# OptiPrep™ Application Sheet C07

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

- OptiPrep™ is a 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

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. The density of the PBMCs from mice, rats and rabbits is apparently slightly higher than that from humans. Some commercial media simply 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].

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 and has now been successfully applied to rat blood using OptiPrep™.

- OptiPrep™ can either be mixed with whole blood directly or if preferred a buffered Working Solution containing 37% (w/v) iodixanol ($\rho$ = 1.199 g/ml) can be added.
- 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 **Application Sheet C01**.

### 2. Solutions required

**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

**D.** Working Solution of 37% iodixanol: mix 3.7 vol. of OptiPrep™ with 2.3 vol. of Solution B (optional).

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 of Tricine stock (for Solutions B or C respectively); 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 (approx 10 ml) by cardiac puncture into a 10 ml syringe containing 1 ml of 3.8% (w/v) citrate as anticoagulant.

2. Mix 5 ml of whole rat blood gently but thoroughly (by repeated inversion) with 0.625-0.63 ml of OptiPrep™ or 1.25-1.26 ml of Solution D, in a suitable capped centrifuge tube (see Note 1).

3. Layer a small volume (0.5 ml) of Solution C on top (see Figure 1 and Note 2).

4. Centrifuge at 1300 *g* for 30 min at 20°C. Collect the PBMCs from the meniscus downwards to about 0.5 cm from the cell pellet (see Figure 1).

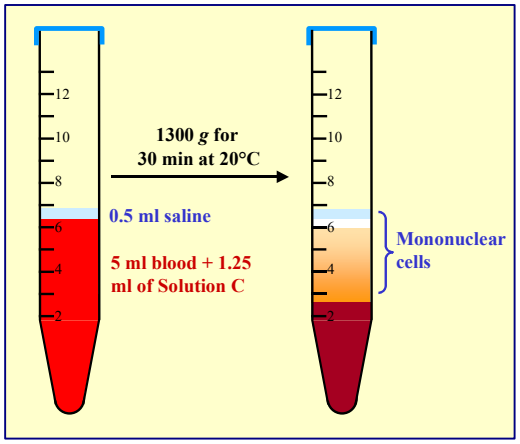


**Figure 1** Flotation of rat mononuclear cells through density-modulated plasma

5. Dilute the collected material with two volumes of buffered-saline and pellet the cells at 250-500 *g* for 5-10 min (see Notes 3 and 4).

## 4. Notes

**1** Larger volumes of blood can be processed if the ratio of blood to added OptiPrep™ is kept constant.

**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.

**3** Recoveries of PBMCs from two experiments at two different blood volumes are given in Table 1.

| Vol. of blood | Vol. of OptiPrep™ | PBMCx$10^{-6}$ |
|---|---|---|
| 5.0 ml | 0.63 ml | 8.25, 13.77 |
| 10.0 ml | 1.25 ml | 25.8, 29.4 |

**Table 1:** PBMCs recovered from 5 ml and 10 ml whole blood in two separations

**4** As with the purification of human PBMCs (see **Application Sheet C04)** 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). A more efficient method for removing platelets from human PBMCs is described in **"Platelets (human)" Application Sheet C13 in index**. 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



serumwerk bernburg
Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwwerk.com • serumwerk.de