OptiPrep™ Application Sheet C46

Isolation of monocytes from a human leukocyte-rich plasma on a density barrier

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density of 1.32 g/ml
- The **OptiPrep™ Application Sheet C03 "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes: a methodological review"** compares all of the currently available methodologies for human leukocytes. See **Applications Sheets C10 and C11** for other monocyte methods.
- A **Reference List (RC01)** of all the published papers reporting the use of OptiPrep™ is also available: to access return to the initial list of Folders and select "**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

The monocytes in human peripheral blood, account for, on average, about 8% of the leukocyte population. They tend to be larger (15-20 µm) than lymphocytes (6-20 µm) and they also have a slightly lower density. These properties allow some scope for their separation by centrifugation. Boyum et al [1,2] introduced a Nycodenz® density barrier (ρ = 1.068 g/ml) for resolving monocytes and lymphocytes from a leukocyte-rich plasma (LRP). It has a slightly raised osmolality (335 mOsm); this enhances the density difference between the monocytes and the osmotically-sensitive lymphocytes, whose density is increased. The method is very effective and the purity of the monocytes is greater than 90% but the monocytes do not form a distinct band; they are concentrated in the upper half of a broad turbid zone within the density barrier (see Figure 1 on page 2).

This medium, which was commercially produced by Axis-Shield as Nycoprep™ 1.068, is no longer available. However a medium of identical density and osmolality can be easily produced from OptiPrep™.

2. Solution preparation (see Section 4, 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-NaOH, pH 7.0

C. Buffered saline: 1.05% (w/v) NaCl, 10 mM Tricine-NaOH, pH 7.0

D. Polysucrose: 6% (w/v) polysucrose (M_r = 400-500 x 10^3) in Solution B (optional, see Step 2 of the Protocol)

Keep Tricine as 100 mM stock solution at 4°C; 1.79g per 100 ml water.
Solutions B and C: Dissolve 0.85g (or 1.05 g) 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

3a. Make up the density barrier

Mix 0.12 vol. of OptiPrep™ 0.48 vol. of Solution D. If an osmometer is available check the osmolality of the density barrier solutions; it should be 335 ± 10 mOsm and the density 1.068 ± 0.001 g/ml. Volumes must be dispensed as accurately as possible; any deviation from the recommended osmolality and/or density will affect the separation. Particular attention must be made to dispensing the OptiPrep™, because of its viscosity.

3b. Prepare the LRP

Always use freshly drawn, whole blood (anti-coagulant 1.5-2.0 mM EDTA). The leukocyte-rich plasma may be prepared as a buffy coat fraction or by polysucrose sedimentation of the erythrocytes.

Buffy coat: Centrifuge the blood at 400 *g* in a swinging-bucket rotor, at about 20°C, for 10-15 min. Harvest the buffy coat layer, on the top of the erythrocytes, in the plasma supernatant (LRP). Some

erythrocytes will also be collected but try to keep them to a minimum. Over 80% of the leukocytes can be recovered in this manner.

Erythrocyte aggregation: Mix 9 vol. of blood with 1 vol. of Solution F by gentle inversions and allow the aggregated erythrocytes to settle to the bottom (20-40 min at room temperature); then aspirate the entire supernatant.

3c. Monocyte separation

In a 15 ml centrifuge tube carefully layer 6 ml of LRP over 3 ml of the density barrier (avoid mixing at the interface). Alternatively the LRP may be underlaid with the density barrier using a syringe and metal cannula (see Section 4, Note 2) and centrifuge at 600 *g* for 15 min at approx. 20°C in a swinging-bucket rotor. Allow the rotor to decelerate without the brake (see Section 4, Note 3).

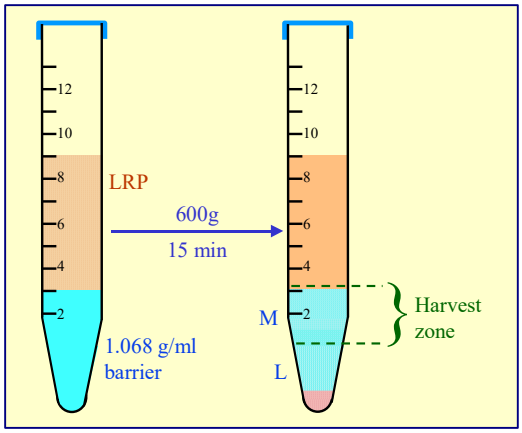

Figure 1 Separation of monocytes from LRP on a density barrier: M = monocytes, L = lymphocytes

After centrifugation the monocytes are contained in the top half of the turbid layer beneath the interface (Figure 1). Aspirate the plasma supernatant to about 3 mm above the barrier interface (Figure 1). Then very slowly aspirate the remaining plasma and the upper half of the turbid layer ("Harvest zone in Figure 1). This is best achieved using a syringe attached to a metal cannula (i.d. 0.8 mm) as the aspiration must be carried out very slowly to avoid drawing up the lymphocytes from the bottom half of the turbid layer (see Section 4, Note 4). The pellet will contain lymphocytes, polymorphonuclear leukocytes and residual erythrocytes.

Dilute the cell harvest with 2 vol. of solution C to reduce the density of the solution; pellet the cells by centrifugation at 400 *g* for 10 min and resuspend the monocyte pellet in Solution B or as required.

4. Notes

1. The Tricine in the saline solutions may be replaced by any suitable organic buffer (e.g. HEPES).
2. For other LRP volumes keep to a ratio of LRP to density barrier of 2:1.
3. To retain the resolution of the monocytes and lymphocytes it is essential that the density barrier layer is disturbed as little as possible after the centrifugation.
4. Flat-tipped metal cannulas can be purchased from many surgical instrument companies.

5. References

1. Bøyum, A., Berg, T. and Blomhoff, R. (1983) *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. (1983) *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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