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ISOLATION OF MONONUCLEAR CELLS BY DENSITY GRADIENT SEPARATION

(McCoy, J.P., 1998. Handling, storage and preparation of human blood cells. *Curr. Protocol. Cytom.* 5:5.1.1-5.1.13)

Tri-lineage separation methods are used when purification of cell populations is required rather than simple removal of erythroid contaminants. Density gradient separation techniques may not yield as many cells as the simple lysis methods, but they have other distinct advantages. Separating cells by Ficoll-Hypaque centrifugation often decreases the cytometry time for acquisition and removal of nonviable cells.

Equipment:

15- and 50-ml conical centrifuge tubes
Centrifuge
Pasteur Pipettes (sterile)

Reagents:

1.077 g/ml Ficoll-Hypaque (Pharmacia Biotech) or Histopaque-1077 (Sigma)
Anticoagulated blood in heparin or EDTA
Phosphate-buffered saline (PBS)
Tissue culture medium (optional)

Method:

1) With a sterile pipet, place the Ficoll-Hypaque solution into a 50-ml conical centrifuge tube, using 2ml of Ficoll-Hypaque per 1ml of blood.

   (The volume of Ficoll-Hypaque may vary with brand used. Consult manufacturer's recommendations.)

2) Mix anticoagulated blood with an equal volume of PBS.
3) Slowly layer the diluted blood over the Ficoll-Hypaque solution by gently pipetting the diluted blood down the side of the tube containing the Ficoll-Hypaque.
4) Centrifuge 40 min at 400 x g, 22ºC, with no brake.
5) Using a sterile Pasteur pipet, carefully remove the mononuclear cells, located at the interface between the plasma (upper layer) and the Ficoll-Hypaque (bottom).
6) Transfer the aspirated mononuclear cells to a 15-ml conical tube. Add 10 ml PBS or tissue culture medium and mix thoroughly. Centrifuge 10 min at 400 x g, 4ºC.
7) Discard the supernatant and repeat wash with PBS or tissue culture medium as needed.

Notes:

- *Some evidence exists to suggest that Eosinophils are better isolated using a 1.088 g/ml density gradient.*