

Whole Blood DNA Extraction Kit

(Solution type)

A fast kit for the isolation of gDNA from whole blood

For laboratory research use only

DP1101 **50 preps**

Kit Content, Storage, and Stability

Content	Storage	50 preps
Erythrocyte Lysis Buffer	RT	50 ml
Nuclear Lysis Buffer	RT	17 ml
Protein Precipitation Buffer	RT	6 ml
DNA Dissolving Buffer	RT	10 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 erythrocyte, then Nuclear Lysis Buffer splits leukocytes and DNA is released. Then Protein precipitation solution precipitates and removes proteins selectively. Finally, the purified DNA is precipitated by isopropanol, and then DNA is dissolved in DNA dissolving solution.

Features:

1. Do not contain 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 150-500 µg of 10 ml whole blood), high purity, the value of OD260/OD280 achieving 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 because of the leukocyte precipitation masses of heparin anti-coagulating and 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 900 μ l Erythrocyte Lysis Buffer to a 1.5ml microcentrifuge tube.
2. Thoroughly mix the anticoagulated blood and add 300 μ l into Erythrocyte Lysis Buffer from the step 1.
3. Let them stay at room temperature for 10min.
4. Centrifuge at 12,000 rpm for 20 sec. Carefully remove red supernatant as possible, leave all leukocyte masses at the bottom of tube and about 10 μ l residual supernatant. White leukocyte masses may appear at the bottom of the tube after centrifugation, and some erythrocyte remains.
Leukocyte masses also may appear, if the most part are red cell masses, because erythrocyte precipitation was not sufficient. Add proper amount Erythrocyte Lysis buffer to resuspend cell masses and repeat step 3 and 4.
5. Resuspend leukocyte masses by vortex for 15 sec, fully disperse leukocyte masses. Leukocyte masses resuspension is important for next step.
6. Add 300 μ l Nuclear Lysis Buffer to resuspend leukocytes. Strongly and quickly beat upon for several times to lyse leukocyte until the mixture appearing viscous because of genomic DNA releasing.
7. **Optional step:** Add RNase A (10mg/ml) into cracking mixture at a final concentration of 30 μ g/ml. Mix thoroughly and incubate for 15 min at 37 $^{\circ}$ C to remove residues of RNA, and then cool to RT.
8. Add 100 μ l protein precipitation and vortex for 20-25 sec, small protein masses appear after mixing.
9. Centrifuge at 13,000rpm for 5 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 300 μ l) to a new 1.5ml microcentrifuge tube.
11. Add equal volume isopropanol (about 300 μ l) and gently reverse 30 times till some white filamentous DNA precipitation appears.
12. Vertically place microcentrifuge tube*, discard supernatant as much as possible and keep the bottom white DNA precipitation. (alternative – instead of placing vertically, for better sedimentation of DNA you can centrifuge the microtube at 12.000 rpm for 1 min.)
13. Add 1 ml 70% ethanol and mix. Centrifuge at 13,000rpm for 1 min. Discard the supernatant.
14. Add 0.5 ml 70% ethanol. Rinse DNA precipitation, centrifuge at 12,000rpm for 1 min, discard supernatant, and air dry for several minutes.
The DNA will be difficult to dissolve because of complete air dry. Don't leave too much ethanol, as it could inhibit the downstream experiments.
15. Add 100 μ 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 $^{\circ}$ C (no more than 1 h), or stay over at RT or 4 $^{\circ}$ C .
16. DNA could be stored at 2-8 $^{\circ}$ C, or stored at -20 $^{\circ}$ 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, re-collecting blood with EDTA, heparin, and citric acid 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.
	Proteinase K is low active or does not work.	Please avoid multiple freeze-thaws.
	Not completely lyse cells or not well mix of isopropanol.	Mix thoroughly after add binding buffer and proteinase K, mix thoroughly after add isopropanol.
	Low elution efficiency.	Make sure the correct operation of the step 8, and carefully read step 9, elute only by EB.
No protein precipitation appear	The mixture not cool to room temperature before add protein precipitation.	Cool to room temperature or incubate for 5 min on ice, then add protein precipitation.
	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 10.
	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 completely, too much ethanol left.	Keep tube open, incubate several minutes at 65°C to volatilize ethanol.