

OptiPrep™ Application Sheets

C7 Isolation of mouse peripheral blood mononuclear cells by flotation (mixer technique)

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

Background

Standard human peripheral blood mononuclear cell (PBMC) isolation media such as Lymphoprep™ or Nycoprep™ 1.077 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 Axis-Shield medium, Nycoprep™ 1.077A (which contains Nycodenz®), 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].

An alternative strategy to the sedimentation of human PBMCs on to a density barrier is to adjust the density of the plasma of whole blood to approx 1.078 g/ml and then allow these cells 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 which 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 mouse blood using OptiPrep™.

Solutions required

- OptiPrep™ (shake gently before use)
- 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

Protocol

- Anaesthetize the animal with CO₂ 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.
- For 0.25-0.5 ml of blood:** To 5.0 ml of TBS 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.
- For 5 ml of blood:** Dilute with 2.5 ml of TBS and then mix with 1.25 ml of OptiPrep™.
- Transfer the blood to a suitable capped tube; layer 0.5 ml TBS on top (see Figure 1) and centrifuge at 1000g_{av} for 30 min at 20°C (see Note 2).
- Collect the PBMCs from the meniscus downwards to about 0.5 cm from the cell pellet as shown in Figure 1.
- Dilute the suspension with two volumes of TBS and pellet the cells at 300-400g for 5-10 min (see Notes 3 -5).

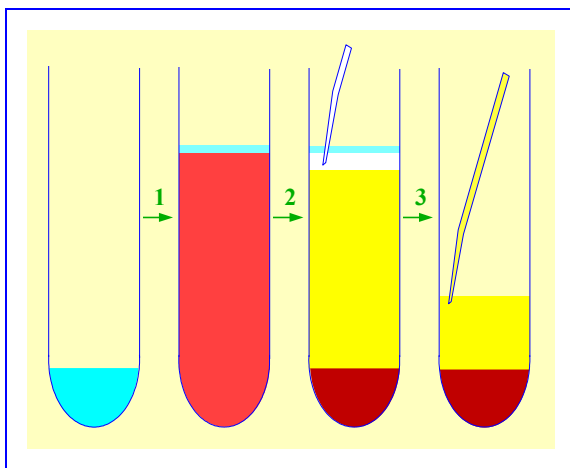


Figure 1 Isolation of PBMCs from mouse blood by flotation. (1) Whole blood mixed with OptiPrep™ (or WS) and TBS layered on top. (2) After centrifugation band of PBMCs is observed at interface. (3) Cells harvested from the meniscus downwards.

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.

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 (see OptiPrep™ Application Sheet C4) 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 (250g for 10 min). The cells can be resuspended in saline and the washing process repeated. Whether this is a satisfactory method for mouse PBMCs has not been tested.

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, $\rho = 1.063 \text{ g/ml}$, (5 vol. OptiPrep™ + 22 vol. Solution B) and centrifugation at 350g 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 OptiPrep™ Application Sheet C12. Whether this is a satisfactory method for mouse PBMCs has not been tested.

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™	PBMC $\times 10^{-5}$
0.50 ml	5.0 ml	12.32, 8.70
0.25 ml	5.0 ml	5.80, 4.10

References

- 1 Boyum, A., Lovhaug, D., Tresland, L. & Nordlie, E.M. (1983) *Scand. J. Immunol.*, **34**, 697-712.
- 2 Ford, T.C. & Rickwood, D. (1990) *J. Immunol. Methods*, **134**, 237-241.