OptiPrep™ Application Sheet C08

Isolation of ruminant and equine peripheral blood mononuclear cells in iodixanol gradients

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **Axis-Shield Mini-Review (MC01)** "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a methodological review" compares all of the currently available methodologies
- **Axis-Shield Mini-Review (MC02)** "Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a bibliographical review" provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™
- To access **MC01 and MC02** return to the initial list of Folders and select "**Mini-Reviews**".
- 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) either Lymphoprep™ or a solution of 14.1% (w/v) Nycodenz®, 0.44% (w/v) NaCl and 5 mM Tricine-NaOH, pH 7.0 have been successfully used. Lymphoprep™ contains the ionic diatrizoate and a polysaccharide, while Nycodenz® is non-ionic and the solution contains no polysaccharide. Both density gradient media have been used for isolating PBMCs from ruminant blood and it seems that the density of PBMCs from ruminants is closer to that of human PBMCs, compared to those from experimental animals such as rodents.

This OptiPrep™ Application Sheet describes two methods for the isolation of PBMCs from ruminants. Protocol A describes the familiar strategy of sedimentation on to a density barrier, while Protocol B presents a strategy in which the density of whole blood is adjusted to a value just greater than that of the PBMCs, which allows them to float to the surface [1]. Although this flotation technique was developed for human blood, it also seems to be rather broadly applicable to the blood of many species. Protocol B was devised for bovine blood, but it is almost certainly applicable to the blood of other ruminants and probably horses.

2. Solutions required (see Note 1)

A. OptiPrep™ (shake gently before use)

B. Diluent: 0.85% (w/v) NaCl, 30 mM Tricine-NaOH, pH 7.4 (Protocol B only)

C. Tricine-buffered saline (TBS): 0.85% NaCl, 20 mM Tricine-NaOH, pH 7.4

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 20 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

3a. Protocol A

1. Collect blood using heparin, citrate or EDTA as anticoagulant and dilute with an equal volume of Solution C (see Note 2).

2. Prepare a 1.078 g/ml solution by diluting 1.4 vol. of Solution A with 4.6 vol. of Solution C.

3. In a suitable centrifuge tube layer 2 vol. of diluted blood over 1 vol. of 1.078 g/ml solution.

4. Centrifuge at 800 *g* for 30 min.

5. Allow the rotor to decelerate without the brake and then collect the PBMCs from the interface.

6. Dilute the collected material with two volumes of Solution C and pellet the cells at 500 *g* for 15 min (see Notes 3-5).

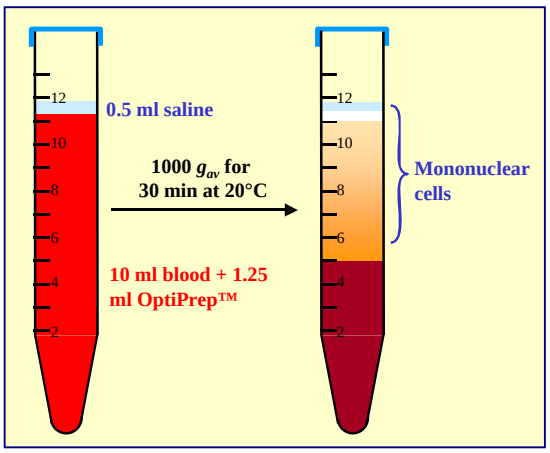

Figure 1 Flotation of equine PBMCs through density-modulated plasma (Protocol B)

3b. Protocol B

1. Collect blood using heparin, citrate or EDTA as anticoagulant (see Note 2)

2. To prepare a 37% (w/v) iodixanol Working Solution: mix 3.7 vol. of OptiPrep™ with 2.3 vol. of Solution B (see Note 6).

3. In a suitable capped centrifuge tube mix 10 ml of whole blood with 1.25 ml of OptiPrep™ or 2.5 ml of Working Solution by repeated inversion and then layer 0.5 ml of Solution C on top (see Note 7).

4. Centrifuge at 1000 g_{av} for 30 min at 20°C (see Note 8).

5. Allow the rotor to decelerate without the brake and then collect the PBMCs from the meniscus downwards to about 0.5 cm from the cell pellet as shown in Figure 1.

6. Dilute the collected material with two volumes of buffered-saline and pellet the cells at 300-400 *g* for 15 min (see Notes 3-5 and 9).

4. Notes

1. Tricine-NaOH buffer is used in the protocol but any suitable buffer may be substituted. Strategies for preparing Working Solutions for cells are described in Application Sheet C01.
2. Choice of the optimal anticoagulant is best determined empirically.
3. In the case of human blood, harvesting PBMCs from the medium is often carried out at 250-300 *g* for 10 min. This is insufficient to pellet all the bovine PBMCs – 300-400 *g* for 15 min recovers all the cells.
4. As with the purification of human PBMCs 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 (250-300 *g* for 10 min). The cells are then resuspended in saline and the washing process repeated. Whether this is a satisfactory method for bovine PBMCs has not been rigorously tested.
5. Complete removal of platelets from human PBMCs can be achieved by dilution with an equal volume of Solution C; layering over an equal volume of iodixanol, ρ = 1.063 g/ml, (5 vol. OptiPrep™ + 22 vol. Solution C) and centrifugation 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. Whether this is a satisfactory method for bovine PBMCs has not been tested. For more details see Application Sheet C12.
6. If addition of unbuffered OptiPrep™ to the blood in Step 3 of Protocol B is regarded as undesirable then use the buffered Working Solution containing 37% (w/v) iodixanol (ρ = 1.199 g/ml).
7. 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.
8. Olsen and Storset [2] used a 35 min rather than a 30 min centrifugation.
9. Total recoveries of PBMCs from two flotation experiments with 10 ml of bovine blood were 10.85 x 10^6 and 12.65 x 10^6.

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 Olsen, I. and Storset, A.K. (2001) *Innate IFN-γ production in cattle in response to MPP14, a secreted protein from Mycobacterium avium subsp. paratuberculosis* Scand. J. Immunol**., 54**, 305-3130

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