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
- **OptiPrep™ Application Sheet C03** "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a methodological review" compares all of the currently available methodologies
- **OptiPrep™ Reference List RC01** "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a bibliographical review" provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™
- To access **C03 and RC01** return to the initial list of Folders and select "**Application Sheets**" or "**Reference Lists**". To access other Application Sheets referred to in the text, return to the Cell Index; key Ctrl "F" and type the C-Number in the Find Box

1. Background

In 1982 Bøyum [1] 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 (ρ = 1.077 ± 0.001 g/ml; osmolality = 295 mOsm) is no longer commercially produced. This solution was subsequently modified [2] to provide a 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 contained 14.1% (w/v) Nycodenz®, 0.30% (w/v) NaCl, 5 mM Tricine-NaOH, pH 7.2 (ρ = 1.077 ± 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. This strategy gives better resolution of the MCs from PMNs than using a barrier of raised density.

This medium, which was commercially produced by Axis-Shield as Nycoprep™ 1.077A, is no longer available. However a medium of identical density and osmolality can be easily produced from OptiPrep™.

2. Solution preparation (see Note 1)

A. OptiPrep™ (60%, w/v iodixanol) – shake the bottle gently before use

B. Buffered saline (isoosmotic): 0.85% (w/v) NaCl, 10 mM Tricine, pH 7.0

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

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

3. Protocol

1. Make up the density barrier: Dilute Solution B with water (volume ratio 2.5:0.5 respectively); this solution has an osmolality of approx 242 mOsm. Dilute OptiPrep™ with this solution using a volume ratio 2.7: 9.3 respectively (see Note 2).

2. Collect the blood by cardiac puncture into a syringe containing anticoagulant; EDTA, citrate, ACD or heparin is usually satisfactory (see Note 3).

3. Dilute the blood with an equal volume of Solution B.

4. 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).

5. Centrifuge at 700 *g* for 20 min at approx. 20°C in a swinging-bucket rotor.

6. After centrifugation the MCs form a sharp band at the interface (see Figure 1).

7. 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).

8. 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

9. Resuspend the MC pellet in Solution B and process as required.

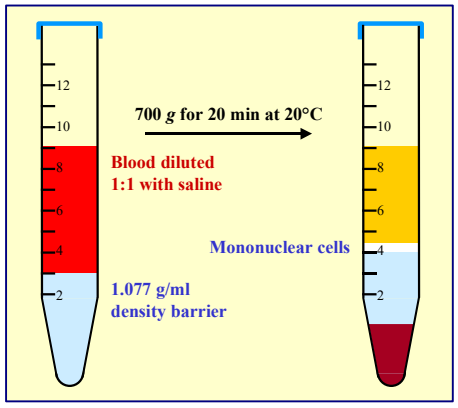

Figure 1 Isolation of mononuclear cells from rodent or rabbit blood on a 1.077 g/ml density barrier

4. Notes

1. The Tricine in the saline solutions may be replaced by any suitable organic buffer (e.g. HEPES)
2. If an osmometer is available check the osmolality of these solutions; it should be 265 mOsm (± 10 mOsm). The dilutions should be prepared as accurately as possible. If there is significant loss of mononuclear cells to the pellet, try increasing the density of the barrier slightly (2.8 vol. of OptiPrep™ and 9.2 vol. of the diluted saline).
3. Rat blood in particular is prone to coagulation and higher concentrations of anticoagulant than those used for human blood may be required. We recommend the use of EDTA and have found 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 **Applications Sheets C07 and C08**

5. References

1. Bøyum, A., Berg, T. and Blomhoff, R. (1982) *Fractionation of mammalian cells* In: Iodinated density gradient media - a practical approach (ed D. Rickwood) IRL Press at Oxford University Press, Oxford, UK, pp 147-171
2. Bøyum, A., Lovhaug, D., Tresland, L. and Nordlie, E.M. (1991) *Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality* Scand. J. Immunol., **34**, 697-712

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