Note: for laboratory research use only

Whole Blood DNA Extraction Kit (Spin-column)

Cat. #: DP1801 (50preps)

DP1802 (100preps)

Signalway Biotechnology

I. Kit Content, Storage, and Stability:

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

All reagents are stable for 12 months at RT if stored properly.

II. Notes：

1. ***Add ethanol to Buffer WB before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Precipitate may occur in buffers under low temperature, and it will affect DNA yield. Incubate to 37°C for a few minutes **until buffer is clear**, and then let the buffer cool to RT before use.
3. ***Proteinase K is provided in freeze-dried powder*** for activity and transportation. Add 1ml sterile water to the tube, mix, and centrifuge a few seconds. Because multiple freeze-thaws may affect enzyme activity, store aliquots under -20℃.
4. Keep all of the reagents lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

III. Principle:

The kit applies the unique buffer to rapidly lyse cell and inactivate cellular nuclease. DNA selectively adsorbs to silicified membrane in high salt solution. Cellular metabolite and proteins etc. are removed by serial of elution and centrifugation steps. Finally, low salt elution buffer elutes purified DNA from silica membrane.

IV. Features:

1. Rapid and convenient. One preparation can be completed in 20 minutes.
2. The kit does not contain poisonous phenol and does not need a step of ethanol precipitation.

3. High-purity; unique membrane absorption and specialized washing for removing protein and other debris.
4. Multi-elution ensures high-purified DNA. The DNA yield is about 3-6 μg from 200 μl whole blood.

V. 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 rotation speed can reach 13,000 rpm, such as Eppendorf 5415C.
2. The DNA yield is about 3-6 μg from 200 μl whole blood (the leukocyte number may vary in different samples, especially in patient blood, so the yield may vary largely).
3. Set water bath to 70℃ before use.
4. For the best result, use fresh blood sample and avoid repeated freezing and thawing.

VI. Procedure:

- ***Add absolute ethanol to Buffer WB before use.***

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

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

described in appendix).

2. Add 200 μl Buffer CB, shake for 15 seconds, and then add 20 μl protease K (20 mg/ml). Mix gently, and then incubate at 72℃ water bath for 10 minutes. Solution should become clear.
3. Add 100 μl isopropanol, and then overturn to mix thoroughly. Flocculated precipitation may appear at this step.

   The proper strength and thorough mix is important for the DNA yield. Gentle 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 10,000 rpm for 30 seconds, and discard the flow-through.
5. Add 500 μl Buffer IR and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
6. Add 700 μl Buffer WB **(please check if ethanol is added!)** and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
7. Add 500 μl Buffer WB and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
8. Put the Spin-column AC back to the collection tube and centrifuge at 13,000 rpm for 2 minutes. Remove rinsing buffer as much as possible. Ethanol will affect the down-stream reaction.
9. Transfer the Spin-column AC to a new microcentrifuge tube and add 100 μl preheated (65-70℃) Buffer EB. Place it at room temperature for 2-5 minutes. Centrifuge at

12,000 rpm for 1 minute. Add the flow-through onto the Spin-column AC and place it at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 minute.

The volume of elution buffer could be adjusted according to needs. Appropriate reduction of elution volume can increase concentration. However, the minimum volume is 20 μl. If the elution volume is less than 20 μl, elution efficiency and DNA yield can be affected.

10. Store DNA at -20℃ or apply to down-stream reactions.

VII. Appendix:

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

1. Add 900 μl erythrocyte lysis buffer (not included; DP1801-A) to a 1.5 ml centrifuge tube or 3 ml erythrocyte lysis buffer to a 15 ml centrifuge tube.
2. Thoroughly mix the anticoagulant blood, add 300 μl/1 ml blood to 1.5 ml/15 ml centrifuge tube respectively, and invert for 6-8 times to make sure a thorough mix.
3. Place them at room temperature for 2-5 minutes.
4. Centrifuge at 12,000 rpm for 20 seconds (for 1.5 ml centrifuge tube)/ 2,000-3,000 rpm for 5 minutes (for 15 ml centrifuge tube) to remove the red supernatant, leave complete leukocyte mass and about 10 μl residual supernatant.
5. ***White leukocyte mass (with a few erythrocytes) should bee seen at the bottom of tube after centrifuge. If the most parts are red cell mass, it indicate that lysis of erythrocyte is not complete. Add 900 μl erythrocyte lysis buffer to a 1.5 ml centrifuge tube or 3 ml erythrocyte lysis buffer to a 15 ml centrifuge tube, resuspend the cell mass, and repeat***

step 3-4 **.**

6. Add 200 μl Buffer BB to suspend and fully disperse the leukocyte masses.

7. Isolate the blood genomic DNA as described in Procedures.

Ⅶ. Troubleshooting:

Problem	Possible reason	Advices
Blood clot appeared in sample	Improper storage of sample; Did not mix thoroughly or use improper anticoagulant collecting tube.	Discard the sample containing blood clot, re-collecting blood using anti-coagulating tube (containing EDTA, heparin, and citric acid).
Incomplete splitting of erythrocyte from white leukocyte	No adjusting to room temperature before sample splitting.	Place sample to room temperature before use.
	Lysis time is not long enough.	Prolonged to 15 minutes.
	No thoroughly mix.	Thoroughly mix the sample with the buffer.
Low DNA yield	Low quantity of leukocyte in the sample.	Increase 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 protease K should be avoided.
	Not completely lysed; not thoroughly mix with isopropanol.	Mix thoroughly after adding binding buffer and protease K. Mix thoroughly after add isopropanol, and then add into column.
	Low elution efficiency.	Make sure the correct operation of the steps 8 and 9; eluting only by EB.

	Some silicon based plasma membrane restrain endonuclease reaction.	Centrifuge genomic DNA solution at 13,000 rpm for 1 minute, carefully harvest supernatant.
DNA size was less than 15 kb	The blood sample is not fresh or improper storaged.	Use fresh blood sample.
	Incorrect operation leading to genome DNA shear.	Avoid vigorously mixing, transfer the samples by large diameter pipette tips.
A260/A280 too high	Silicon membrane interferes the value of A260/A280.	Centrifuge genomic DNA solution at 13,000 rpm for 1 minute, carefully harvest supernatant.
DNA remains slight colored after elution	Not enough washing.	1. After the step 7, if the flow-through is colored, repeat step 7 until the flow-through has no color. 2. Use the eluted DNA as starting materials, and repeat the experiment again (skip the protease K digestion and 70℃ incubation step.