# OptiPrep™ Application Sheet C08

## Isolation of mononuclear cells from mouse blood by flotation (iodixanol mixer technique)

- 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" 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

Standard human peripheral blood mononuclear cell (PBMC) isolation media such as Lymphoprep™ are less effective for the isolation of these cells from the blood of certain experimental animals because of the slightly higher density of the PBMCs from mice, rats and rabbits. Some commercial media address this problem by having a correspondingly raised density. This simple solution however fails to address the simultaneous problem that the density of the polymorphonuclear leukocytes (PMNs) is the same. Thus although recoveries of PBMCs are satisfactory, contamination from PMNs can be significant. The alternative strategy solves this problem by maintaining the density at 1.077 g/ml, while reducing the osmolality of the medium from 295 mOsm to 265 mOsm. The density of the osmotically-sensitive PBMCs is thus reduced to a value less than 1.077 g/ml, while the density of the other cells is unaffected. In this manner, the difference in density between the PBMCs and the PMNs is enhanced and the cells behave essentially the same as those from human blood [1]. For more details see **Application Sheet C43**.

Human PBMCs may also be isolated by flotation: the method involves adjustment of the density of the plasma of whole blood to approx 1.078 g/ml by addition of a dense solution, which allows cells with a density lower than 1.078 g/ml to float to the surface during the centrifugation [2]. Initially this method was carried out using Nycodenz® but was subsequently adapted to the use of OptiPrep™. This flotation strategy, for reasons that are not clear, allows satisfactory separation of PBMCs and PMNs from other species without modulation of the osmolality; it seems not to be species-sensitive.

### 2. Solutions required

**A.** OptiPrep™ (shake gently before use)

**B.** Tricine-buffered saline (TBS): 0.85% NaCl, 10 mM Tricine-NaOH, pH 7.4 (see Note 1)

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

Solution B: Dissolve 0.85 g NaCl in 50 ml water; add 10 ml of Tricine stock; adjust to pH 7.4 with 1 M NaOH and make up to 100 ml

### 3. Protocol

1. Anaesthetize the animal with $CO_2$ and collect the blood (0.5-1.0 ml) by cardiac puncture into a 2 ml syringe containing 0.1 ml of 3.8% (w/v) citrate as anticoagulant.

2. For 0.25-0.5 ml of blood: To 5.0 ml of Solution B, add 1.5 ml of OptiPrep™, and mix well. Then add 5.0 ml of this medium to the mouse blood by gentle and repeated inversion.

3. For 5 ml of blood: Dilute with 2.5 ml of Solution B and then mix with 1.25 ml of OptiPrep™.

4. Transfer the blood to a suitable capped tube; layer 0.5 ml Solution B on top (see Figure 1) and centrifuge at 1000 $g_{av}$ for 30 min at 20°C (see Note 2).

5. Collect the PBMCs from the meniscus downwards to about 0.5 cm from the cell pellet (Figure 1).
6. Dilute the suspension with two volumes of Solution B and pellet the cells at 300-400 *g* for 5-10 min (see Notes 3 -5).

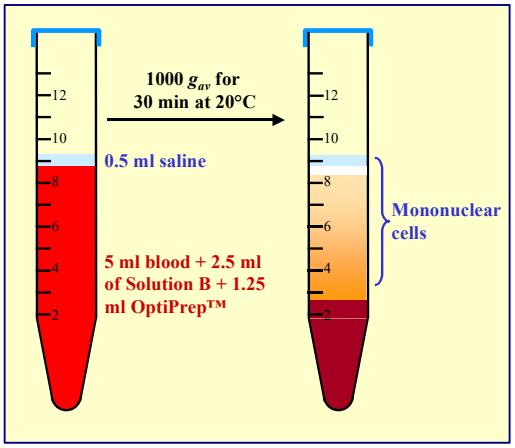


**Figure 1** Isolation of mononuclear cells from 5 ml of mouse blood

## 4. Notes

**1** Any suitable buffer may be used, but Tricine is the buffer of choice for many cell types.

**2** The small volume of saline on top of the sample is not required for the fractionation, but it facilitates harvesting the PBMCs, from the top of the plasma. It also prevents the cells from collecting at, and adhering to, the walls of the tube at the meniscus.

**Table 1 PBMCs recovered from 0.5 ml and 0.25 ml of whole blood in two separations**

| Vol. of blood | Vol. of Saline-OptiPrep™ | PBMCx$10^{-5}$ |
|---|---|---|
| 0.50 ml | 5.0 ml | 12.32, 8.70 |
| 0.25 ml | 5.0 ml | 5.80, 4.10 |

**3** Total recoveries of PBMCs from two experiments at two different blood volumes (from single animals) are given in Table 1.

**4** As with the purification of human PBMCs by this method the cells will be contaminated with platelets in the plasma. 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). The cells can be resuspended in saline and the washing process repeated. Whether this is a satisfactory method for mouse PBMCs is not clear.

**5** Complete removal of platelets from human PBMCs can be achieved by dilution with an equal volume of Solution B; layering over an equal volume of iodixanol, ρ = 1.063 g/ml, (5 vol. OptiPrep™ + 22 vol. Solution B) and centrifugation at 350 *g* for 15 min at 20°C. The platelets form a wide band just below the interface; the entire liquid is aspirated and the PBMC pellet resuspended in a suitable medium. For more details see Application Sheet C12. The method has been successfully used with rodent cells.

## 5. References

1 Boyum, 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

2 Ford, T. C. and Rickwood, D. (1990) *A new one-step method for the isolation of human mononuclear cells* J. Immunol. Meth., **134**, 237-241