

Purification of human mononuclear cells and neutrophils

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Purpose

Materials

- 10ml 6% dextran + 7ml citrate/citric acid
 - Dextran: T500 --> 6g+100ml PBS
 - Citrate solution: 25g Na Citrate + 8g citric acid + 500 ml PBS
- 43 ml blood
- 12 ml RT Histopaque 1077
- 18 ml cold H₂O
- 2 ml 10x PBS
- M199 for HUVECs: 1L powder packet M199 + 35g NaHCO₃ + 25mM Hepes + 5 mM glutamine + 50 ug/ml Gentamycin.
- 1M Hepes: 119.1 g Hepes + 500 ml dH₂O.
- Media A: 1X HBSS (10X:50ml) + 10 mM Hepes (1M: 5 ml) + 5 mM EDTA (0.5M: 5ml) + 2.5 % FBS (12.5 ml) in 500 ml.
- Media B: RPMI1640 470 ml + Gent (1000X) 0.5 ml + Glutamine (100X) 5 ml + 5% FBS (25 ml)

Procedure

1. Draw venous blood into 60 ml syringe with 10ml 6% dextran + 7 ml citrate/citric acid. Fill to 60 ml total (blood 43 ml).
2. Mix and sediment 30 min at RT (white blood cells now with serum).
3. Transfer serum/white blood cells to 50 ml tube, underlay 12 ml RT Histopaque 1077.
4. Spin 2500 rpm/30min at RT.

To take mononuclear cells:

5. Take off serum above band. (See attached figure, in preparation)
6. Take mononuclear band + some Ficoll into two 15ml tubes.
7. Add cold media A up to 15ml.
8. Spin at 1800 rpm 4°C for 5min.
9. Pool pellet into one 15ml tube and add cold media A.
10. Wash cells three times (1400 rpm, 4°C, for 5 min) to remove platelets.
11. Add media B and count cells.

To take neutrophils:

5. Remove mononuclear band and serum with suction. (See attached figure, in preparation)
6. Wash in PBS (or media A) to remove Histopaque (spin 1800 rpm/5 min at 4°C)
7. Remove PBS (or media A) , add 18 ml cold H₂O to lyse RBC, and add 2 ml 10x PBS.
8. Spin 1400 rpm/5min at 4°C.
9. Wash as needed, resuspend, and count cells.