OptiPrep™ Application Sheet C13

Isolation (removal) of human platelets (thrombocytes) on an iodixanol barrier

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **The OptiPrep™ Reference List (RC02)** "The purification of platelets from whole blood and their removal from blood leukocyte preparations" provides a comprehensive list of all the relevant published papers: to access 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

Although platelet-rich plasma (PRP) is relatively easy to produce by centrifugation of whole blood, yields of platelets may be variable, because many of them are trapped within the erythrocyte layer. Although they can be recovered by washing these cells with isotonic saline, it is a general rule that to avoid activation of the platelets, the number of centrifugations and resuspensions should be kept to a minimum. Another problem is that aspiration of the PRP must be performed carefully to avoid contamination from leucocytes in the buffy coat which lies atop the erythrocytes.

To provide a highly purified platelet fraction from human blood Ford et al [1] layered whole blood over a density barrier of Nycodenz® (ρ = 1.063 g/ml) that allowed the erythrocytes and leucocytes to pellet during centrifugation at 350g. The platelets, because of their small size, sediment much more slowly; they form a broad band extending into the density barrier from just above the interface. The platelets recovered from this density barrier method have been used directly in aggregation studies; the Nycodenz® did not interfere with this process [1]. An iodixanol barrier of the same density can be substituted for the Nycodenz®; this has no effect on the separation or yield of platelets.

- Recently the purity of the iodixanol-isolated platelets has been validated by flow cytometry and their functional integrity confirmed [2,3].

2. Solutions required

A. OptiPrep™ (shake the bottle gently before use)

B. Diluent: 0.85% (w/v) NaCl, 20 mM HEPES-NaOH, pH 7.4, 1 mM EDTA

Keep the following stock solutions at 4°C
100 mM HEPES (free acid) 2.38 g per 100 ml water
100 mM EDTA (Na_2•$2H_2O$) 3.72 g per 100 ml water

Solution B: Dissolve 0.85 g NaCl in 50 ml water, add 20 ml and 1 ml of buffer and EDTA stock solutions respectively; adjust to pH 7.4 with 1 M NaOH; make up to 100 ml.

3. Protocol

1. Collect blood by venepuncture into a suitable anti-coagulant (EDTA or citrate).

2. Produce the ρ = 1.063 g/ml density barrier by mixing 5 vol OptiPrep™ with 22 vol of Solution B (see Note 1).

3. In a centrifuge tube layer 1 vol of blood over 1 vol of density barrier (see Figure 1 and Notes 2 and 3) and centrifuge at 350 *g* for 15 min at 20°C in a swinging-bucket rotor and allow the rotor to decelerate without the brake.

4. Harvest the autologous plasma and the platelet-containing band as shown in Figure 1.

5. Towards the bottom (lowest 2-3 mm) of the platelet band there will be a slight increase in the contamination by leucocytes and erythrocytes (up to 3-5%) while the contamination in the bulk of the platelet band is <1%.

4. Notes

1 For more information on preparing density solutions see **Application Sheet C01**.

2 The separation of the platelets is based on their slow rate of sedimentation, so it is very important that the centrifugation speed and time is carefully adhered to. Higher speeds and longer times will result in the platelet band moving closer to the cell pellet.

3 To permit an adequate linear separation of the platelets from the pellet the density barrier column needs to be approx 5 cm, thus in a 15 ml centrifuge tube there should be a minimum of 5 ml of density barrier. For small volumes (1-5 ml of blood) use 5 ml of barrier; for larger volumes of blood, use an equal volume of barrier. The column height of the density barrier in larger volume tubes should be maintained.

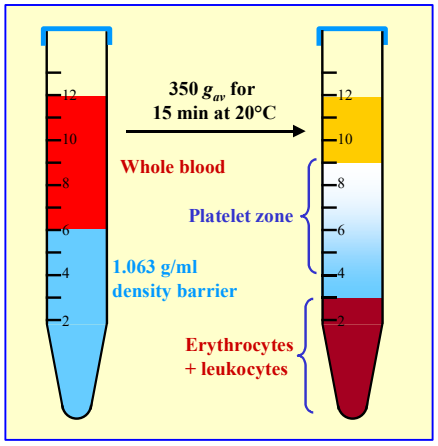

Figure 1: Isolation of platelets from whole human blood. Equal volumes of blood and density barrier layered in tube. After centrifugation platelets harvested from the broad turbid band below the interface

- The method can be used both to prepare platelets for analysis and to remove them from other cell types such as peripheral blood mononuclear cell suspensions.

5. Methodological variation

A three-layer iodixanol gradient has been used for the very successful production of highly-purified functional platelets by Birschmann et al [4,5] from platelet concentrates (ex blood bank). The washed platelets were layered on top of 14, 14 and 15 ml respectively of 6%, 7.8% and 10.2% (w/v) iodixanol (equivalent to 10, 13 and 17% (v/v) OptiPrep™) and centrifuged at 300 *g* for 20 min. After discarding the top 7.5 ml, the highly-purified platelets were recovered in the next 12.5 ml.

6. References

1. Ford, T.C., Graham, J. and Rickwood, D. (1990) *A new, rapid, one-step method for the isolation of platelets from human blood* Clin. Chim. Acta, **192**, 115-120
2. Bagamery, K., Kvell, K., Barnet, M., Landau, R. and Graham, J. (2005) *Are platelets activated after a rapid, one-step density gradient centrifugation? Evidence from flow cytometric analysis* Clin. Lab. Haem., **27**, 75-77
3. Bagamery, K., Kvell, K., Landau, R. and Graham, J. (2005) *Flow cytometric analysis of CD41-labeled platelets isolated by the rapid, one-step OptiPrep method from human blood* Cytometry Part A, **65A**, 84-87
4. Birschmann, I., Mietner, S., Dittrich, M., Pfrang, J., Dandekar, T. and Walter, U. (2008) *Use of functional highly purified human platelets for the identification of new proteins of the IPP signaling pathway* Thromb. Res., **122**, 59–68
5. Gambaryan, S., Kobsar, A., Hartmann, S., Birschmann, I., Kuhlencordt, P.J., Müller-Esterl, W., Lohmann, S.M. and Walter, U. (2008) *NO-synthase-/NO-independent regulation of human and murine platelet soluble guanylyl cyclase activity* J. Thromb. Haemost., **6**, 1376-1384