

OptiPrep™ Application Sheet C11

Isolation of a monocyte-rich fraction from whole human blood by iodixanol barrier flotation

- ◆ 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

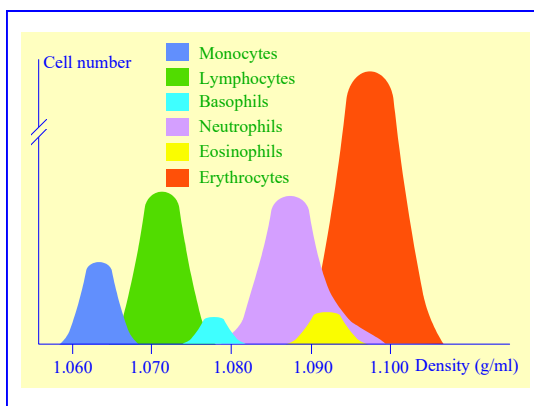


Figure 1 Density of human blood cells

1. Background

Monocytes in human peripheral blood, account for, on average, about 8% of the leukocyte population. They tend to be larger (15-20 μm) than lymphocytes (6-20 μm) and they also have a slightly lower density (Figure 1).

A method developed by Graziani-Bowering et al [1] permits the separation of monocytes from lymphocytes on the basis of rate of flotation from a leukocyte-rich plasma (LRP). The method provides highly purified and viable monocytes [1]; see **Application Sheet C10: “Separation of monocytes from a leukocyte-rich plasma by flotation through a discontinuous iodixanol gradient”**.

However because of the requirement to prepare a buffy-coat fraction from whole blood, the yield of monocytes is always compromised by the inevitable loss of leukocytes that occurs during the preparation of such a fraction. This step also adds to the overall time of preparation. Moreover the platelets in the blood also sediment into the buffy coat fraction and the close juxtaposition of platelets and monocytes may lead to activation of the latter. The method originally devised by Graziani-Bowering et al [1] has therefore been modified in an attempt to reduce any activation of the monocytes (by platelets or by the isolation procedure itself) to a minimum.

The method described in this Application Sheet relies on the same principle of separation as that described in **Application Sheet C10** (i.e. the more rapid rate of flotation of monocytes compared to lymphocytes) and in both cases the vast majority of the platelets remains in the high-density sample zone and do not co-band with the monocytes. The method described in this OptiPrep™ Application Sheet is carried out at 4°C; this tends to reduce activation and minimizes the vesiculation within the cytoplasm of the monocytes that is sometimes observed when the separations are carried out at room temperature. When this vesiculation occurs yields are very low.

- ◆ The monocytes produced by the methods described in this OptiPrep™ Application Sheet have been quantified by esterase and Sudan black staining. Purity of monocytes isolated by this method is not as high as that of cells isolated by the C10 method; recoveries however are more reproducible.

- ◆ In accordance with the observations of Filion et al [2], the OptiPrep™ used to increase the density of the blood sample is “spiked” with NaCl in order to avoid the decrease of ionic strength that occurs if neat OptiPrep™ is used. This improves the recovery and function of the monocytes.
- ◆ See Section 5 for a new mixer strategy for the isolation of monocytes from whole blood.

2. Solutions required (see Note 1)

- OptiPrep™
- 8.5% (w/v) NaCl
- Diluent: Routine culture medium (e.g. RPMI or DMEM) containing 10% serum.

3. Protocol

- ◆ Use polypropylene tubes for all operations. Take care to mix the OptiPrep™ gently before removing an aliquot.

- Collect 10 ml of blood using EDTA (2 mM final concentration as anticoagulant).
- Prepare a 1.070 g/ml OR 1.072 g/ml OR 1.074 g/ml density barrier solution by mixing Solution A with Solution C at one of the following volume ratios: 11.8 + 48.2, 12.2 + 47.8 or 12.6 + 47.4 respectively (see Notes 2-4).

- Mix 5.4 vol. of Solution A with 0.6 vol. of Solution B to make a 54% (w/v) iodixanol solution (see Note 5).
- Mix 2 ml of the 54% iodixanol with 10 ml of whole blood by several very gentle inversions.

- In a 15 ml centrifuge tube, layer 6 ml of the chosen density barrier solution over 4 ml of blood, and then layer approx 0.5 ml of Solution C on top (see Notes 6 and 7).

- Centrifuge at 700 g in a swinging-bucket rotor for 30 min at 20°C. Do not use the brake during deceleration (see Note 8).

- Collect the monocytes that float to the top of the 1.072 or 1.074 g/ml layer (Figure 2). The band may be quite diffuse and occupy 2-3 ml.

- Dilute the collected cells with 2 vol of Solution C and harvest by centrifugation and resuspend the pellet gently in any medium as required (see Note 9).

4. Notes

- It has currently not been ascertained if improved yields or purity might be achieved if the ionic strength of Solution C was increased in line with that of the OptiPrep™ (see Step 3 of the protocol)
- The choice of density for the low-density barrier depends to some extent on the operator's requirements. Use of a 1.072 g/ml barrier will give a monocyte preparation that is approx. 85-90% pure (as estimated by esterase staining) but the yields are only approx. 30%. A 1.074 g/ml density barrier will permit the recovery of more monocytes (approx. 60% of the total) but the contamination by lymphocytes is proportionately greater (approx. 80-85% pure). The 1.070 g/ml barrier has not been investigated.
- For rat blood, a density for the upper layer of 1.076 g/ml is suggested.

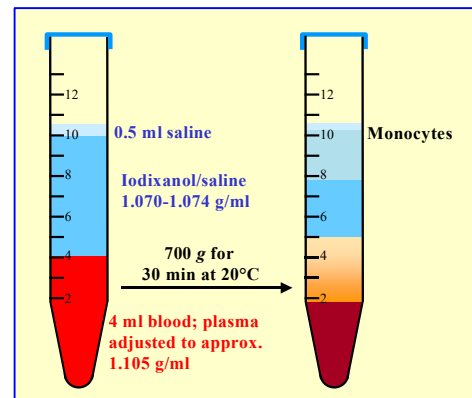


Figure 2: Separation of monocytes by flotation through a low-density barrier from whole human blood

4. The protocol describes the use of a single low-density layer prepared by dilution of OptiPrep™ with a routine culture medium containing 10% fetal calf serum, but a two-layer format of approx 1.084 g/ml and 1.068 g/ml described in **Application Sheet C10** may give better monocyte purity.
5. The method only works satisfactorily on fresh blood (used within 2 h of drawing) from healthy individuals and with EDTA as anticoagulant.
6. The uppermost layer of medium is not required for the separation process, but it avoids the banding of cells at a liquid/air interface and also prevents the cells from adhering to the walls of the tube at the meniscus.
7. There are a number of possible variants to this method that might be investigated. For example: (1) using a 50 ml centrifuge tube so that the radial distance occupied by the sample is less, which might be accompanied by increasing the volume of the low-density barrier, or (2) using a two-layer gradient format as in **Application Sheet C10**.
8. Increasing the centrifugation time to 40 min may improve the yield of monocytes with the lower density barriers.
9. The purity of the monocytes is not as high as that obtained by using the method described in **Application Sheet C10**. Nevertheless this might be regarded as a very useful preliminary step prior to the more economical use of antibody-coated beads to remove residual lymphocytes by negative selection.

5. Mixer strategy for whole blood monocyte preparations

Yin et al [3] have reported a simple mixer technology for the isolation of monocytes from whole human blood. It resembles the methodology described in **Application Sheet C05** for the isolation of PBMCs. Yin et al [3] mixed whole blood and OptiPrep™ in the ratio of 8:1; layered a small volume of buffered saline and top and centrifuged at 1500 g for 30 min. The cells recovered from the interface between the saline and the plasma contained >90% monocytes.

6. References

1. Graziani-Bowering, G.M., Graham, J. and Filion, L.G. (1997) *A quick, easy and inexpensive method for the isolation of human peripheral blood monocytes* J. Immunol. Meth., **207**, 157-168
2. Filion, L.G., Graziani-Bowering, G., Matusевичius, D. and Freedman, M.S. (2003) *Monocyte-derived cytokines in multiple sclerosis* Clin. Exp. Immunol., **131**, 324-334
3. Yin, K., Deng, X., Mo, Z-C., Zhao, G-J., Jiang, J., Cui, L-B., Tan, C-Z., Wen, G-B., Fu, Y. and Tang, C-K. (2011) *Tristetraprolin-dependent post-transcriptional regulation of inflammatory cytokine mRNA expression by apolipoprotein A-I Role of ATP-binding membrane cassette transporter A1 and signal transducer and activator of transcription 3* J. Biol. Chem., **286**, 13834–13845

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