OptiPrep™ Application Sheet C25

Isolation of cells from pulmonary tissue
Endothelial from epithelial cells, type I epithelial cells and macrophages

- 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
- For other pulmonary cells methods **see the companion Application Sheet C26**

1. Background

Nycodenz® gradients have been used very successfully for the isolation of Type II pneumocytes [1,2] and Viscardi et al [2] pointed out the advantages of using this medium and the potential problems of using the colloidal silica medium Percoll® for this task. Alveolar macrophages have also been purified using Nycodenz® gradients [3].

OptiPrep™ has been employed, as a continuous gradient, in the separation of endothelial and epithelial cells [4] and also of alveolar macrophages [5] and as a discontinuous gradient in the isolation of Type I epithelial cells [6]. Pu et al [4] studied the regulation of calcitonin secretion in pulmonary neuroendocrine cells, which banded with the epithelial cells. Borok et al [6] were able to confirm the involvement of Type I cells in transalveolar Na^+ transport. Starr et al [5] studied the effects of bronchofibrinous pneumonia in cattle. All three separations are described in this OptiPrep™ Application Sheet.

2. Separation of endothelial and epithelial cells (adapted from ref 4)

2a. Solutions required

A. OptiPrep™ (shake the bottle gently before use)
B. Culture medium, e.g. RPMI-1640 or DMEM (see Note 1)
C. 1% (w/v) collagenase II in Solution B
D. Solution B + 10% fetal bovine serum

2b. Protocol

1. Finely mince the dissected lung tissue, using scissors, into 1 mm^3 pieces

2. Incubate in 10 ml Solution C at 37° for 2-3 h (see Notes 2 and 3).

3. Add an equal volume of Solution D and centrifuge the cell suspension at 600 *g* for 20 min.

4. Resuspend the cell pellet in 5 ml of Solution D.

5. Mix 0.5 ml of OptiPrep™ with 4.5 ml of the cell suspension to make a final density of 1.04 g/ml (see Note 4).

6. Make a solution of density 1.15 g/ml by mixing 3 ml of Solution D and 2.5 ml of OptiPrep™ (see Note 4).

7. Prepare a continuous gradient from 5 ml each of the cell suspension (1.04 g/ml) and the 1.15 g/ml solutions using a Gradient Master™ (see Note 5).

8. After centrifuging at 600 *g* for 20 min, harvest the banded material from the gradient using a syringe and metal cannula (see Note 6).

3. Isolation of alveolar epithelial type I cells (adapted from ref 6)

3a. Solutions required

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

B. Culture medium, e.g. RPMI-1640 or DMEM (see Note 1)

3b. Protocol

♦ For details of lavage procedure and tissue disaggregation with elastase see ref 6.

1. Suspend the crude single cell suspension in Solution B.

2. Produce four solution of density 1.012, 1.033, 1.047 and 1.068 g/ml by diluting OptiPrep™ with Solution B using, respectively, the following volume ratios: 2 + 58, 5 + 55, 8 + 52 and 12 + 48.

3. Layer 2 ml of each gradient solution, followed by the crude cell suspension in a 15 ml centrifuge tube and centrifuge at 600 *g* for 15 min.

4. Harvest the type I cells that band in the 1.033 g/ml layer.

4. Isolation of alveolar macrophages (adapted from ref 5)

4a. Solutions required

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

B. Lavage medium: phosphate-buffered saline (PBS)

C. Cell suspension medium: Ca^{2+}/Mg^{2+} - free Hank's Balanced Salt Solution (see Note 1)

4b. Protocol

1. Prepare two iodixanol solutions of 5% (w/v) and 30% (w/v) iodixanol by diluting OptiPrep™ with Solution C using, respectively, the following volumes ratios: 5 + 55 and 1 + 1.

2. Lavage the lungs using Solution B (see Note 8)

3. Pass the lavage through a 22 μm pore-size nylon membrane filter.

4. Collect the cells by centrifugation at 500 *g* for 15 min and resuspend in Solution C.

5. During the centrifugation produce a linear gradient in a 15 ml tube from 5 ml each of the 5% and 30% iodixanol solutions using a two-chamber gradient maker or a Gradient Master™ (see Note 9).

6. Layer the cell suspension on top and centrifuge at 800 *g* for 20 min.

7. Collect the macrophages that band at approx 15% iodixanol.

5. Notes

1. Any suitable isoosmotic culture medium or balanced salt solution may be used.
2. The time required for tissue disaggregation may need modification depending on the animal species being used; Syrian golden hamsters were used in the study by Pu et al [4].
3. Alternative more elaborate techniques for tissue disaggregation involving lavage of the lungs prior to use of elastase rather than collagenase might be used (see ref 6).
4. The ratio of OptiPrep™ to diluent will depend on whether fetal bovine serum (FBS) is included; with FBS (as in this case) the diluent will have a density of approx 1.009 g/ml, without FBS the density will be approx 1.006 g/ml. For more information on the preparation of density solutions see **Application Sheet C01**.
5. If a Gradient Master™ is unavailable then a two-chamber gradient maker might be used, but to avoid vigorous stirring of the cell suspension. It might be more suitable to make the 1.04 g/ml solution from Solution A and Solution D (i.e. a cell-free solution) and then to layer the crude cell

suspension (in Solution D) on top. Alternatively the continuous gradient might be produced by diffusion from a discontinuous gradient using 2.5 ml each of 1.040 g/ml (containing cells), 1.075g/ml, 1.110 g/ml and 1.145 g/ml (equivalent to approx 6%, 13%, 20% and 27% (w/v) iodixanol). For information on the preparation of continuous gradients for cell separations see **Application Sheet C02**.

6. Endothelial cells + macrophages make up the least dense band (approx 1.049 g/ml), epithelial cells band at 1.059-1/062 g/ml, fibroblasts and erythrocytes at 1.069-1.096 g/ml and the densest band (1.113 g/ml) contained fibroblasts + non-viable cells + cell debris.
7. For more information see ref 5.
8. Alternatively the continuous gradient might be produced by diffusion from a discontinuous gradient using 2.5 ml each of 5%, 10%, 15%, 20% and 30% (w/v) iodixanol. For information on the preparation of continuous gradients for cell separations see **Application Sheet C02**.

6. References

1. Schultz, C.J., Torres, E., Londos, C. and Torday, J. S. (2002) *Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells* Am. J. Physiol. Lung Cell Mol. Physiol., **283**, L288-L296
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3. Killingsworth, C.R., Shore, S.A., Alessandrini, F., Dey, R.D. and Paulauskis, J.D. (1997) *Rat alveolar macrophages express preprotachykinin gene-I mRNA-wncoding tachykinins* Am. J. Physiol. Lung Cell Mol. Physiol., **273**, L1073-L1081
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5. Starr, A.E., Dan, T., Minhas, K., Shewen, P.E. and Coomber, B.L. (2004) *Potential involvement of gelatinases and their inhibitors in Mannheimia haemolytica pneumonia in cattle* Infect. Immun., **72**, 4393-4400
6. Borok, Z., Liebler, J.M., Lubman, R.L., Foster, M.J., Zhou, B., Li, X., Zabski, S. M., Kim, K-J. and Crandall, E.D. (2002) *Alveolar epithelial ion and fluid transport Na transport proteins are expressed by rat alveolar epithelial type I cells* Am. J. Physiol. Lung Cell Mol. Physiol., **282**, L599-L608

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