

DNA Extraction Protocol

Thaw frozen whole blood samples quickly in a 37° C water bath. Immediately place on ice until proceeding with the DNA purification procedure.

Cell Lysis

1. Add 0.7x of Lab RBC Lysis Soln to 45ml mark of 50ml conical tube for 6-10ml of blood, and to 50ml mark for more blood ~12ml.
2. Invert to mix and let stand at RTP for 5-10 min.
3. Centrifuge at 2000x g for 10 min.
4. Carefully pour off supernatant containing lysed RBC, leaving little soln ~0.1-0.2ml behind.
5. Vortex well to resuspend the WBC pellet.
6. Add 10ml of Cell Lysis Solution to pellet. Pipette up and down with a cut pipette tip to lyse cells.

Protein removal, DNA Precipitation

7. (Keep samples on ice) Add 4ml of PureGene Protein Precipitation Soln to precipitate out proteins. Invert tube, vortex well 5-10 sec.
8. Shake or vortex samples well again before centrifuge at 2000x g for 10 min.
9. Pour off supernatant to 50ml tube containing 10ml of cold Isopropanol and 133µl of 20mg/ml glycogen.
10. Invert tube to mix many times until DNA precipitate into compact thread and centrifuge at 2000x g for 5-10 min.
11. Carefully pour off supernatant, leaving behind the DNA pellet and wash with 10ml of 70% ethanol by inverting tube up and down.
12. Centrifuge at 2000x g for 5-10 min, and carefully pour off supernatant and leaving behind the DNA pellet. Be careful pellet may be a bit loose.
13. Invert tube to dry on clean paper towel for 10min or so. Be careful not to loose pellet.
14. Add 300-600µl of 1x TE (10mM Tris, 1mM EDTA, pH 8) or PureGene DNA Hydration Soln to DNA pellet based on pellet size; normally 500-600µl is fine.

15. Heat samples at 65°C for ~1-1:30 hours by tapping the tube to dissolve the DNA once in a while.
16. Once the DNA is all dissolved, centrifuge at 2000x g for 1min to collect sample. Then aliquot sample to 1.5ml Eppendorf tube, keep at 4°C until Pico Green reading.

Notes: If more clumps exist at step 6, then leave samples at RTP for a while. Sample mixture can be kept at RTP at this step for a long time.

Samples can be kept on ice if break time is needed at step 7.

If there is debris or blood foam after step 8, vortex samples well at full speed and shake them before a repeat of centrifuge again. You can increase time and centrifuge forces like 2350x g for 12 min; this will clear up the supernatant.

Only PureGene Protein Precipitation Soln is used in this protocol, everything else is from our lab reagents.

Our Lab's Reagents and Solutions

A. Red Blood Cell Lysis Solution: 10x

- 1.45M NH₄Cl (MCB crystal cat # AX1270-1, FW=53.5)
- 5mM EDTA (Free acid, anhydrous, Sigma #EDS, FW 292.2)
- 0.1M KHCO₃ (Mallinckroft cat # 6748, FW= 100.12)
- KOH (Any kind of reagent or grade)

Prepare as follows for 2L:

1. Add 155g NH₄Cl to ~1.5L of ddH₂O, stir until DISSOLVED.
2. Add 20g KHCO₃ and stir until DISSOLVED.
3. Add 100ml of 100mM EDTA soln, pH 8 (1/20 final volume).
4. Bring to ~ 1980ml with ddH₂O.
5. Recheck the pH with meter, should be between 7.2 and 7.8.
If > 7.8 decrease pH with 100mM HCl added drop by drop.
If < 7.2 increase pH with 100mM KOH added drop by drop.
6. Filter sterilize through a 0.2 or 0.45 micron filter.

B. White Blood Cell Lysis Solution: 1x for 1L

<u>[Stock]</u>	<u>Stock Volume</u>	<u>[Final]</u>
1M Tris-Cl	10ml	10mM
0.5M EDTA	50ml	25mM
10% SDS	50ml	0.5%
ddH ₂ O	~885ml	up to 1L