Note: for ***in vitro*** laboratory research use only

Virus genomic DNA isolation kit
(Spin Column)

Cat # **DP3201（20preps）**
DP3202（50preps）

ABigen corporation

www.abigen.com Tel:+86-01-13683383835 E-mail: info@abigen.com

Kit Content Storage and Stability:

Component	Storage	20preps （DP3201）	50preps （DP3202）
Buffer VB	RT	3ml	5 ml
Binding buffer CB	RT	6 ml	15 ml
Inhibitor removing buffer IR	RT	11 ml	27 ml
Washing buffer WB	RT	6ml	15ml
		Diluted with absolute ethanol before first use	
Eluting buffer EB	RT	15 ml	20 ml
Isopropanol	RT	3 ml	7 ml
Protease K （20mg/ml） （only for II type）	-20℃	8mg Dry powder	20mg Dry powder
Spin column AC	RT	20	50
Collection tube（2ml）	RT	20	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 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. Protease K is provided in freeze-dried powder for activity and transportation. On receiving it, add 1ml sterile water after transient centrifugation. Then stored in per dose under -20℃
4. Please ensure the bottles of buffer tightly capped when no in use, preventing reagents evaporating, oxidation and PH changing.

Principle introduction:

The Kit apply the unique binding buffer/ Protease K to rapidly lyses cell and inactivate cellualar nuclease , then DNA selectively adsorbed to silica membrane in high salt solution. cellular metabolite and proteins etc. are removed by serial of elution- centrifuge step .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 ensure 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 Centrifugal machine that the rotational speed can reach 13,000rpm,such as Eppendorf 5415C and others .it could easily precipitation in low temperature, you can dissolute in 65℃ water bath.
2. It need water bath to 70℃ 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 200µl blood/serum/plasma including virus into 1.5ml centrifuge tube .**if the initial volume is less than 200µl ,please add up to 200µl by buffer VB , if the initial volume is between 200µl-300µl ,it should increase the solution dosage in the next step .if the initial volume is between 300µl -1ml ,it need erythrocyte splitting(appendix).**
2. Add 200µl binding buffer CB ,shaking for 15s and then add 20µl protease K (20mg/ml) solution , mixed by soft overturn , 72℃ water bath for 10 min ,solution should appear clarification .
3. Add 100µl isopropyl alcohol, then overturn to mix thoroughly. Flocculated precipitation may appear in this step.

   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 a absorption column AC ,(Insert a Column AC into a collection tube) ,centrifuge at 10,000rpm for 30s ,discard the waste liquid of collecting tube .
5. Add 500µl Buffer IR and centrifuge at 12,000rpm for 30s.Discard the waste liquid.
6. Add 700µl Buffer WB**(please diluted with absolute ethanol before use)** and centrifuge at 12,000rpm for 30s.Discard the waste liquid.
7. Add 500µl Buffer WB and centrifuge at 12,000rpm for 30s.Discard the waste liquid.
8. Put the column AC back to the collection tube and centrifuge at 13,000rpm for 2 min .Remove rinsing buffer as possible ,or else the left ethanol will affect the next reaction .
9. Transfer the column AC to a new collection tube and add 30-50µl preheated (65℃-70℃)Buffer EB. Let it sit at room temperature for 2-5min. Centrifuge at 12,000rpm for 1min.Add the flow-through onto Column AC and let it sit at room temperature for 2min. Centrifuge at 12,000rpm for 1min.

The volume of elution buffer could be adjusted according to needs. Appropriately reduce elution volume can increase concentration. But the minimum volume is 20μl, too less will decrease the elution efficiency and the DNA yield

10. Store at 2-8℃ .(-20℃ for long term storage)