AccuPrep® Viral RNA Extraction Kit (K-3033)

Ⅰ Before You Begin

1) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
2) Dissolve **Poly (A)** with **500 µl** of **ER Buffer**. Gently mix with vortex mixer. Mix dissolved Poly (A) solution into **VB buffer**. Shake it thoroughly.
3) Add correct amount of **absolute ethanol** to **VW1 Buffer**.
4) Before starting extraction process, heat the **ER Buffer** at **56~60°C**.
5) The protective seal in BST Solution should be completely removed. BST Solution may be discolored, but it does not affect nucleic acid extraction.

Ⅱ Viral RNA Extraction

1) Add **10 µl** of **Proteinase K** to a 1.5 ml or 2 ml tube.
2) Add **200 µl** of **Serum, Plasma, Urine** or **CSF** to the tube. If the sample is swab, then add 1X PBS (phosphate buffered saline) and vortexing it to use only the supernatant.
3) Add **300 µl** of **VB Buffer** in the tube and mix by vortexing for 10 sec. To ensure efficient lysis, the sample should be mixed thoroughly with VB Buffer.
4) Incubate at **56~60°C** for **10 min**.
5) Add **300 µl** of **isopropanol**, lightly vortex for about 10 sec. Spin down for 5 sec to down the lysate clinging to the walls and lid of the tube.
6) Add **100 µl** of **BST Solution** to the Binding column tube (fit in a collection tube) and centrifuge for **30 sec** at **13,000 rpm**.
7) Discard the solution from the collection tube and reuse the collection tube.
8) Transfer the lysate into the Binding column tube not getting the lid wet.
9) Close the tube and centrifuge at **13,000 rpm** for **1 min**.
(Option) If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.
10) Discard the solution from the collection tube and reuse the collection tube.
11) Add **500 µl** of **VW1 Buffer** to the column, close the lid, and centrifuge at **13,000 rpm** for **1min**.
12) Discard the solution from the collection tube and reuse the collection tube.
13) Add **600 µl** of **RWA2 Buffer**, to the column, close the lid, and centrifuge at **13,000 rpm** for **1min**.
14) Discard the solution from the collection tube and reuse the collection tube.

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15) Centrifuge once more at **13,000 rpm** for **1 min** to remove ethanol completely.
(Caution) Make sure that there is no droplet hanging from the bottom of the Binding column. Residual RWA2 Buffer left in the Binding column may cause problems in later applications.
16) Transfer the Binding column to a new 1.5 ml tube for elution, add **100 µl** of **ER Buffer**, and let stand for 1 min to allow the buffer to permeate the column.
(Option) We recommend letting stand for about 5 min to increase RNA yield. You can also increase yield by heating the ER Buffer at about 60°C before adding to the column.
17) Elute by centrifuge at **13,000 rpm** for **1 min**. The eluted RNA solution can directly be used, or stored at -70°C for longer storage.

Ⅲ Viral DNA Extraction

1) Add **10 µl** of **Proteinase K** to a 1.5 ml or 2 ml tube.
2) Add **200 µl** of **Serum, Plasma, Urine** or **CSF** to the tube. If the sample is swab, then add 1X PBS (phosphate buffered saline) and vortexing it to use only the supernatant.
3) Add **200 µl** of **VB Buffer** in the tube and mix by vortexing for 10 sec. To ensure efficient lysis, the sample should be mixed thoroughly with VB Buffer.
4) Incubate at **56~60°C** for **10 min**.
5) Add **400 µl** of **isopropanol**, lightly vortex for about 10 sec. Spin down for 5 sec to down the lysate clinging to the walls and lid of the tube.
6) Add **100 µl** of **BST Solution** to the Binding column tube (fit in a collection tube) and centrifuge for **30 sec** at **13,000 rpm**.
7) Discard the solution from the collection tube and reuse the collection tube.
8) Go to **step 8** of "Viral RNA Extraction" in page 1 and continue the instructions accordingly.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.