Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

DNA extraction from Blood

PSR

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6/12/2016

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Solutions

- Red blood cells lysing buffer (RLB): 2M Tris-HCl pH 7.6, 1M MgCl2, 3M NaCl.
- White blood cells lysing buffer (WLB): 2M Tris pH 7.6, 0.4 M disodium salt of ethylenediaminetetra acetic acid (Na_2EDTA), pH 8, 3M NaCl, 2% cetyl trimethyl ammonium bromide (CTAB)
- Chloroform: isoamyl alcohol 24:1

Detailed procedure

1. Transfer 2 mL of fresh blood sample into a 2 mL tube.
2. Centrifuge the sample at 6000 rpm at 4°C for 10 min.
3. Aspirate the plasma without touching the leukocyte layer.
4. Add 1 mL RLB and mix gently.
5. Centrifuge the sample for 5 min at 3000 rpm.
6. Remove the supernatant.

Note: If red blood cells still remain, re-suspend the cellular pellet in 1 mL of RLB and repeat steps 4-6 for 3 or 4 times until only the white pellet appears.

7. Add 1 ml of WLB and mix with white blood cells.
8. Incubate the tube at 65 °C for 30 min.
9. Centrifuge the tube at 12,000 rpm for 5 min and transfer the supernatant to a new clean tube and discard the pellet.
10. Add an equal volume of Chloroform-Isoamilalcohol solution to supernatant.
11. Centrifuge the tube at 12000 rpm for 8 min and transfer the supernatant to a new tube.
12. Add an equal volume of chilled Isopropanol.
13. Keep the sample in -20 °C for 30 min.
14. Centrifuge the tube at 4 °C, 12000 rpm for 10 min.

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15. Discard the supernatant and add 300 µL of chilled 90% Ethanol. Centrifuge the tube at 4 °C, 12000 rpm for 5 min.

16. Repeat the 15 and 16 steps with chilled 70% Ethanol.

17. Discard the supernatant and let pellet to be dried at room temperature.

18. Dissolve the pellet in 100 µL of TE buffer or ddH2O and store DNA solution at -20 °C.

Notification:

- For frozen blood samples start the procedure from step 4.
- pFor clotted blood samples, start the procedure from step 7 by adding1 mL of WLB and incubating at 65 °C for 1 hour (shaken every 10 min). Then continue by step10.

Reference

Samadi Shams, S., Zununi Vahed, S., Soltanzad, F., Kafil, V., Barzegari, A., Atashpaz, S., *et al.* (**2011**) Highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples. *Bioimpacts* **1**: 183-7.

Good luck!

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