OptiPrep™ Application Sheet C05

Isolation of human peripheral blood mononuclear cells by flotation (low density iodixanol density barrier)

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **Axis-Shield Mini-Review (MC01)** "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a methodological review" compares all of the currently available methodologies
- **Axis-Shield Mini-Review (MC02)** "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 **MC01 and MC02** return to the initial list of Folders and select "**Mini-Reviews**".
- 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 isolation of human peripheral blood mononuclear cells (PBMCs) presented in OptiPrep™ Application Sheets C03 and C04 represent two approaches to PBMC purification in a non-ionic medium without polysaccharide. Application Sheet C03 describes a "traditional" approach of layering the blood over a ρ = 1.077 g/ml density barrier. Application Sheet C04 describes a simpler ("mixer") approach in which the blood is adjusted to ρ = 1.077-1.078 g/ml and the PBMCs allowed to float to the surface. This Application Sheet presents a third alternative in which the blood is adjusted to a density considerably higher than that of the PBMCs (ρ = 1.095 g/ml) and layered beneath a ρ = 1.078 g/ml density barrier. As with the mixer technique, the PBMCs float to the surface, but this is the only system in which the cells do not band adjacent to the plasma-containing sample layer. The low-density barrier acts as a "buffer-zone" which "washes" the PBMCs free of soluble plasma proteins and particulate contaminants such as platelets at the same time as they are purified from other blood cells.

2. Solutions required (see Note 1)

A. OptiPrep™ (shake gently before use)

B. Diluent: 0.85% (w/v) NaCl, 30 mM Tricine-NaOH, pH 7.4 (for Working Solution only)

C. Tricine-buffered saline (TBS): 0.85% NaCl, 10 mM Tricine-NaOH, pH 7.4

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

Dissolve 0.85 g NaCl in 50 ml water; add 30 ml or 10 ml Tricine stock (for Solution B or C respectively); adjust to pH 7.4 with 1 M NaOH and make up to 100 ml

3. Protocol

1. Make a Working Solution of 40% (w/v) iodixanol: mix 4 ml of OptiPrep™ and 2 ml of Solution B.

2. Adjust the plasma of whole blood to approx ρ = 1.095 g/ml by adding 2.7 ml of the Working Solution to 10 ml of whole undiluted blood (see Notes 2 and 3).

3. Prepare the ρ = 1.078 g/ml density barrier solution by diluting 5 ml of Working Solution with 9.6 ml of Solution C.

4. Using a syringe and metal cannula underlayer 5 ml of the density barrier with 5 ml of blood in a 15 ml centrifuge tube (see Note 4).

5. Layer approx 0.5 ml of Solution C on top (see Note 5) and centrifuge at 700 g_{av} for 20 min at 20°C.

6. The PBMCs band on the top of the 1.078 g/ml barrier (see Figure 1). Remove the band with a pipette or syringe and metal cannula.

7. To pellet the cells, dilute the suspension with an equal volume of Solution C and centrifuge at 400 *g* for 10 min (see Notes 6 and 7).

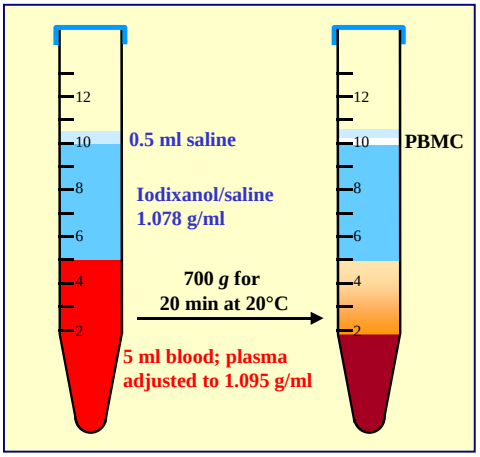

Figure 1 Isolation of human PBMCs by flotation through a low-density barrier

4. Notes

1. The composition of the diluents can be tailored to suit the operator's own requirements so long as the density remains approx 1.006 g/ml. Tricine-NaOH buffers are used in the protocol but any suitable buffer may be substituted. Strategies for preparing Working Solutions for cells are described in Application Sheet C01.
2. OptiPrep™ can be mixed with whole blood directly, but a buffered Working Solution containing 40% (w/v) iodixanol (ρ = 1.216 g/ml) is the recommended option.
3. A minor modification to this method has been investigated [1] in which the blood plasma was adjusted to 1.1 g/ml rather than 1.095 g/ml. This seemed beneficial to the recovery of PBMCs, but only from those samples whose erythrocytes sedimented at this higher density. If most of the erythrocytes floated up to the bottom of the 1.078 g/ml layer, then the recovery of PBMCs was marginally worse.
4. For more information on layering of gradient solutions see Application Sheet C02.
5. It is recommended that a small volume of saline be layered on top of the 1.078g/ml layer: this facilitates harvesting of the PBMCs and avoids their banding at a water/air interface. It is not however critical in any way to the separation.
6. In an in-depth survey of PBMC isolation methods for proteomic analysis Roos et al [2] reported that the yield of PBMCs by this method was as good as the standard density-barrier sedimentation and the contamination by platelets the lowest.
7. If contamination from lymphocytes is not a problem, this flotation method is sometimes used in studies of monocyte function (e.g. see ref 3)

5. References

1. Ahmed, Y., Walton, L. J. and Graham, J. M. (2004) *An improved method for isolation of mononuclear cells from peripheral blood* 12th Int. Congr. Immunol., Abstr. 1758
2. De Roos, B., Duthie, S.J., Polley, A.C.J., Mulholland, F., Bouwman, F.G., Heim, C., Rucklidge, G.J., Johnson, I.T., Mariman, E.C., Daniel, H. and Elliott, R.M. (2008) *Proteomic methodological recommendations for studies involving human plasma, platelets and peripheral blood mononuclear cells* J. Proteome Res., **7**, 2280-2290
3. Hartrick, C.T. (2002) *Increased production of nitric oxide stimulated by interferon-γ from peripheral blood monocytes in patients with complex regional pain syndrome* Neurosci. Lett., **323**, 75-77.

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