OptiPrep™ Application Sheet C37

Fractionation of coelomocyte cell populations from sea urchins

- 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

The coelomocytes of echinoderms, such as the purple sea urchin *Stronglyocentrotus purpuratus*, which are free-floating cells in the coelomic fluid, have been divided into at least four types, amoeboid phagocytes, red spherule cells, colourless spherule cells and vibratile cells [1,2]. A variety of discontinuous gradients have been used to fractionate these cells, including sucrose [3] sodium metrizoate [4] and Percoll® [5]. All of these suffer from serious disadvantages:

- Sucrose gradients pose the problem of high osmolality
- The silica particles of Percoll® may be engulfed by the potentially phagocytic cells [1]
- Both polysucrose and silica particles have a tendency to adhere to cell surfaces
- The extreme sensitivity of coelomocytes to lipopolysaccharide [1] makes Percoll® less than ideal, in view of the significant levels of endotoxin in this medium

OptiPrep™ offers some important advantages over these other gradient media for the fractionation of coelomocytes; it is a true solute and not a colloid, it has the lowest endotoxin levels (< 1 EU/ml) of any commercial density gradient medium and, as the solution contains only iodixanol and no other solutes, it is easy to prepare solutions which are compatible with non-mammalian cells.

The following protocol is adapted from ref 1. Iodixanol gradients have also been used by Johnston et al [6] for cell fractionation of the sea anemone *Aiptasia pallida*.

2. Solutions required

A. OptiPrep™ (shake the bottle gently before use)
B. Ca^{2+}/Mg^{2+}-free sea water containing 70 mM EDTA, 50 mM imidazole, pH 7.5

3. Protocol

1. Prepare the following solutions by diluting OptiPrep™ with Solution B: 3%, 6%, 12%, 18% and 42% (w/v) iodixanol and keep at 4°C (see Notes 1 and 2).

2. Introduce (through the peristomeum) into the coelomic cavity of the sea urchin, a 23-gauge needle attached to a syringe charged with chilled Solution B. Use a volume equal to that of the recoverable coelomic fluid (see next step).

3. Withdraw the coelomic fluid into the syringe.

4. Prepare a discontinuous gradient from 5 ml each of the five iodixanol solutions (see Notes 3-6).

5. Layer 5 ml of the coelomocyte-containing fluid on top of the gradient and centrifuge at 1500 *g* for 30 min at 4°C (see Note 7).

4. Recover the banded material (see Figure 1) using a syringe attached to a metal cannula and if required dilute with 2-3 volumes of Solution B before pelleting and resuspending in Solution B (see Notes 8-11).

4. Notes

1. In a more recent paper by Li et al [7] a similar density gradient, which replaced the densest 42% (w/v) iodixanol layer with a 36% (w/v) iodixanol layer, was used for the fractionation of cells from the green sea urchin *Strongylocentrotus droebachiensis*. The same centrifugation conditions were used as described in Section 3.
2. A gradient for fractionating *Paracentrous lividus* coelomocytes comprised three layers of 6%, 12% and 18% (w/v) iodixanol in 0.5 M NaCl, 10 mM EDTA [8].
3. For more information about preparing gradients see **Application Sheet C02**.

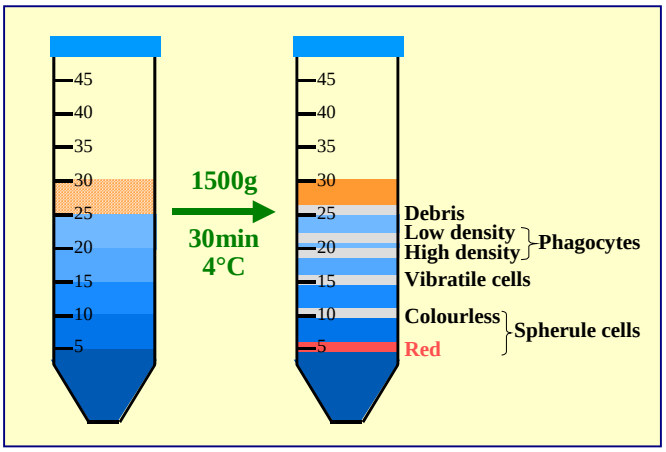

Figure 1: Distribution of coelomocytes in iodixanol gradient. Adapted from ref 1.

4. The volumes described are for a standard 50 ml centrifuge tube. The method can be scaled up or down proportionately – for example in a 15 ml tube use 2 ml each of the coelomic fluid and iodixanol solutions.
5. If the primary interest is the low and high-density phagocytes, a revised gradient of 3%, 5%, 7.5%, 10% (w/v) iodixanol might be investigated.
6. With other types of echinoderm, it may be necessary to modulate the density of one or more of the layers.
7. To avoid disturbance to the gradient layers use a programmed slow-acceleration if available, and to avoid disturbance to the banded cells during deceleration, turn off the brake.
8. Visible clumping of cells at one or more of the interfaces will lead to poor resolution; this is usually due to the use of too high a cell concentration. This problem may be exaggerated if other species of sea urchin or sea anemone are used. If this problem persists (in spite of reducing the cell concentration), it might be alleviated by adjusting the sample to 2.5% (w/v) iodixanol, so that the cells and, in particular the debris, sediment to the first interface more slowly.
9. Arizza et al [8] used 800 *g* for 30 min at 7°C.
10. Either aspirate observable bands of material or unload the entire gradient by careful aspiration from the meniscus; use a flat-tipped metal cannula (0.8 mm i.d.) attached to a 1-2 ml syringe. Most gradient unloaders are designed for use with flexible thin-walled tubes and not the screw-cap thick-walled tubes routinely used for cells. For more information regarding the harvesting of gradients **see Application Sheet S08, accessed from the Subcellular membranes index**
11. Figure 1 shows the expected distribution of cell types in the gradient. The four interfaces of the Arizza et al [8] gradient, in order of increasing density, contained 90% amoebocytes, 84% vibratile cells, 90% uncoloured spherulocytes and 91% red cells.

5. References

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8. Arizza, V., Giaramita, F.T., Parrinello, D., Cammarata, M. and Parrinello, N. (2007) *Cell cooperation in coelomocyte cytotoxic activity of Paracentrotus lividua coelomocytes* Comp. Biochem. Physiol. Part A, **147**, 389-394

6. Acknowledgements

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