

# Viral DNA/RNA Rapid Extraction Kit

## (Spin-Column)

**DNP 5201 (50 preps)**

### Kit Content, Storage Condition and Stability

Content	Storage	50 preps
Buffer VB	RT	5 ml
Binding Buffer CB	RT	15 ml
Carrier	-20°C	200 ul
Inhibitor Removing Buffer IR	RT	27 ml
Washing Buffer WB *	RT	15 ml
Eluting Buffer	RT	15 ml
Isopropanol	RT	7 ml
Proteinase K (20 mg/ml)	-20°C	20 mg
RNase-free Spin Column AC	RT	50
Collection Tubes 2 ml	RT	50

All reagents, when stored properly, are stable for 12 months.

#### \*Note:

1. Dilute Buffer WB with four volumes absolute ethanol before starting and mix thoroughly. Please mark it on the bottle label to avoid repeated addition.
2. Binding buffer CB and IR may form precipitation due to low storage temperatures. If necessary, dissolve the precipitation by 37°C water-bath and then cool to room temperature before use.
3. Proteinase K is provided in freeze-dried powder for activity and transportation. On receiving it, add 1ml sterile water after transient centrifugation. Then store in per dose under -20°C.
4. Please ensure the bottles of buffers are tightly capped when no in use, preventing reagents evaporating, oxidation and pH changing.

### Principle introduction

The kit applies the unique binding buffer/Proteinase K for rapid cell lysis and inactivation of cellular nucleases, then DNA is selectively adsorbed to silica membrane in high salt solution. Cellular metabolites and proteins are removed by serial of elution - centrifuge steps. Finally, purified genomic DNA from silica membrane is washed by low salt elution buffer.

#### Features:

1. No need poisonous phenol and ethanol precipitation.
2. Simple and rapid. One preparation can be completed in 20 min.
3. Multi-elution ensures high-purified DNA.

#### Notes:

**Please read this section before your experiment.**

1. All the centrifugation steps can be performed at room temperature. Use a traditional centrifuge that the rotational speed can reach 13,000 rpm. It could easily precipitation in low temperature, you can dissolve in 65°C water bath.
2. It needs water bath to 70°C before use.
3. For the best result, you'd better use fresh liquid sample and avoid repeated freezing and thawing.

## Procedure

**Add 60 ml absolute ethanol to 15 ml buffer WB by instruction prior to first use.**

1. Add 200  $\mu$ l blood/serum/plasma including virus into 1.5ml centrifuge tube.  
*If the initial volume is less than 200  $\mu$ l, please add up to 200  $\mu$ l by buffer VB, if the initial volume is between 200  $\mu$ l-300  $\mu$ l, it should increase the solution dosage in the next step. If the initial volume is between 300  $\mu$ l -1 ml, it is needed erythrocyte splitting.*
2. Add 200  $\mu$ l binding buffer CB, shaking for 15 s and then add 20 $\mu$ l proteinase K (20mg/ml) solution, mix by soft overturn. After 72°C water bath for 10 min solution should appear clear.
3. Add 100  $\mu$ l isopropyl alcohol to the RT cooled mixture, then overturn to mix thoroughly. Flocculated precipitation may appear in this step. Add 4  $\mu$ l Carrier, if the sample is micro.  
*The proper strength and thoroughly mix is important for the DNA yield. It could use vortex agitation if necessary, but can't seriously agitate by hand to avoid shearing DNA.*
4. Add the harvest solution and flocculated precipitation into an Spin column AC, (insert a Column AC into a collection tube), centrifuge at 10,000 rpm for 30 s, discard the waste liquid from collection tube.
5. Add 500  $\mu$ l Buffer IR and centrifuge at 12,000 rpm for 30 s. Discard the waste liquid.
6. Add 700  $\mu$ l Buffer WB (**please dilute with absolute ethanol before use**) and centrifuge at 12,000 rpm for 30 s. Discard the waste liquid.
7. Add 500  $\mu$ l Buffer WB and centrifuge at 12,000 rpm for 30 s. Discard the waste liquid.
8. Put the Spin column AC back to the collection tube and centrifuge at 13,000 rpm for 2 min. Remove rinsing buffer as possible, as the left ethanol can affect next reactions .
9. Transfer the column AC to a new collection tube and add 30-50  $\mu$ l preheated (65°C -70°C) Buffer EB. Let it stay at room temperature for 2-5 min and centrifuge at 12,000 rpm for 1 min. Add the flow-through into Column AC and let it stay at room temperature for 2 min. Centrifuge at 12,000 rpm for 1 min. The volume of elution buffer could be adjusted according to needs. Reduce elution volume can increase final concentration. But the minimum volume is 20  $\mu$ l, too low volume will decrease the elution efficiency and the final DNA yield.
10. Store at 2-8 °C. (-20 °C for long term storage).