

# Whole Blood DNA Maxi Preparation Kit

## (Solution type)

*A fast kit for maxi-isolation of gDNA from whole blood*

For laboratory research use only

**DP2202**      **32 preps, each per 10 ml of whole blood**

### Kit Content, Storage, and Stability

Content	Storage	32 preps
Erythrocyte Lysis Buffer (10 x concentrated)	RT	200 ml
Nuclear Lysis Buffer	RT	350 ml
Protein Precipitation Buffer	RT	120 ml
DNA Dissolving Buffer	RT	30 ml

All reagents are stable for 18 months at RT.

### Note:

1. Nuclear Lysis buffer may form precipitation due to low storage temperatures. If necessary, dissolve the Nuclear Lysis buffer by 37°C water-bath and then cool to the room temperature before use.
2. Protein precipitation solution may form precipitation. Dissolve the precipitation buffer by 37°C water-bath.
3. Please ensure the bottles of buffer tightly capped when not in use, preventing reagents evaporating, oxidation and pH change.

### Principle:

Whole Blood DNA Extraction Kit is developed for rapid DNA isolation. First, Erythrocyte Lysis Buffer removes DNA-free erythrocytes, then Nuclear Lysis Buffer splits leukocytes and DNA is released. Protein precipitation solution precipitates and removes proteins selectively. Finally, the purified DNA is precipitated by isopropanol and DNA is dissolved in DNA dissolving solution.

### Features:

1. Without phenol or other poisonous compounds.
2. Simple and rapid. One preparation can be completed in 1 hour.
3. Stable and high yield (the typical yield is 150-500 µg of 10 ml whole blood), high purity, the value of OD260/OD280 achieves 1.7-1.9. The length of the genomic DNA is 50kb-150 kb, which can be applied to PCR, Southern-blot and digestions directly.

### Notes:

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

1. All the centrifugation steps can be performed at room temperature.
2. Prepare 70% ethanol.
3. The typical yield of 300 µl whole blood is 5-15 µg genomic DNA (DNA yield depends on kind of blood).
4. This kit is solution type, which can be proportionally amplified or narrowed to samples (20 µl-10 ml).

5. This kit can apply to kinds of whole blood of anticoagulant, such as EDTA, citric acid, heparin anti-coagulating. It's hard to resuspend leukocyte precipitation masses by heparin anti-coagulating. Moreover, heparin will affect cell lysis and DNA yield. We recommend using heparin sodium free anticoagulant to collect samples.
6. In order to achieve the best data, it is better to use fresh blood sample. Do not use the sample after repeated freezing and thawing for more than 3 times, as the yield will significantly decrease.

### Procedure:

1. Add 30 ml Erythrocyte Lysis Buffer to a 50 ml tube.
2. Thoroughly mix the anticoagulated blood and add 10 ml into Erythrocyte Lysis Buffer from the step 1.
3. Let them stay at room temperature for 10 min.
4. Centrifuge at 2000 g for 10 min. Carefully remove red supernatant as possible, leave all leukocyte mass at the bottom of the tube and about 10  $\mu$ l residual supernatant. White leukocyte mass appear at the bottom of the tube after centrifugation and some erythrocyte can remain.  
Leukocyte mass also may appear, if the most part is red cell mass, because erythrocyte precipitation was not sufficient. If so, add proper amount (10-15 ml) of Erythrocyte Lysis Buffer to resuspend cell mass and repeat step 3 and 4.
5. Resuspend leukocyte mass by vortex for 15 sec, fully disperse leukocytes. Leukocyte mass resuspension is important for next step.
6. Add 10 ml Nuclear Lysis Buffer to resuspend leukocytes. Strongly and quickly beat upon for several times to lyse leukocyte mass until the mixture appears viscous because of genomic DNA releasing.
7. **Optional step:** Add RNase A (10mg/ml) into cracking mixture to the final concentration 30  $\mu$ g/ml. Mix thoroughly and incubate for 15 min at 37°C to remove residues of RNA and then cool to RT.
8. Add 3,3 ml Protein Precipitation Buffer and vortex for 20-25 sec, small protein mass appears after mixing.
9. Centrifuge at 2.000 g for 10 min (adjust the centrifugal force by centrifuge effect). Brown protein precipitation at the bottom of tube or on the surface of liquid will appear.
10. Carefully transfer supernatant (about 10 ml) to a new 50 ml microcentrifuge tube.
11. Add equal volume of isopropanol (about 10 ml) and gently reverse 30 times till some white filamentous DNA precipitation appears.
12. Centrifuge the tube at 2.000 g for 1 min, discard supernatant.
13. Add 10 ml 70% ethanol and mix. Centrifuge at 2.000 g for 3-5 min. Discard the supernatant.
14. Add 3 ml 70% ethanol. Rinse DNA precipitation, centrifuge at 2000 g for 1 min, discard supernatant and air dry for several minutes.  
The DNA will be difficult to dissolve if complete air dry.  
Don't leave too much ethanol, as it could inhibit the downstream experiments.
15. Add 600  $\mu$ l DNA (or adjust by concentration) Dissolving Buffer to dissolve DNA precipitation, mix by tap the tube wall and incubate for 30-60 min at 65°C (no more than 1 h) or stay at RT or 4°C till DNA is fully dissolved.
16. DNA could be stored at 2-8°C, or stored at -20°C for long term storage.

## Troubleshooting:

Problem	Possible causes	Advices
Blood clots in sample	Improper storage of sample; mix not well, or not use proper anticoagulant collecting tube.	Discard sample containing blood clots, recollecting blood with suitable anticoagulating tubes.
Erythrocyte lysis not complete	Not adjust to RT before sample lysis.	Place it to RT before use.
	Pyrolysis not enough.	Extend to 15 min.
	Not mix in the course of pyrolysis.	Mix several times in the course of pyrolysis.
Low DNA yield	Low quantity leukocyte of the sample.	Increase the initial quantity of blood.
	Sample degraded.	Use the fresh sample.
	Not completely lyse cells or not well mix of isopropanol.	Mix thoroughly after adding of buffers, mix thoroughly after adding of isopropanol.
	Low elution efficiency.	Make sure the correct operation of the all steps.
No protein precipitation appear	The mixture not cool to room temperature before adding of protein precipitation buffer.	Cool to room temperature or incubate for 5 min on ice, then add protein precipitation buffer.
	Not thoroughly mix protein precipitation solutions and cracking mixture	Vortex for 25 sec to mix solutions.
DNA length less than 30kb	The blood sample is not fresh or in improper storage.	Use fresh blood sample.
	Incorrect operations damage genomic DNA.	Gently mix, transfer solutions by large diameter pipette tips or mix DNA.
A260/A280 <1.6	Protein contamination	Refer to "No protein precipitation appear "and step 9.
	Dilute DNA with water when measure the value of A260/A280	Use TE buffer instead of water, keep pH value >8.0
DNA precipitation difficult to re-dissolve	Completely air dry of DNA precipitation	Not air dry DNA completely.
Downstream digestion inhibited	DNA does not dry fully, too much ethanol left.	Keep tube open, incubate several minutes at 65°C to volatilize ethanol.