNA (Cat #MB891e) is provided lyophilized. Prior to use for the first time, centrifuge the vial at 1,500x g for 1 minute. Add **300 µL** of nuclease-free water (Cat # MB891d) to make carrier RNA stock solution. Aliquot as needed. Store at -20°C. Avoid repeated freeze-thaw cycles.

Procedures

Note: Avoid touching the RNA spin column membrane with the tip of a pipette, as membrane can be easily damaged.

1. For each sample (such as cell culture media, plasma, serum, saliva, bronchoalveolar lavage, nasal/nasopharyngeal/oropharyngeal swab specimens, and other body fluids) with up to 75 µL to purify, prepare one 1.7 mL microcentrifuge tube with 300 µL of buffer VL (Cat #MB891a).
   Note: If the sample volume is larger than 75 µL, increase the amount of buffer VL (Cat #MB891a) proportionally and use a larger tube if necessary. For example, a 150 µL sample will require 600 µL of buffer VL (Cat #MB891a).

2. Add 3 µL of carrier RNA stock solution to the microcentrifuge tube.
   Note: It is not necessary to increase the volume of carrier RNA stock solution if the starting sample volume is larger than 75 µL.

3. Add the sample to the microcentrifuge tube. Mix by pulse-vortexing.

4. Incubate at room temperature for 10 minutes.

5. Add 300 µL of ethanol (96-100%) to the sample and mix by pulse-vortexing.
   Note: If the starting sample volume is larger than 75 µL, increase the amount of ethanol (96-100%) proportionally. For example, a 150 µL starting sample will require 600 µL of ethanol.

6. Transfer the mixture (up to 650 µL each time) into an RNA spin column in a wash tube (Cat #MB891f). Close the cap and centrifuge at ≥ 8,000 x g for 1 minute. Discard the filtrate and put the spin column back to the same wash tube. Repeat this step until all of the mixture has been applied to the spin column.

7. Apply 500 µL of complete buffer VW1 to the spin column. Close the cap and centrifuge at ≥ 8,000 x g for 1 minute. Discard the filtrate and put the spin column back to the same wash tube.
   Note: It is not necessary to increase the volume of buffer VW1 if the starting sample volume is larger than 75 µL.
   Note: Host cell DNA may be co-purified with this procedure. If DNA-free RNA is desired, instead of performing Step 7 above, follow Steps *O7.1-O7.4* in Section "Optional: On-column DNase Digestion" below, then resume performing Steps 8-12.

8. Apply 500 µL of complete buffer VW2 to the spin column. Close the cap and centrifuge at ≥ 8,000 x g for 1 minute. Discard the wash tube containing the filtrate and transfer the spin column into a new wash tube (Cat #MB891g)
   Note: It is not necessary to increase the volume of buffer VW2 if the starting sample volume is larger than 75 µL.

9. Centrifuge at ≥ 15,000 x g for 2 minutes. Discard the wash tube containing the filtrate and transfer the spin column into a new 1.7 mL microcentrifuge tube.

10. Add 30 µL of nuclease-free water (Cat #MB891d) to the center of the spin column membrane. Close the cap and incubate at room temperature for 1 minute. Centrifuge at ≥ 15,000 x g for 1 minute.

11. ***Optional:*** If a higher yield of viral RNA is required, repeat step 10 with another 30 µL of nuclease-free water (Cat #MB891d) and combine the two eluates. The final RNA concentration will be lower.

12. Discard the spin column and save the eluate containing the viral RNA. The eluted viral RNA can be used immediately or stored at -80 °C.

Optional: On-column DNase Digestion

Host cell DNA may be co-purified with this procedure. If DNA-free RNA is desired, we recommend performing on-column DNase digestion using RNase-Free DNase Set (Qiagen, Cat #79254). Instead of performing Step 7 in Section "Procedures" above, follow Steps *O7.1-O7.4* below, then resume performing Steps 8-12 outlined in Section "Procedures".

O7.1. Apply 300 µL of complete buffer VW1 to the spin column. Close the cap and centrifuge at ≥ 8,000 x g for 1 minute. Discard the filtrate and put the spin column back to the same wash tube.

O7.2. In a 1.7 mL microcentrifuge tube, mix 10 µL of DNase I stock solution (prepared according to the Product Sheet of RNase-Free DNase Set, Qiagen, Cat #79254) with 70 µL Buffer RDD (supplied with RNase-Free DNase Set, Qiagen, Cat #79254). Mix by gently pipetting up and down a few times.

O7.3. Carefully apply the DNase I mixture directly to the spin column membrane, and incubate at room temperature for 15 minutes.

O7.4. Apply 300 µL of complete buffer VW1 to the spin column. Close the cap and centrifuge at ≥ 8000 x g for 1 minute. Discard the filtrate and put the spin column back to the same wash tube.