OptiPrep™ Application Sheet C13

Removal of non-viable cells from a cell suspension

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review (MC04)** "Viable/non-viable cell separation" provides a bibliography of all the published papers reporting the use of OptiPrep™: to access 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

Isolation of cells from a lavage of a body cavity or from the mechanical or enzymic dissociation of a tissue will inevitably render a number of cells non-viable, which must be removed prior to further processing. Other important scenarios are the removal of non-viable cells after electroporation and the retrieval of viable cells from a valuable line of cultured cells after an incubator failure. Non-viable cells may also release intracellular components, for example hydrolytic enzymes and DNA, into the suspension; these also need to be removed from the aqueous environment of any recovered viable cells.

Non-viable cells, which no longer enclose an osmotic space, are significantly denser than non-viable cells and thus should be easily separated from them across a density barrier. The actual density of the non-viable cells will be partly related to that of their viable counterparts.

Strategy 1

A simple method developed with Nycodenz®, and subsequently extended to OptiPrep™, is to centrifuge the cell suspension over a solution whose density is higher than that of the viable cells; the non-viable cells pellet and the viable ones are recovered from the interface (Figure 1). Once the cells have lost their osmotic competence and become leaky to the gradient solute, their density should increase significantly and theoretically their density should exceed approx 1.15 g/ml. With this strategy however there is no need to use such a high density for the barrier as long as it is of a sufficient density to retain the viable cells. Consequently the density of barriers used to effect this separation varies from approx 1.060 to 1.15 g/ml, although in the majority of cases the density is at least 1.080 g/ml.

Strategy 2

A drawback of Strategy 1 is that the viable cells band adjacent to the sample zone and hence will remain in contact with released intracellular macromolecules (Figure 1). In Strategy 2, the cell suspension is adjusted to a density of 1.15-1.16 g/ml, layered beneath a solution whose density is greater than that of the viable cells, thus allowing these viable cells to float away from the sample zone (Figure 2) and any released macromolecules. Suspending the cells in a high concentration of the gradient solute will also be a more efficient way for the solute to enter the intracellular space of the non-viable cells. A density of 1.12 g/ml has been chosen for the upper layer, as this is likely to be high enough to allow the flotation of any viable mammalian or non-mammalian cell. It might however be replaced by a solution of any density higher than that of the cell of interest.

- Strategy 2 has been used very successfully for recovering a small number of viable cells from a vast preponderance of non-viable cultured cells.
- The following protocols use OptiPrep™ rather than Nycodenz® because of the easier solution preparation with the former.

2. Solutions required

A. OptiPrep™ (shake the bottle gently before use)
B. OptiPrep™ Diluent: culture medium (RPMI or DMEM) + 10% serum (see Note 1).

3. Protocol

3a. Strategy 1

1. Make a suitable density barrier solution by diluting OptiPrep™ with Solution B. See Notes 2-4 for detailed information on choice of the correct density solution.

2. Put a small volume of the density barrier in 15 ml (approx. 2 ml) or 50 ml (approx 5 ml), conical plastic centrifuge tubes and carefully layer the cell suspension on top.

3. Centrifuge in a swinging bucket rotor at approx 800 *g* for 15-20 min (see Note 5).

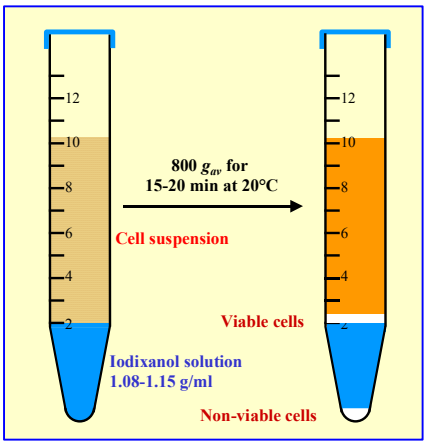

Figure 1: Removal of non-viable cells (Strategy 1)

4. Non-viable cells will pellet and any macromolecular material, and residual enzymes from any enzymically-digested tissue, will mostly remain in the supernatant, above the interfacial band of viable cells (see Figure 1).

5. Remove as much of the supernatant as possible: harvest the cell band; dilute with two volumes of Solution B (or any balanced salt solution); pellet the cells at 200-400 *g* for 10 min and resuspend them in a suitable medium.

3b. Strategy 2

1. Prepare a 40% (w/v) iodixanol working solution (WS) by diluting 2 vol. of OptiPrep™ with 1 vol. of Solution B. Dilute the WS further with Solution B to give a 22% (w/v) iodixanol solution (approx ρ = 1.12 g/ml). See Notes 2-4 for more information on density solution preparation.

2. Carefully mix 1 vol. of WS with 0.45 vol. of cell suspension by gentle repeated inversion and transfer to a centrifuge tube (up to 6.0 ml in a 15 ml centrifuge tube).

3. Overlay this with 3.0 ml of the 22% (w/v) iodixanol (see Note 6) and 0.5 ml of culture medium or Solution B (scale up or down as appropriate).

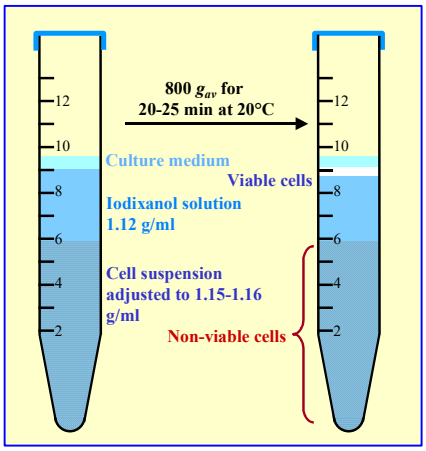

Figure 2: Removal of non-viable cells (Strategy 2)

4. Centrifuge at 800 *g* for 20-25 min (see Note 6).

5. Collect the viable cells from the top interface (see Figure 2); dilute with 2 vol. of culture medium or Solution B and harvest the cells at 200-400 *g* for 10 min.

4. Notes

1. Solution B may be any suitable balanced salt solution, or a Tricine or HEPES-buffered saline (with or without serum) may be substituted for the culture medium. Low concentrations of Mg^{2+} and/or Ca^{2+} and glucose may be included in the saline solution.
2. To recover all the viable cells from a mixed cell population containing cells with a broad range of densities, a density barrier of at least 1.11 g/ml should be chosen. If the requirement is to recover a single cell type of known density, then the density of the barrier might be reduced. Viable cells that have a relatively low density (e.g. lymphocytes, thymocytes, some bone marrow progenitor cells) can be separated from non-viable cells on density barrier of approx 1.09 g/ml.

Table 1 Preparation of density gradient solutions

Density (g/ml)	OptiPrep™ : Solution B Volume Ratio	
	Solution B containing 10% serum	Solution B containing no serum
1.15	0.45 : 0.55	0.46 : 0.54
1.12	0.36 : 0.64	0.36 : 0.64
1.11	0.33 : 0.67	0.34 : 0.66
1.09	0.26 : 0.74	0.27 : 0.73

3. The precise amounts of OptiPrep™ and Solution B will vary with the density of the latter but the values given in Table 1 will cover most cases. If the barrier is ineffective in removing the non-viable cells its density must be decreased; if viable cells are lost into the pellet its density must be increased. For more information about preparing iodixanol density gradient solutions and more extensive density tables see **Application Sheet C01**.
4. Table 2 lists some of the cell types processed using OptiPrep™, together with the centrifugation conditions (if given) in the text.
5. It may be necessary to increase either the g-force or the time of centrifugation to achieve a satisfactory result.
6. It is permissible to omit the layer of ρ = 1.12 g/ml but this provides a useful "clean zone" to separate the viable cells from all of the non-viable cells plus any released cytosolic components plus any residual digestive enzymes (if these were used for tissue disaggregation). All of these remain in the load zone.

Table 2 Iodixanol density barriers for removing non-viable cells: method variations

Cell type	Gradient conditions (if stated in text)	Ref. #
Amphibian skin	1.11 g/ml density barrier, 1700 rpm for 20 min	1
Human liver cells (postnatal)	Large scale in Cobe 2991 centrifuge, 500 *g* for 5 min	2
Multiple myeloma (post- electroporation)	Cells in 1.08g/ml density barrier, 1400 *g* for 5 min	3
Myoblast	1.15 g/ml density barrier, 800 *g* for 20 min	4
Promyelocytic leukemia cells (HL60)	1.068 or 1.057 g/ml density barrier, 450 *g* 30 min	5

5. References

1. Cox, T.C. (1999) *Calcium and ATP regulation of ion transport in larval frog skin* J. Comp. Physiol. B, **169**, 344-350
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