

OptiPrep™ Application Sheet C04

Isolation of mononuclear cells from human blood by sedimentation on to a density barrier

- ◆ OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 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” provides a comprehensive list of all the published papers reporting the use of OptiPrep™
- ◆ To access **RC01** 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

A simple, effective method for the isolation of peripheral blood mononuclear cells (PBMCs) from human blood was first reported by Boyum in the mid-sixties [1]. Since then, the commercial medium known as Lymphoprep™, which contains sodium diatrizoate (9.6% w/v) and a polysaccharide (5.6% w/v), has been widely used for isolating the PBMCs. This simple isoosmotic density barrier (1.077 g/ml), separates the mononuclear cells from the denser polymorphonuclear leukocytes and erythrocytes. The polysaccharide aggregates the erythrocytes to increase their rate of sedimentation.

It is however well established that the polysaccharide may interact with the surface of lymphocytes. Moreover, the presence of an impermeant ion (diatrizoate) in the medium may also affect the Gibbs-Donnan equilibrium of ions across the membrane. A non-ionic derivative of diatrizoate (Nycodenz®), was therefore developed. An iso-osmotic solution of 14.1% (w/v) Nycodenz®, 0.44% NaCl and 5 mM Tricine-NaOH, pH 7.0 [2,3], with the same density as Lymphoprep™ separates the PBMCs in exactly the same manner. Omission of a polysaccharide requires a slightly longer centrifugation time to achieve satisfactory pelleting of the erythrocytes.

Identical separations can be obtained by replacing Nycodenz® with iodixanol. Because iodixanol is available as a 60% (w/v) solution in water ($\rho = 1.32$ g/ml) with no additives (OptiPrep™), the 1.077-1.078 g/ml density barrier can be made up in the operator’s own choice of buffer and additives. The routine OptiPrep™ diluent for cells is usually 0.85% (w/v) NaCl containing 10 mM of a suitable buffer; this is normally either Hepes-NaOH or Tricine-NaOH.

In an alternative strategy for the isolation of human PBMCs, the plasma itself is adjusted to a density of 1.077 g/ml cells; consequently during the centrifugation the PBMCs float to the surface of the plasma. The efficacy of this technique appears to be less species-sensitive than the density barrier strategy. This technique is described in **Application Sheet C05**. A modification of this flotation strategy allows the isolation of human PBMCs that are contaminated neither by platelets nor plasma. This is described in **Application Sheet C06**.

- ◆ This Application Sheet describes the preparation of the 1.077 g/ml barrier from OptiPrep™.

2. Choice of anticoagulant

EDTA (final concentration 1.5-2.0 mM) is the anticoagulant of choice. Both citrate and heparin are acceptable but, for reasons that are unclear, heparin is more likely to cause less than optimal separations with some blood samples.

3. Solution preparation

- A. OptiPrep™ (60%, w/v iodixanol)
- B. Saline solution: 0.85% (w/v) NaCl, 10 mM Hepes (or Tricine) at pH 7.0-7.6 (see Note 1)

Keep Hepes (free acid) or Tricine as a 100 mM stock solution at 4°C; Hepes (2.38g) or Tricine (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 7.4 with 1 M NaOH and make up to 100 ml.

Shake the OptiPrep™ bottle gently before use and make up the density barrier using 5 vol. of OptiPrep™ + 17 vol. of Solution B (see Note 2).

4. Protocol

1. Collect human blood by venepuncture into a suitable anticoagulant; e.g. mix 10 ml of blood gently with 150µl of 100 mM di-potassium EDTA. Then dilute with an equal volume of Solution B (see Note 3).
2. Deliver 3 ml of the barrier solution into a 15 ml conical tube; then layer 6 ml of the diluted blood on top. To achieve a sharp interface, tilt the tube and deliver the blood from a 10 ml plastic syringe attached to a metal filling cannula (see Notes 4 and 5 and Figure 1).
3. Centrifuge at 700 g for 20 min at 20°C (see Notes 6-8).
4. Harvest the PBMCs from the interface (see Figure 2 and Notes 9 and 10).

5. Notes

1. Any balanced salt solution or culture medium may be used as Solution B.
2. OptiPrep™ is quite viscous; when withdrawing an aliquot into an automatic pipette do this slowly and likewise, expel it slowly into the mixing vessel.
3. High yields (>95%) of PBMCs are only obtained if the whole blood is diluted with saline. With undiluted blood yields are reduced to <85% because the interface between the sample and the medium is less stable and there is a tendency for the blood cells to "stream" through the medium, carrying erythrocytes and mononuclear cells into the pellet.
4. Wide-bore stainless-steel filling cannulas (i.d. approx 0.8 mm) are readily available from surgical equipment supplies companies. By tilting the tube and positioning the tip of the cannula 1-2 cm above the density barrier, a more or less continuous stream of blood can be maintained, thus producing a sharp interface (see Figure 1).
5. Larger volumes of diluted blood (e.g. 8-9 ml) are permissible, but it may be necessary to increase the centrifugation time by approx. 5 min; the cells at the top of the sample will be exposed to a lower g-force than in the 3+6 ml format. In a 50 ml tube, use 10 ml of barrier and 20 ml of diluted blood.
6. It is recommended that Lymphoprep™ separations be carried out at 800 g for 20 min; with this iodixanol barrier 700 g is sufficient; the presence of a polysaccharide in Lymphoprep™ makes the solution more viscous, hence the higher recommended g-force.
7. The separation may be carried out equally effectively at 4°C, but it may be necessary to increase the centrifugation time by 5 min to overcome the slightly raised viscosity of the density barrier at the lower temperature.
8. Do not use the brake to decelerate the rotor. Rapid changes in the rpm create a vortex in the liquid and "swirling" of the pellet and banded cells.

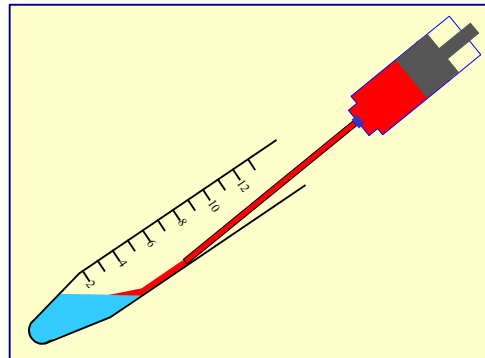


Figure 1. Layering of blood from a syringe on to a density barrier

9. The cells will be contaminated with platelets from the plasma above the cells. Partial removal of platelets from human PBMCs can be carried out by pelleting the cells preferentially at a low RCF, 250-300 g for 10 min (no brake). The cells can be resuspended in saline and the washing process repeated. At these low g-forces the pellet is very loosely-packed and great care must be taken during aspiration of the supernatant to avoid losing cells. Moreover pelleting and resuspending any cells is potentially damaging to the cells and should be avoided if possible..
10. If complete removal of platelets is important, the PBMCs harvested from the barrier interface should be diluted with an equal volume of Solution B (or the plasma) and the platelets separated on a 1.063 g/ml density barrier (see [Application Sheet C13](#)).

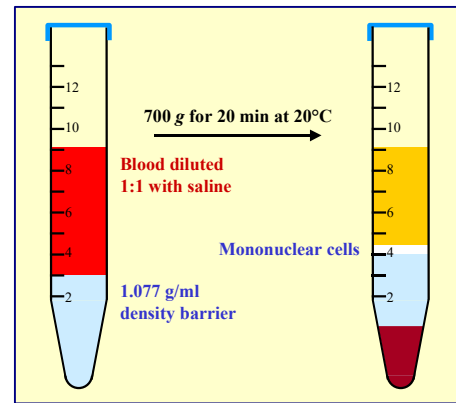


Figure 2: Isolation of PBMCs: diluted blood layered on top of iodixanol density barrier

6. References

1. Boyum, A. (1968) *Isolation of mononuclear cells and granulocytes from human blood: Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1g* Scand. J. Clin. Lab. Invest., **21** (Suppl. 97), 77-89
2. Ford, T. C. and Rickwood, D. (1982) *Formation of isotonic Nycodenz gradients for cell separations* Anal. Biochem. **124**, 293-298
3. 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

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