

OptiPrep™ Application Sheet C05

Isolation of human peripheral blood mononuclear cells by flotation (iodixanol mixer technique)

- ◆ OptiPrep™ is a sterile 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

The most commonly used technique for the isolation of peripheral blood mononuclear cells (PBMCs) from human blood is to centrifuge whole blood (diluted 1:1 with saline) over an isoosmotic 1.077 g/ml density barrier. For more information see “**Mononuclear cells**” - **Application Sheet C04 in index**. An alternative strategy devised by Ford and Rickwood [1] simplifies the procedure. A 19% (w/v) Nycodenz® solution ($\rho = 1.100$ g/ml), produced commercially as NycoPrep™ Mixer, was added to an equal volume of whole blood to raise the density of the plasma to 1.077 g/ml. During centrifugation at 1500 g for 30 min at 20°C the erythrocytes and polymorphonuclear leukocytes (PMNs) sediment while the PBMCs float to the top and are recovered from the meniscus and the medium below it. NycoPrep™ Mixer is no longer available but the technique has been adapted very successfully to the use of OptiPrep™ and is described below.

- ◆ OptiPrep™ can 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. Strategies for preparing Working Solutions for cells are described in Application Sheet C01.

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

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

Table 1 Volumes of blood and OptiPrep or WS

Blood vol.	OptiPrep	WS
5 ml	0.625 ml	1.25 ml
10 ml	1.25 ml	2.5 ml
20 ml	2.5 ml	5.0 ml

3. Protocol

1. If using a 37% (w/v) iodixanol Working Solution: mix 3.7 vol of OptiPrep™ with 2.3 vol of Solution B.
2. Mix whole blood gently but thoroughly (by repeated inversion) with OptiPrep™ or the Working Solution (WS) according to Table 1 (see Notes 1-3) in a suitable capped centrifuge tube (e.g. 15 ml tubes for 5-12 ml samples).
3. Layer approx 0.5 ml of Solution C on top and centrifuge at 1500 g for 30 min at 20°C (see Figure 1 and Note 4).

4. Collect the PBMCs from the meniscus downwards to about 1 cm from the cell pellet (see Figure 1).
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 5-8).

4. Notes

1. The mixer based on Nycodenz® was formulated so that equal volumes of blood and medium were mixed together to produce the required density. By using solutions of higher density (either OptiPrep™ or the Working Solution prepared from it) blood sample volumes are increased by only 12.5% or 25% (respectively) after mixing, thus the environment of the cells is closer to that of the original plasma and larger blood volumes are easier to handle (see Note 9).
2. The actual increase in density of the plasma will depend of the haematocrit of the blood and the density of the plasma. The volumes given in Table 1 assume that the hematocrit is approx. 46% (adult male average) and the plasma density is approx 1.022 g/ml. The hematocrit of normal adult female blood tends to be lower, approx 43%. If contamination of the PBMCs by PMNs is routinely unacceptable, the amount of OptiPrep™ or WS added should be reduced.
3. If the aim is to isolate monocytes from the mononuclear cells, and if addition of OptiPrep™ to the blood is chosen, rather than the 37% iodixanol working solution, it is beneficial to spike the OptiPrep™ with 8.5% NaCl, 10 mM Hepes-NaOH, pH 7.4 (volume ratio of 1:0.01).
4. The layer of TBS on top of the blood is not critical to the separation, but it facilitates the harvesting of the PBMCs from the meniscus.
5. Table 2 shows the numbers of PBMCs recovered from 5 ml or 20 ml of blood from eight healthy donors. They represent recoveries of 92-98% from the original blood sample. Recoveries are volume independent, approx four times the number of cells being recovered from four times the blood volume. No granulocytes were observed in any of the PBMC harvests and the erythrocyte contamination was 1-3% of total cells.
6. Recoveries and purity of PBMCs isolated by flotation in iodixanol are almost identical to those obtained with Lymphoprep™. The ease of operation however makes the mixer-flotation technique the method of choice especially when handling large numbers of potentially pathogenic samples. The results are in line with those of Kaden et al [2] who compared Lymphoprep™ with a mixer based on Nycodenz®; these workers found that the PBMC harvests were essentially identical by both techniques.
7. The cells will be contaminated with platelets from the plasma. Partial removal of platelets from human PBMCs can be carried out by pelleting the cells preferentially at a low RCF (250 g for 10 min). The cells can be resuspended in saline and the washing process repeated. However pelleting and resuspending any cells is potentially damaging to the cells and should be avoided.
8. If complete removal of platelets is important, the PBMCs should be diluted with an equal volume of Solution C; layered over an equal volume of iodixanol, $\rho = 1.063 \text{ g/ml}$, (5 vol OptiPrep™ + 22 vol Solution C) and centrifuged 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 **“Platelets (human)” Application Sheet C13 in index.**
9. If the blood has to be stored before fractionation then it is useful to note that if the density of the blood is raised by addition of the dense medium immediately after drawing, then the loss of

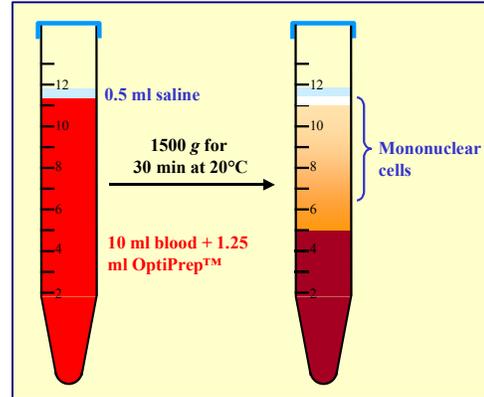


Figure 1: Mixer flotation of mononuclear cells
After centrifugation, recover liquid down to 6 ml mark

Table 2 PBMCs recovered from 5 ml and 20 ml whole blood in four separations

5 ml blood	20 ml blood
9.77×10^6	3.8×10^7
10.01×10^6	4.1×10^7
8.95×10^6	3.2×10^7
9.30×10^6	2.9×10^7

recovery and purity of the PBMCs that is observed with density barrier techniques, is much less marked. This is probably related to the fact that once the density of the plasma has been raised, the PBMCs do not settle out upon standing [3].

5. References

1. 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
2. Kaden, J., Schönemann, C., Leverenz, S. and Koch, B. (1994) *Optimized lymphocyte isolation. One-step procedure for isolation of human lymphocytes by means of NycoPrep Mixer Recovery and purity in comparison with other separation media* Allergologie Jahrgang, **17**, 429-433
3. Ford, T. C. and Rickwood, D. (1992) *Improved isolation of mononuclear cells from stored blood* Clin. Chim. Acta, **206**, 249-252

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