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EasySC Viral RNA Mini Kit

Catalog Number: D143-1, D143-2

Table 1. Kit Components and Storage

Kit Component	D143-1 (50 preps)	D143-2 (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	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

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

Product Description

EasySC 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 EasySC Viral RNA Mini Kit can be fully automated on Qiagen QIAcube Connect. 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 spin column membrane, and the sample is loaded onto the Mini spin column. The RNA binds to the membrane, and contaminants are efficiently washed away using washing buffers. High-quality RNA is eluted for direct use or safe storage. The purified RNA is free of protein, nucleases and other contaminants and inhibitors, and 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 and loading the Mini column multiple times, 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 using a microcentrifuge. The protocol can be adapted for using with vacuum manifold. For automated purification of viral RNA using the EasySC Viral RNA Mini Kit on QIAcube Connect, refer to the *QIAcube Connect User Manual* and the relevant protocol sheet.

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 560 µL absolute ethanol to the sample, and vortex for 15 second. After mixing, briefly centrifuge the tube to remove drops from inside the lid.
   Note: To ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution. If the sample volume is greater than 140 µL, increase the amount of ethanol proportionally (e.g., a 280 µL sample will require 1120 µL ethanol).
6. Insert a RNA Mini Column into a 2 mL Collection Tube. Transfer 630 µL of the solution from step 5 to the RNA Mini Column. Close the cap, and centrifuge at 8,000 x g for 1 min.
7. Discard the filtrate and reuse the collection tube. Carefully open the Mini column, and repeat step 6. If the sample volume was greater than 140 µL, repeat this step until all of the lysate has been loaded onto the spin column.
8. Discard the filtrate and reuse the collection tube. Carefully open the Mini column, and add 500 µL of Buffer AW1. Close the cap, then centrifuge at 8,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Carefully open the Mini column, and add 500 µL of Buffer AW2. Close the cap, then centrifuge at 8,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Discard the filtrate and reuse the collection tube. Centrifuge the Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
11. Transfer the RNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube. Carefully open the Mini column, add 60 µL RNase-Free ddH_2O equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min, then centrifuge at 8,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
12. Discard the column and store the viral RNA at -20-80ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column clogged	• Cryoprecipitates have formed in plasma due to repeated freezing and thawing. Do not use plasma that has been frozen and thawed more than once.
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 ethanol 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: Incubate the column at RT for 5 min before elution to dry the membrane completely. • Reduced sensitivity: Determine the maximum volume of eluate suitable for your RT-PCR. Reduce the volume of elute added to the RT-PCR.