Note: for laboratory research use only

Viral DNA/RNA Fast Extraction Kit (Spin-column)

Cat. #: DP5201（50 preps）

Signalway Biotechnology

Ⅰ.Kit Content, Storage and Stability

Content	Storage	50 preps（DP5201）
Buffer VB	RT	5 ml
Binding Buffer CB	RT	15 ml
Carrier RNA	－20℃	200μl
Inhibitor Removing Buffer IR	RT	27 ml
Rinsing Buffer WB	RT	15 ml
		Add 60 ml ethanol before first use
Elution Buffer EB	RT	15 ml
Isopropanol	RT	7 ml
Protease K（20mg/ml）	－20℃	20mg (dried powder)
RNase-free Spin-column AC	RT	50
Collection Tube（2ml）	RT	50

All reagents are stable for 12 months when stored properly.

Reminder:

1. ***Add ethanol to Buffer WB before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Buffer CB and IR may precipitate under low temperature. Incubate to 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
3. Protease K is provided in freeze-dried powder for activity and transportation. When receiving it, **add 1ml sterile water** after transient centrifugation. Then stored in per dose under -20℃.
4. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

Ⅱ. Principle:

The kit applies the unique binding buffer/ Protease K to rapidly lyse cells and to inactivate cellular nucleases, then DNA selectively adsorbs to silica membrane in high salt solution. Cellular metabolite and proteins are removed by serial of elution-centrifugation steps. Finally use low salt to elute purified viral DNA/RNA from silica membrane.

Ⅲ. Features:

1. Rapid and convenient. One preparation can be completed in 20 minutes.
2. Do not contain poisonous phenol and not need carrying ethanol precipitation.
3. This kit is used for viral DNA/RNA isolation from cryogenic plasma/serum/whole blood /other body fluid without cells. Do not repeat freezing and thawing for cryogenic sample. The harvest sample can directly used for PCR, restriction enzyme and hybridization.

Ⅳ. Notes

Please read this section before your experiment.

1. All the centrifugation can be performed at room temperature.
2. Set water bath to 60-70℃ before use.
3. For the best result, use fresh liquid sample and avoid repeated freezing and thawing.

Ⅴ. Procedure

Before Starting

- ***Dilute Buffer WB with 60ml absolute ethanol, vortex adequately, then mark the check box, avoid multi-adding!***

1. Add 200 µl virus liquid sample (plasma**,** serum, whole blood) into 1.5ml centrifuge tube. **If the liquid volume is less than 200 µl, please add to 200 µl by Buffer VB, if the liquid volume is 200-300 µl; please increase the solution volume in the next step.**
2. Add 200 µl binding buffer CB, shake for 15 seconds, mix thoroughly, and then

add 20 µl protease K (20mg/ml) solution, mix gently, incubate at 72℃ water bath for 10 minutes. Solution should appear clarification.

3. Add 100 µl isopropyl alcohol and 4 µl Carrier RNA after cool to RT, and then overturn to mix thoroughly. Flocculated precipitate may appear in this step.
4. Place a Spin-column AC into a collection tube and transfer all the mixture from step 3 in to the Spin-column AC. Centrifuge at 10,000rpm for 30 seconds. Discard the flow-through.
5. Add 500 µl Buffer IR and centrifuge at 12,000rpm for 30 seconds. Discard the flow-through.
6. Add 700 µl Buffer WB **(Please diluted with absolute ethanol before use)** and centrifuge at 12,000rpm for 30 seconds. Discard the flow-through.
7. Add 500 µl Buffer WB and centrifuge at 12,000rpm for 30 seconds. Discard the flow-through.
8. Put the Spin-column AC back to the collection tube, centrifuge at 13,000rpm for 2 minutes, remove rinsing buffer as possible, or else the left ethanol will affect the next reaction.
9. Transfer the Spin-column AC to a new 1.5ml centrifuge tube and add 100µl preheated (65℃-70℃) Buffer EB. Let it sit at room temperature for 2-5 minutes. Centrifuge at 12,000rpm for 1min. Add the flow-through onto Spin-column AC and let it sit at room temperature for 2 minutes. Centrifuge at 12,000rpm for 1 minute.

   The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. But the minimum volume is 20 µl; too less will decrease the elution efficiency and the DNA yield.
10. Keep DNA at -20℃ or apply to down-stream reactions.