



### *Introduction*

When drawing venous blood use sodium heparin as an anti-coagulant. Mix thoroughly and process samples within 30 hours. If storage is necessary prior to processing, store the blood at room temperature, shielded from light, and on a rocker. DO NOT refrigerate the cells.

All procedures should be carried out a room temperature.

### *Materials and Equipment*

- Human blood sample
- Disposable Pasteur pipettes (sterile)
- Sterile tubes (30ml and 50ml)
- Serological pipettes of appropriate volumes (sterile)
- Density gradient media (e.g. Ficoll-Paque or Lymphoprep), at room temperature
- Buffered saline (e.g. PBS) or RPMI-1640, at room temperature
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers
- Hemacytometer and microscope for cell counting

*Procedure (may be scaled up or down according to requirements)*

- 1. Take 50 ml fresh blood and split into 2 x 50ml Falcon tubes. Add 12.5ml PBS/RPMI-1640 to each and mix thoroughly.**
- 2. Add 10ml density gradient media to each of 4 x 50 ml universal tubes.**
- 3. Carefully overlay 25ml diluted blood on top - avoid mixing the two phases.**
- 4. Centrifuge at  $800 \times g$  for 30 minutes at room temperature with no brake.** Ensure the brake is off so that deceleration does not disrupt the density gradient.
- 5. Remove and discard the top plasma layer (~15ml) using a plastic Pasteur pipette or serological pipette.**
- 6. Draw off the lymphocyte layer at the density gradient interphase using a plastic Pasteur pipette.** Take care to minimize drawing of the density gradient media. **Transfer this fraction to fresh 30 ml tubes (one tube per original tube) and top up tube with PBS/RPMI-1640.**
- 7. Wash cells twice by centrifuging at  $400 \times g$  for 10 minutes**
- 8. Resuspend cells in culture medium or appropriate buffer for your application**
- 9. Count live cells using a hemacytometer and light microscope.** The cell count should be in the region of 1 million PBMCs for each ml of blood drawn, though this number will vary between individuals.

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