OptiPrep™ Application Sheet C45

Isolation of peripheral blood mononuclear cells from non-human primates

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

For the isolation of human peripheral blood mononuclear cells (PBMCs) from whole blood, sedimentation onto a barrier of density 1.077 g/ml is probably the most widely used technique. There are two commercially available media from Axis-Shield PoC AS; Lymphoprep™ (the most widely used medium) contains diatrizoate and a polysaccharide and Nycoprep™ 1.077, which contains a buffered solution of Nycodenz®. This density barrier method for human PBMCs has also been adapted to the use of OptiPrep™ (**see Application Sheet C04**). Published papers report the use of both Lymphoprep™ and Nycoprep™ 1.077 for the isolation of PBMCs from many types of higher primate but there have been no detailed comparative studies between different primates. The method described in this Application Sheet (Section 2), devised by Stittelaar et al [1,2] was designed specifically for macaque PBMCs (see also ref 3), but it has now been extended to mandrills [4].

An alternative "mixer" technique was devised for human PBMCs in 1990 [5], which has also been adapted to the use of OptiPrep™ technology (**see Application Sheet C05**); this has now been extended to cynomolgus macaques and is described in Section 3.

2. Density barrier method

2a. Solutions required

A. OptiPrep™ (shake gently before use)
B. Ten-times concentrated phosphate buffered saline (10xPBS).
C. Polysaccharide solution: 6% (w/v) polysucrose 400 (MWt 400,000) in water.

2b. Protocol (adapted from ref 1, see Section 2c)

1. Prepare the density barrier by mixing Solutions A, B and C in the following volume ratio: 16.7:8.3: 75.

2. In a centrifuge tube layer 2 volumes of heparinized blood over 1 volume of the density barrier (see Figure 1 and Section 2c).

3. Centrifuge at 600 *g* for 20 min; allow the rotor to decelerate without the brake.

4. Remove the PBMC that band at the interface (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.

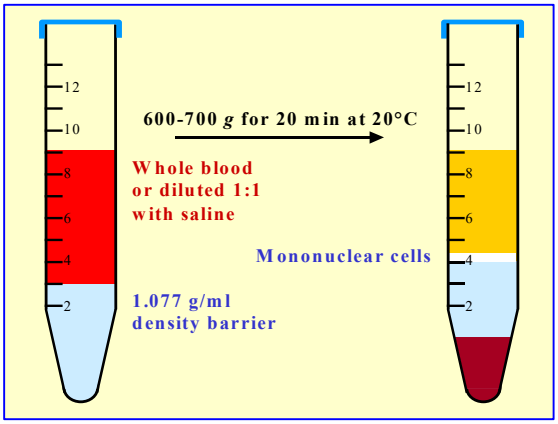

Figure 1: Isolation of Macaque PBMCs on a density barrier. Two vol. of blood layered over 1 vol. of medium. After centrifugation the PBMC band is harvested by aspiration (see text for details)

2c. Notes

Sittelar et al [1,2] specified the use of heparinized blood and used the blood undiluted. Human blood is normally diluted with an equal volume of saline before applying to the density barrier. It is not entirely

clear from other publications which strategy was adopted [3,4,6]. Use whichever method provides the optimal results.

3. Mixer strategy

3a. Solutions required

OptiPrep™ (shake gently before use)
Buffered saline

3b. Protocol

Mix 10 ml of blood with 1.25 ml of OptiPrep™ by several GENTLE inversions and proceed as described in Figure 2.

3c. Notes

Guo et al [7] who investigated this method concluded that the mixer strategy described here gave consistent yields, which are described here, that were higher than any of the established alternative procedures.

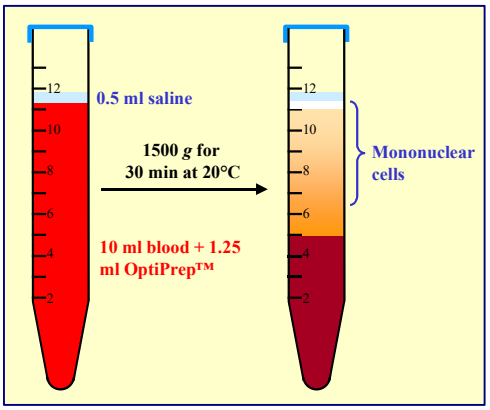

Figure 2: Mixer flotation of mononuclear cells
After centrifugation, recover liquid down to 6 ml mark

4. References

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