

# OptiPrep™ Application Sheet

## C43 Isolation of mononuclear cells from rat, mouse and rabbit blood on a density barrier

- ◆ **OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density of 1.32 g/ml**
- ◆ For links to other relevant files click on the double blue arrow in the following text

### 1. Background

Using a mixture of sodium metrizoate and polysaccharide ( $\rho = 1.077$  g/ml), Boyum [1] developed a one-step density-barrier technique for the isolation of human PBMCs. Subsequently the metrizoate was replaced by diatrizoate and this solution of diatrizoate and polysucrose is commercially produced by Axis-Shield as Lymphoprep™. Polysaccharides are however known to alter the mitogen responsiveness of lymphocytes [2] and for this reason an alternative medium, which omitted polysaccharide, was sought. In 1982 Bøyum [3] published a new method for the isolation of human PBMCs that employed a medium of the same density as Lymphoprep™ containing no polysaccharide. This medium containing 14.1% (w/v) Nycodenz®, 0.44% NaCl, 5 mM Tricine-NaOH, pH 7.0 ( $\rho = 1.077 \pm 0.001$  g/ml; osmolality = 295 mOsm) is commercially produced by Axis-Shield as Nycoprep™ 1.077.

This solution was subsequently modified [4] to provide a reliable and simple method for the preparation of mononuclear cells (MCs) from rodent and rabbit blood, which have a higher density than those from human blood. The solution contains 14.1 (w/v) Nycodenz®, 0.30% (w/v) NaCl, 5 mM Tricine-NaOH, pH 7.2 ( $\rho = 1.077 \pm 0.001$  g/ml; osmolality 265 mOsm). Reducing the osmolality of the solution causes the MCs, but not the polymorphonuclear leukocytes (PMNs), to gain water, thus their density decreases. Experiments showed that this strategy gives a much better resolution of the MCs from PMNs than simply using a density barrier of raised density.

This medium, called Nycoprep™ 1.077A, is no longer commercially available from Axis-Shield. However a medium of identical density and osmolality can be easily produced from OptiPrep™ and experiments have shown that the separation of the MCs is identical.

### 2. Solutions required

- A. OptiPrep™ (60%, w/v iodixanol)
- B. Buffered saline: 0.85% (w/v) NaCl, 10 mM Tricine, pH 7.0 (see Note 1)

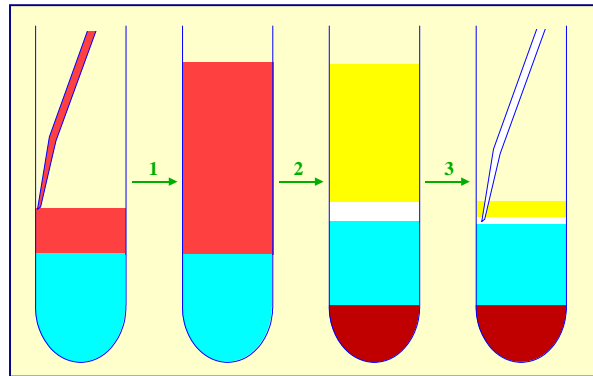
Keep Tricine as 100 mM stock solution at 4°C; 1.79g per 100 ml water.

Solution B: Dissolve 0.85g NaCl in 50 ml water; add 10 ml of Hepes or Tricine stock solution; adjust to pH 7.0 with 1 M NaOH and make up to 100 ml.

### 3. Protocol

1. Dilute Solution B with water (volume ratio 2.5:0.5 respectively); this solution has an osmolality of approx 242 mOsm (see Note 2).
2. Produce the density barrier solution by diluting OptiPrep™ with the 242 mOsm solution (volume ratio 2.7: 9.3 respectively); this solution has a density of 1.077 g/ml and osmolality approx 265 mOsm (see Note 2).
3. Collect the blood by cardiac puncture into a syringe anticoagulant; EDTA, citrate, ACD or heparin is usually satisfactory (see Note 3).
4. Dilute the blood with an equal volume of Solution B.
5. In a 15 ml centrifuge tube carefully layer 6 ml of diluted blood over 3 ml of the density barrier (avoid mixing at the interface). Alternatively the blood may be underlaid with the density barrier using a syringe and metal cannula (see Notes 4 and 5).

6. Centrifuge at 700 g for 20 min at approx. 20°C in a swinging-bucket rotor.
7. After centrifugation the MCs form a sharp band at the interface (see Figure 1).
8. Remove the plasma layer to just above MC band (Figure 1) and then recover the band of MCs. This is best achieved using a syringe attached to a metal cannula (i.d. 0.8 mm).
9. Dilute the cell harvest with 2 vol. of solution B to reduce the density of the solution; pellet the cells by centrifugation at 400 g for 10 min
10. Resuspend the MC pellet in Solution B and process as required.



**Figure 1** Isolation of mononuclear cells from rodent or rabbit blood on a 1.077 g/ml density barrier: diluted blood layered on top of iodixanol (1); after centrifugation at 700g for 20 min mononuclear cells band at interface (2) and are harvested using a pipette (3)

#### 4. Notes

1. The Tricine may be replaced by any suitable organic buffer (e.g. Hepes)
  2. If an osmometer is available check the osmolality of these solutions. The dilutions should be prepared as accurately as possible. If the density of the barrier solution is too high poor sedimentation of the erythrocytes may be observed.
  3. Rat blood in particular is prone to coagulation and higher concentrations of anticoagulant than those used for human blood may be required. We have found for example that the final concentration of EDTA should be 3-4 mM.
  4. For other blood volumes keep to a ratio of diluted blood to density barrier of 2:1. For small volumes of mouse blood use a smaller volume (narrower) tube.
  5. Flat-tipped metal cannulas can be purchased from many surgical instrument companies.
- ◆ MCs from rat and mouse blood may also be isolated by flotation using OptiPrep™, see respectively OptiPrep™ Applications Sheets C6 →→ and C7 →→
  - ◆ Sedimentation of MCs onto a 1.077 g/ml barrier with a reduced-osmolality has been widely reported for rat blood [5-14] and mouse blood [15-24] but rather less for rabbit blood [25].

#### 6. References

- ◆ Refs. 1-4 describe background methodology.  
To access abstracts of refs 1-25 (file CA43) click on the double blue arrow →→
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