Note: for laboratory research use only

Whole Blood DNA Extraction Kit (Spin-column)

Cat. #: **DP1801（50preps）**

DP1802（100preps）

DP1803（200preps）

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Ⅰ.Kit Content, Storage and Stability:

Component	Storage	50preps （DP1801）	100preps （DP1802）	200preps （DP1803）
Buffer BB	RT	15 ml	30 ml	60 ml
Binding Buffer CB	RT	15 ml	30 ml	60 ml
Inhibitor Removing Buffer IR	RT	27 ml	50 ml	100ml
Washing Buffer WB	RT	15 ml	25ml	50ml
		Add ration ethanol before use.		
Eluting Buffer EB	RT	15 ml	20 ml	40 ml
Isopropanol	RT	7 ml	15 ml	30 ml
Protease K （20mg/ml） （only for type II）	-20℃	20mg Dry powder	20mg×2 Dry powder	20mg×4 Dry powder
Spin-column AC	RT	50 pcs	100 pcs	200 pcs
Collection Tube （2ml）	RT	50 pcs	100 pcs	200 pcs

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

Notes:

1. ***Dilute 15ml Buffer WB with 60ml absolute ethanol before first use and mix thoroughly. Please mark it to avoid repeat add.***
2. 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 tightly capped when not in use, preventing reagents evaporating, oxidation and pH changing.

II. Principle:

The kit applies the unique binding buffer/ Protease K to rapidly lyse cell and inactivate cellualar nuclease, then DNA selectively adsorbs to silicified membrane in high salt solution. Cellular metabolite and proteins etc. are removed by serial of elution- centrifugation steps. Finally purified DNA from silica membrane is eluted by low salt elution buffer.

III.Features:

1. No need of harmful phenol and ethanol precipitation.
2. Simple and rapid. One preparation can be completed in 20 min.
3. Multi-elution ensures high-purified DNA. The DNA yield achieving 3-6μg from 200μl whole blood.

Ⅳ.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.
2. The genomic DNA typical yield is 3-6 μg genomic DNA from 200μl whole blood (the leukocyte count may vary in different samples especially in disease ones, so the individual yield may have large difference).
3. Set water bath to 70℃ before use.
4. For the best result, you'd better use fresh liquid sample and avoid repeat freezing and thawing.

Ⅴ.Procedure:

Add 60 ml absolute ethanol to 15 ml Buffer WB before use.

1. Add 200ul fresh / cryogenic / anticoagulant blood into 1.5ml centrifuge tube.

   If the initial volume is less than 200μl, please add up to 200μl Buffer BB; if the initial volume is between 200μl-300μl, increase the solution dosage in the next step. If the initial volume is between 300μl -1ml, please lyse erythrocyte first (appendix).

2. Add 200μl Buffer CB, shake for 15s and then add 20μl protease K (20mg/ml), mix gently, incubate at 72℃ water bath for 10min, solution should become clear .

3. Add 100μl isopropanol, and then overturn to mix thoroughly. Flocculated precipitation may appear in this step.

   The proper strength and thoroughly mix is important for the DNA yield. Vortex if necessary, but do not seriously agitate by hand to avoid shear DNA.

4. Transfer the solution and flocculated precipitation into a Spin-column AC (Insert a spin-column AC into a collection tube), centrifuge at 10,000rpm for 30s, discard filtrate.

5. Add 500μl Buffer IR and centrifuge at 12,000rpm for 30s. Discard filtrate.

6. Add 700μl Buffer WB **(please check absolute ethanol added!)** and centrifuge at 12,000rpm for 30s. Discard filtrate.

7. Add 500μl Buffer WB and centrifuge at 12,000rpm for 30s. Discard filtrate.

8. Put the Spin-column AC back to the collection tube and centrifuge at 13,000rpm for 2 min. Remove rinsing buffer as possible, or left ethanol will affect the next reaction.

9. Transfer the Spin-column AC to a new collection tube and add 100μl preheated (65℃-70℃) Buffer EB. Place it at room temperature for 2-5min. Centrifuge at 12,000rpm for 1min.Add the flow-through onto the Spin-column AC and place it 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)

Ⅵ.Appendix:

(If sample volume is around 300μl-1ml, please lyse erythrocyte first!)

1. Add 900μl erythrocyte lysis buffer to a 1.5 ml centrifuge tube or 3ml erythrocyte lysis buffer to a 15ml centrifuge tube.
2. Thoroughly mix the anticoagulant blood, add 300μl /1ml blood to 1.5ml /15ml centrifuge tube respectively ,invert for 6-8 times to make sure thoroughly mix .
3. Place them at room temperature for 2-5min.
4. Centrifuge at 12,000rpm for 20s(for 1.5ml centrifuge tube)/ 2,000-3,000rpm for 5 min (for 15ml centrifuge tube) to remove the red supernatant, leave complete leukocyte mass and about 10μl left supernatant.

   It may see white leukocyte mass in the bottom tube after centrifuge, or a few erythrocyte leavings and leukocyte mass, if the most part are red cell mass, it show that cracking erythrocyte is not enough, it should add erythrocyte splitting liquor and re-suspend cell masses, repeat step 3 and 4 .
5. Add 200μl Buffer BB to suspend and fully disperse the leukocyte masses.
6. Isolate the blood genomic DNA by operation steps.

Ⅶ.Troubleshooting:

Problem	Possible reason	Advices
Blood clot appeared in sample	Improper storage of sample; Do not mix thoroughly or improper anticoagulant collecting tube used	Discard sample of containing blood clot, re-collecting blood by EDTA , heparin, and citric acid anticoagulating tube
Erythrocyte splitting not enough.	No adjust to RT before sample splitting	Place it to RT before use
	Pyrolysis time not enough	Prolonged to 15 min
	No multiple mix in the course of pyrolysis	multiple mix in the course of pyrolysis
Low DNA yield	Low quantity Leukocyte of the sample itself	Add the initial quantity of blood
	The storage time is too long	Use the fresh sample
	Failure of protease K	The repeated melt and freeze of the serum must be avoided
	Not completely lysed ; not well mix of isopropanol	Mix thoroughly after add binding buffer and protease K, it should mix thoroughly after add isopropanol and fragments ,and then add into column
	Low elution efficiency	Make sure the correct operation of the step 8,and carefully read step 9,eluting only by EB
Downstream digestion inhibited	If skip over the step 8, ethanol restrain the endonuclease reaction	Do the step 8 and air drying for min, let the remaining ethanol volatilize.
	Some silicon based plasma membrane restrain	Centrifuge genomic DNA at 13,000rpm for 1 min, carefully

	endonuclease reaction	harvest supernatant
DNA length were less than 15kb	The blood sample is not fresh or improper storage	Use fresh blood sample
	Incorrect operation lead to genome DNA shear	It should not too violent when mixed ,transfer it by large diameter pipette tips or mix DNA
A260/A280 too high	Some silicon based plasma membrane interfere the value of A260/A280	Centrifuge genomic DNA at 13,000rpm for 1 min, carefully harvest supernatant
DNA remains slight color after eluting	The washing not enough	1. Washing until transparent after centrifugation. 2. Refer eluting buffer as starting materials, and repeat the experiment again, neglect the protease K digestion and 70℃ incubation step.