

# Whole Blood DNA Extraction Kit (Spin-column)

**DP1801      50 preps**

Note: for laboratory research use only

**Kit Content, Storage and Stability:**

Component	Storage	50 preps
Buffer BB	RT	15 ml
Binding Buffer CB	RT	15 ml
Inhibitor Removing Buffer IR	RT	27 ml
Washing Buffer WB	RT	15 ml
		Add ration ethanol before use
Eluting Buffer EB	RT	15 ml
Isopropanol	RT	7 ml
Proteinase K (20mg/ml)	-20°C	20 mg (Dry powder)
Spin-column AC	RT	50 pcs
Collection Tube 2ml	RT	50 pcs

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

**Notes:**

1. Dilute 15 ml Buffer WB with 60 ml absolute ethanol before first use, mix thoroughly and mark it to avoid repeat adding.
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. Proteinase K is provided in freeze-dried powder for activity and transportation. On receiving it, add 1 ml sterile water after transient centrifugation. Then stored in per dose under -20°C.
4. Please ensure the bottles tightly capped when not in use, preventing reagents evaporating, oxidation and pH changing.

**Principle:**

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

**Features:**

1. No need of poisonous 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 achieves 3-6 µg from 200µl whole blood.

**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. Buffers could easily precipitate in low temperature; you can dissolve them in 65°C water bath and cool to the RT.

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

### **Procedure:**

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

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

If the initial volume is less than 200 µl, please add up to 200 µl Buffer BB.

*If the initial volume is between 200-300µl, it should increase the solution dosage in the next step. If the initial volume is between 300 µl -1 ml, it needs erythrocyte splitting (see appendix).*

2. Add 200 µl Buffer CB, shake for 15 sec and then add 20 µl proteinase K (20mg/ml) solution, mix by soft overturn, 72°C water bath incubation for 10 min. Solution should appear clear.

3. Add 100 µl 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. 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 Spin-column AC (Insert a Column AC into a collection tube), centrifuge at 10,000 rpm for 30 sec, discard eluate from collecting tube.

5. Add 500 µl Buffer IR and centrifuge at 12,000 rpm for 30 sec. Discard the eluate.

6. Add 700 µl Buffer WB (**please diluted with absolute ethanol before first use**) and centrifuge at 12,000 rpm for 30 sec. Discard the waste liquid.

7. Add 500 µl Buffer WB and centrifuge at 12,000 rpm for 30 sec. Discard the eluate.

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 will affect the next reactions.

9. Transfer the Spin-column AC to a new collection tube and add 100 µl preheated (65°C -70°C) Buffer EB. Place Spin-column AC at room temperature for 2-5 min and centrifuge at 12,000 rpm for 1 min. Add the flow-through onto the Spin-column AC and place it 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. Appropriately reduce elution volume can increase concentration. But the minimum volume is 20 µl, too low elution volume will decrease the elution efficiency and the final DNA yield.

10. Store DNA at 2-8°C (-20°C for long term storage).

### **Appendix:**

**(Take 300 µl /1 ml whole blood for example to show the operation of erythrocyte splitting)**

1. Add 900 µl **erythrocyte splitting liquor\*** to a 1.5 ml centrifuge tube or 3 ml erythrocyte splitting liquor to a 15 ml centrifuge tube. (\* = **3.735 g NH<sub>4</sub>Cl, 1.3 g Tris - diluted in 500ml ddH<sub>2</sub>O**)

2. Thoroughly mix the anticoagulant blood, add 300 µl /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 2-5 min.

4. Centrifuge at 12,000 rpm for 20 sec (for 1.5 ml centrifuge tube)/ 2,000-3,000 rpm for 5 min (for 15ml centrifuge tube) to remove the red supernatant and carefully harvest supernatant as much as possible, leave complete leukocyte mass and about 10  $\mu$ l 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 is red cells mass, it show that cracking erythrocyte is not enough, it should be added erythrocyte splitting liquor and re-suspended cell masses, repeat step 3 and 4.

5. Add 200  $\mu$ l Buffer BB to suspend and fully disperse the leukocyte masses.

6. Isolate the blood genomic DNA by operation steps (point 2 from general procedure)

### Troubleshooting:

Problem	Possible reason	Advices
<b>Blood clot appeared in sample</b>	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
<b>Erythrocyte splitting not enough.</b>	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
<b>Low DNA yield</b>	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
<b>Downstream digestion inhibited</b>	Low elution efficiency	Make sure the correct operation of the step 8 and carefully read step 9, eluting only by EB
	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
<b>DNA length were less than 15 kb</b>	Some silicon based plasma membrane restrain endonuclease reaction	Centrifuge genomic DNA at 13,000 rpm for 1 min, carefully harvest supernatant
	The blood sample is not fresh or improper storage	Use fresh blood sample
<b>A260/A280 too high</b>	Incorrect operation lead to genome DNA shear	It should not too violent when mixed ,transfer it by large diameter pipette tips or mix DNA
	Some silicon based plasma membrane interfere the value of A260/A280	Centrifuge genomic DNA at 13,000 rpm for 1 min, carefully harvest supernatant
<b>DNA remains slight color after eluting</b>	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°C incubation step