

OptiPrep™ Application Sheets

C5 Isolation of mononuclear cells from peripheral blood and from bone marrow by flotation through a density barrier

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml.

Background

The isolation of human peripheral blood mononuclear cells (PBMCs) presented in OptiPrep™ Application Sheets C3 and C4 represent two approaches to PBMC purification in a non-ionic medium without polysaccharide - C3 is a traditional approach of layering the blood over a $\rho = 1.077$ g/ml density barrier and C4 is a simpler approach in which the blood is adjusted to $\rho = 1.077$ - 1.078 g/ml (mixer) 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 ($\rho = 1.090$ g/ml) and layered beneath a $\rho = 1.078$ g/ml density barrier. As with the mixer, 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. The method [1] has also been adapted to bone marrow (see Note 1).

- ◆ OptiPrep™ can be mixed with whole blood directly, but a buffered Working Solution containing 40% (w/v) iodixanol ($\rho = 1.216$ g/ml) is the recommended option.
- ◆ Tricine-NaOH buffer is used in the protocol but any suitable buffer may be substituted. Strategies for preparing Working Solutions for cells are described in OptiPrep™ Application Sheet C1.

Solutions required

- OptiPrep™ (shake gently before use)
- Diluent: 0.85% (w/v) NaCl, 30 mM Tricine-NaOH, pH 7.4 (for Working Solution only)
- Tricine-buffered saline (TBS): 0.85% NaCl, 10 mM Tricine-NaOH, pH 7.4 (see Note 2)

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

Protocol

- Make a Working Solution of 40% (w/v) iodixanol: dilute 4 ml of OptiPrep™ with 2 ml of Solution B.
- Adjust the plasma of whole blood to approx $\rho = 1.095$ g/ml by adding 2.7 ml of the Working Solution to 10 ml of whole undiluted blood (see Notes 3 and 4).
- Prepare the $\rho = 1.078$ g/ml density barrier solution by diluting 5 ml of Working Solution with 9.6 ml of Solution C.
- 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 5).
- Layer approx 0.5 ml of TBS on top (see Note 6) and centrifuge at $700g_{av}$ for 20 min at 20°C.
- The PBMCs band on the top of the 1.078 g/ml barrier (see Figure 1). Remove the band with a pipette (see Note 6).
- To pellet the cells, dilute the suspension with an equal volume of TBS and centrifuge at 400g for 10 min.

Notes

1 An identical flotation strategy has been developed by Ruijs [1] for the isolation of mononuclear cells from bone marrow and found to be superior to the routine Percoll® method. The bone marrow sample was adjusted to 1.085 g/ml and a 1.073 g/ml solution and isotonic buffer layered on top. Although Ruijs [1] used a commercial preparation of iodixanol which also contained polysucrose, there is no reason to suppose that OptiPrep™ could not be used as the iodixanol source.

2 The composition of the diluent can be tailored to suit the operator's own requirements so long as its density remains approx 1.006 g/ml.

3 See OptiPrep™ Application Sheet C1 for more information about the dilution of OptiPrep™ for the preparation of density solutions and adding to cell suspensions.

4 A minor modification to this method has been investigated [2] 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.

5 For more information on the layering of gradient solutions see “Types of Centrifugal Separations” in the main Axis-Shield Catalogue “Applications and Products” and ref 3.

6 It is recommended that a small volume of saline is 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.

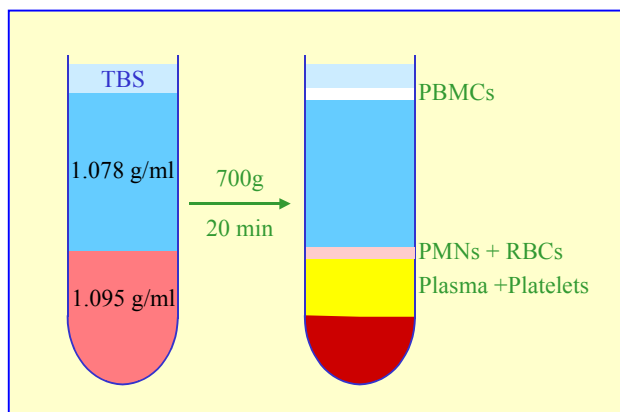


Figure 1 Isolation of human PBMCs by flotation through a low density barrier. PBMC = peripheral blood mononuclear cells, PMN = polymorphonuclear leukocyte, RBC = red blood cell

References

1. Ruijs, W. P. M. (2000) *Int. Soc. Hematother. Graft. Eng., San Diego 2000*, Abstr. #024
2. Ahmed, Y., Walton, L. J. & Graham, J. M. (2004) *12th International Congress of Immunology, Montreal* Abstr. #1758.
3. Graham, J. M. (2001) in *Biological Centrifugation*, pp 61-84. Taylor and Francis Books Ltd, Oxford, UK.