AccuPrep® Viral RNA Extraction Kit (K-3033G)

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 (not provided)** to **VW1 Buffer (see bottle label).**
4) Before starting extraction process, heat the **ER Buffer** at **56-60°C.**
5) The protective seal in BSTB Solution should be completely removed. BSTB Solution may be discolored, but it does not affect nucleic acid extraction.
6) Add correct amount of **absolute ethanol (not provided)** to **RWB2 Buffer** and **BSTB Solution**, respectively (see bottle label).

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,** or **Urine sample** to the tube. If the sample is swab, add 1X PBS (phosphate buffered saline, not provided) and vortex 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 (not provided),** 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 **BSTB Solution** to the Binding column tube (fitted 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**.
   (Note) If the liquid has not completely passed the column after 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 **RWB2 Buffer**, to the column, close the lid, and centrifuge at **13,000 rpm** for **1min**.

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14) Discard the solution from the collection tube and reuse the collection tube.
15) Centrifuge once more at **13,000 rpm** for **1 min** to remove ethanol completely.
(Note) Make sure that there is no droplet hanging from the bottom of the Binding column. Residual RWB2 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(supplied with a kit), add **100 µl** of **ER Buffer**, and let stand for 1 min to allow the buffer to permeate the column.
(Note) We recommend letting it to stand for about 5 min to increase RNA yield. You can also increase yield by heating the ER Buffer at about **56-60°C** before adding to the column.
17) Elute by centrifuging 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,** or **Urine sample** to the tube. If the sample is swab, then add 1X PBS (phosphate buffered saline, not provided) and vortex 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 (not provided)**, 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 **BSTB Solution** to the Binding column tube (fitted 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 Guide of this kit.