EZ-press Viral RNA Purification Kit

Catalog No.: EZB-VRN1

Description

The *EZ*Bioscience® **EZ-press Viral RNA Purification Kit** provides a simple, rapid and efficient method to simultaneously purify viral RNA from fresh or frozen cell-free biological fluids (plasma, serum, cerebrospinal fluid, etc.) and cell culture supernatants. The purified Viral RNA is suitable for use in a variety of downstream applications, including RT-PCR, qRT-PCR, and qPCR, and can be used for viral load monitoring, viral detection and genotyping. Cell-free samples are first lysed and homogenized in a strong denaturant and phenol containing buffer, which immediately inactivates RNase to ensure isolation of intact RNA. After homogenization, Buffer A is mixed with the lysate and incubated. And then the mixture is centrifuged for phase separation; the upper-layer supernatant is collected and mixed with Supplemental Reagent and ethanol. The liquid is then passed through the RNA binding Spin Column containing a silica-based membrane to which the RNA binds. Impurities are effectively removed by subsequent washing. The purified total RNA is then eluted with Elution Buffer.

Components

Components	EZB-VRN1 (100 Preps)
Lysis Buffer	60 ml
Buffer A	22 ml
Supplemental Reagent	1100 µl
Wash Buffer 1*	13 ml
Wash Buffer 2*	13 ml
Elution Buffer	25 ml
Spin Columns (with Collection Tubes)	100 Preps

***:** Before using for the first time, add 52 ml of 100% ethanol to the Wash Buffer 1, 52 ml of 100% ethanol to the Wash Buffer 2.

Storage

Store the Lysis Buffer and Buffer A at 2 ~ 8°C, protect from light. Store the Supplemental Reagent at -20°C. Store other components at room temperature (When using these buffers, be careful to avoid of contamination). Divide the Elution Buffer into small aliquots upon reception is suggested.

Precautions

1. Handle all viruses in compliance with established institutional guidelines. Since safety requirements for use and handling of viruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.
2. Be sure to take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling viral samples.

Protocol

Starting materials

Collect the sample (such as plasma or serum) and proceed immediately to the purification protocol. If desired, you can store the sample at –80ºC or –20ºC for long-term storage. Do not freeze-thaw the plasma or serum sample more than once. Remove any visible cryoprecipitates from samples by centrifugation at ~7,000 × g for 2 ~ 3 minutes. Use the clear supernatant immediately for purification.

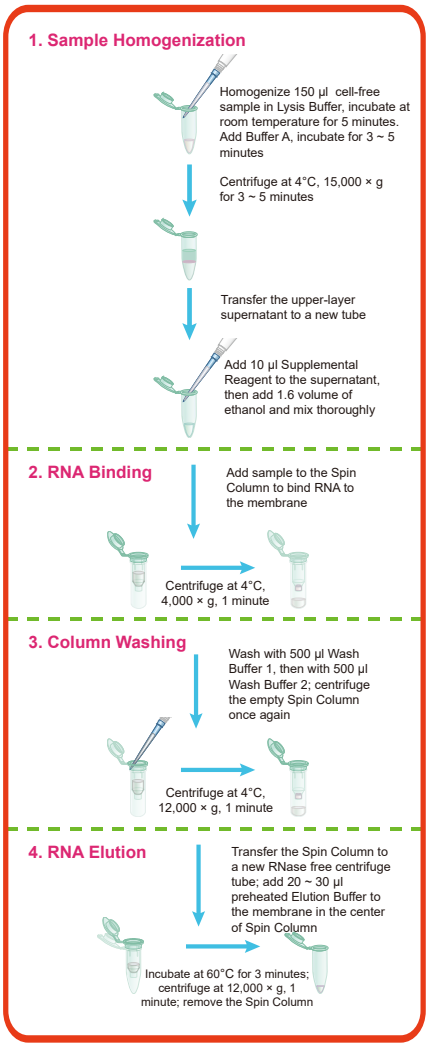
Sample Lysis

1. Place 150 ul cell-free sample in a 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer. Pipette up and down for 10 times to mix thoroughly.
2. After mixed, Incubate the mixture at room temperature for 5 minutes to lysis thoroughly.
3. Add 150 µl Buffer A to the lysates, mix by pipetting and hand-shaking. Incubate at room temperature for 3 ~ 5 minutes.
4. Centrifuge at 15,000 × g, 4°C for 3 ~ 5 minutes. Then the mixture is separated into three phases. Transfer the upper-layer supernatant (about 300 µl) to a new RNase free 1.5 ml centrifuge tube (be careful to avoid disturbing the middle or bottom layer).

RNA Binding

5. Add 10 µl Supplemental Reagent to each volume of supernatant, mix thoroughly and then add 1.6 volume of 100% ethanol to each volume of the above solution (supernatant + Supplemental Reagent).
6. Invert the centrifuge tube for several times or pipette up and down for 10 times to mix thoroughly, and transfer the mixture to the Spin Column. Centrifuge at 4,000 × g, 4°C for 1 minute. Pour off the liquid.

Experimental Procedure Overview



Column Washing

7. Add 500 µl of Wash Buffer 1 to the column. Centrifuge at 12,000 × g, 4°C for 1 minute (be careful to avoid of contacting the bottom of the column with the liquid when taking out of the column). Pour off the liquid.
8. Add 500 µl of Wash Buffer 2 to the column. Centrifuge at 12,000 × g, 4°C for 1 minute.
9. Pour off the liquid and eliminate the residual liquid using towel paper. Place the empty column back on the collection tube and Centrifuge at 12,000 × g, 4°C for 1 minute.
10. Don't need to pour off the liquid, directly transfer the column to an RNase free 1.5 ml centrifuge tube, open the lid and keep in the air for 2 minutes.

RNA Elution

11. Preheat the Elution Buffer to 60°C, add 20 ~ 30 µl to the membrane in the center of the Spin Column, and then place the EP tube with the Spin Column in a water bath or metal bath at 60°C for 3 minutes to dissolve the RNA.
12. Centrifuge at 12,000 × g, 4°C for 1 minute to elute Viral RNA (transfer the elution back to the column, incubate at room temperature for 5 minutes and centrifuge once more will get more viral RNA).
13. Store purified viral RNA at -80°C or use RNA for the desired downstream application.

Representative Results

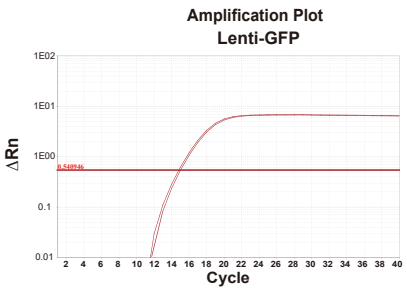


Figure1. RNA isolated from Lentivirus RNA sample using *EZ*Bioscience® EZ-press Viral RNA Purification Kit. The qPCR detection result shows that this kit can be well used for the purification of viral RNA.

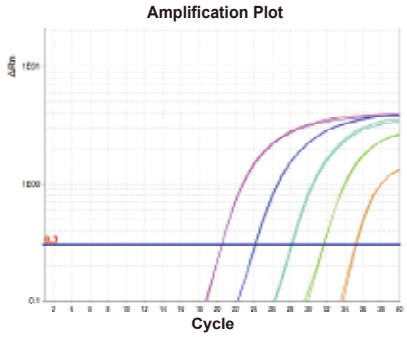


Figure 2. The N gene of SARS-CoV-2 Virus expression level in equivalently diluted RNA purified from SARS-CoV-2 Pseudovirus were detected. The amplification plots above indicate that, the target gene could be amplified efficiently and specifically. And the linear relationship within the detection range is very good.

Trouble shooting

The quantity of viral RNA purified by this Kit is too small, or the qPCR results is not so good, the Ct value is too large, or some specific genes can not be amplified normally.

Suggestions:

a. **Examine whether any of the reagents in the Kit is contaminated**: use a new Kit as positive control to confirm whether the reagents are contaminated (If the reagents are contaminated, the results between these two will be different, obviously). Therefore, **it is recommended to divide the reagents into 3 ~ 4 parts, each part in a 15 ml/50 ml centrifuge tube.** Be careful when taking the reagents out of the bottles to avoid of contamination.

b. The gene specific primers for qPCR should be divided to aliquots and stored at -20°C, to decrease the possibility of degradation or contamination.

c. **Examine whether the experiment is carried out correctly.**

e.g.:

1. During the whole process of RNA purification by this Kit, **it must be manipulated at room temperature (unless specially required) but not on ice** (until the RNA is eluted after the centrifugation), to avoid of blocking the membrane in the Spin Column by the water-insoluble substances formed during ice cooling.
2. Before using this Kit for the first time, **add 32 ml 100% ethanol to each bottle of the Wash Buffer** and mix thoroughly.
3. Transfer the upper-layer supernatant to a new tube after phase separation. **Avoid transferring any of the middle or bottom layer into the pipette when removing the upper-layer**.
4. **Add 10 µl Supplemental Reagent to the supernatant, then add 1.6 volume of ethanol** and mix thoroughly, then load onto the column.
5. After each column washing, the column should be taken out carefully to avoid of contacting the bottom of column with liquid.
6. The column must be dried in air for 2 minutes after the centrifugation of empty column.
7. When doing the step of RNA binding and RNA elution, **load the flow-through of centrifugation to the column and repeat the centrifugation once again will get more RNA.**