OptiPrep™ Application Sheet S57

Isolation of organelles from algae and fungi

- 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 Subcellular Membranes Index; key Ctrl "F" and type the S-Number in the Find Box.
- **Yeast organelles are covered in other Application Sheets; see Subcellular Membrane Index**
- This Application Sheet summarizes all of the known OptiPrep™ applications for algae and fungi; a detailed methodology is only provided in a few instances.
- **Some of the more recent papers are summarized in Section 7.**

1. *Chlamydomonas reinhardtii*

Gradients that are used primarily for the isolation of acidocalcisomes also resolve mitochondria; **see Application Sheet 48**.

Membrane vesicles can be shed from flagella by shaking in 25 mM KCl, 5 mM $MgSO_4$, 1 mM DTT, 0.5 mM EDTA, 10 mM Hepes, pH 7.2 containing 0.1% NP40 (plus protease inhibitors). Axonemes are then removed by repeated centrifugation at 16,000 *g* for 10 min and the vesicles harvested from the supernatant at 228,000 *g* for 30 min. The crude vesicles in 1 ml of the medium are adjusted to 15% (w/v) iodixanol; underlaid with an equal column of 30% iodixanol and centrifuged at 431,000 *g* for approx 1 h. The vesicles band in the middle of the gradient [1].

In a study of the development of cilia in Wood and Rosenbaum in *Chlamydomonas reinhardtii* [2] sCytoplasmic extract was adjusted to 30% w/v iodixanol and overlaid by layers of 25%, 20%, 15%, 12%, 10%, and 5% and centrifuged for 3 hr at 200,000 *g*. The gradients were used to study the attachment of proteins to cytoplasmic membranes during flagellar development.

2. *Cladopsorium resinae*

The LMF in a routine buffered 0.25 M sucrose solution containing 1 mM EDTA was adjusted to 35% (w/v) iodixanol and a gradient formed by self-generation in near vertical NVT65 rotor centrifuged at 202,000 *g* for 4 h [3]. The organelle distribution is shown diagrammatically in Figure 1A; if the overlapping fractions of mitochondria and lysosomes are collected and adjusted to 25% iodixanol and re-centrifuged then a complete separation of these two organelles is obtained (Figure 1B).

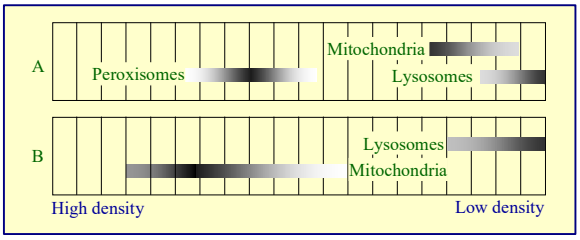

Figure 1 Sequential separation of *Cladosporium* organelles in two self-generated gradients: A, 35% iodixanol, B 25% iodixanol. For more information see text.

3. *Cyanidioschyzon merolae*

3.1 Microtubule-linked organelles

Cells were lysed in a microtubule stabilizing medium: 1.2 M sorbitol, 2 mM $MgSO_4$, 2 mM KCl, 1 mM DTT, 4 mM EGTA, 10 mM Tris-HCl, pH 7.2, containing, 50µM paclitaxel and 0.1% BSA, plus protease inhibitors [4,5]. The homogenate was mixed with an equal volume of 10% (w/v) iodixanol in 0.6 M sorbitol, 2 mM $MgSO_4$, 2 mM KCl, 1 mM DTT, 4 mM EGTA, 10 mM Tris-HCl, pH 7.2, containing, 10µM paclitaxel (plus protease inhibitors). A discontinuous gradient of 15%, 25% and 40% (w/v) iodixanol (8 ml, 8 ml and 4 ml respectively), in the same buffer, were overlaid with 15 ml of 300 *g* supernatant and centrifuged at 141,000 *g* for 30 min. The microtubule-linked organelles banded at the lowest interface.

3.2 Polyphosphate vacuoles

Yagisawa et al [6] isolated these polyphosphate-rich vacuoles from *Cyanidioschyzon merolae* and identified a number of important proteins. The discontinuous gradient used by the authors was an

unusual one, comprising three layer one of 27%, 62% and 80% (w/v) iodixanol. To achieve the latter two solutions, the authors first produced a very high density stock by evaporating the water from OptiPrep™. **This is not a procedure that the manufacturers of OptiPrep™ can recommend**. Since however the vacuoles banded at the 27%/62% iodixanol interface we suggest that the densest layer is omitted and the gradient formed from OptiPrep™ and 27% (w/v) iodixanol. To maintain the other solution properties used by the authors first prepare a diluent of 62% (w/w) sucrose solution containing 40 mM MgCl 2, 100 mM KCl, 400 mM Tris-HCl, pH 7.6. For the dense gradient solution add 1 ml of the diluent to 20 ml of OptiPrep™. From this prepare a 27% (w/v) iodixanol solution by diluting 2.7 vol. with 3.3 ml of 0.25 M sucrose, 2 mM MgCl 2, 5 mM KCl, 20 mM Tris-HCl, pH 7.6. Use the latter to resuspend the total membrane pellet. Layer 0.6 ml of this on to 2 ml of the dense solution and overlay with 1.4 ml of the lysis solution and centrifuge at 50,000 g for 1 h at 8°C. The vacuoles band at the lower interface.

- More recently, iodixanol gradients have been used to study peroxisomes from this algae [7].

4. *Cryptococcus neoformans* extracellular vesicles (EVs)

The glucuronoxylomannan of this fungus appears to be transported into the extracellular space in a membrane vesicle. This exocytic process is akin to that which occurs in mammalian cells and the shallow 6-18% (w/v) iodixanol gradients used to purify the EVs released from such cells have also been used for similar studies with *Cryptococcus neoformans*. The gradients were centrifuged at 250,000 *g* for 75 min [8]. A complex banding pattern of the glucuronoxylomannan and sterol suggest a heterogeneity of the budded vesicles. Discontinuous iodixanol gradients have been used by Wolf et al [9] comprising 3 ml of 30% and 2 ml each of 25%, 20%, 15% and 10% (w/v) iodixanol. The crude membrane vesicles in 5% (w/v) iodixanol were layered on top and centrifuged at 140 000 *g*, at 4°C, for 15 h. The peak of EVs occurred just below the mid-point of the gradient and interestingly the lightest ones were deficient in glucuronoxylomannan.

5. *Neurospora crassa*

Glyoxysomes have been prepared from an *N. crassa* post-nuclear supernatant (PNS) using a gradient similar [10] to that developed for mammalian peroxisomes **see Application Sheet S12**. The following method is adapted from ref 10.

Keep the following stock solutions at 4°C:

1 M MOPS	20.9 g per 100 ml water
100 mM EDTA (Na_2•$2H_2O$)	3.72 g per 100 ml water

Solution B: Dissolve 17 g sucrose in 100 ml water; add 2 ml, 20 ml and 20 ml respectively of ethanol, EDTA stock and Mops stock; adjust to pH 7.2 with 1 M NaOH and make up to 200 ml.

Solution D: Dissolve 17 g sucrose in 100 ml water; add 0.2 ml, 2 ml and 2 ml respectively of ethanol, EDTA stock and Mops stock; adjust to pH 7.2 with 1 M NaOH and make up to 200 ml.

5.1 Solutions required

A. OptiPrep™
B. OptiPrep™ diluent: 0.25 M sucrose, 10 mM EDTA, 100 mM MOPS-NaOH pH 7.2, 1% (v/v) ethanol (see Box)
C. 54% (w/v) Iodixanol stock solution: 9 vol. of Solution A + 1 vol. Solution B.
D. Homogenization medium: 0.25 M sucrose, 1 mM EDTA, 10 mM MOPS-NaOH, pH 7.2, containing 0.1% (v/v) ethanol.
E. Gradient solutions of 47% and 35% (w/v) iodixanol: dilute Solution C with Solution D.

5.2 Ultracentrifuge rotor

Sorvall TH641 or Beckman SW41 or equivalent rotor with approx. 30 ml tubes

5.3 Protocol

1. Prepare an homogenate of *N crassa* in Solution D.

2. Centrifuge the homogenate at 500 *g* for 5 min and repeat this three times to remove all of the abrasive powder and nuclei.

3. Prepare a crude organelle pellet was by centrifugation of the PNS at 25,000 *g* for 30 min and resuspend the pellet in Solution D.

4. Mix the resuspended pellet with an equal volume of the 47% (w/v) iodixanol solution.

5. Transfer 9-10 ml of the crude organelle suspension in 23.5% iodixanol and underlayer with 1-2 ml of the 35% iodixanol solution. If necessary top up the tubes to the required filling volume by layering Solution D on top.

6. Centrifuge at 110,000 *g* for 2 h. The glyoxysomes band at the interface between the two iodixanol solutions.

6. *Paracoccidioides brasiliensis*

The mitochondria and peroxisomes from the yeast phase of *Paracoccidioides brasiliensis* have been isolated using a gradient identical to that used for preparing mitochondria from *S. cerevisiae*. **Section 4 of Application Sheet S17** describes the discontinuous gradient that is used [11]; the densest band contains the peroxisomes. See also **Application Sheet S55** for more information of the isolation of peroxisomes from *S. cerevisiae*.

7. Recent publications

A CsCl gradient was used primarily to separate flagella, transition zones, and empty flagellar collars from *Chlamydomonas elegans* . To improve the ourity of the transition zone fraction, it was diluted with buffer and harvested by centrifugation at approx 190,000 g_{av} for 20 min. The pellet was made was resuspended in buffer; adjusted to 30% iodixanol and made part of a discontinuous 60%, 30%, 20% (w/v) iodixanol step gradient After centrifugation for 3 hr at approx 165,000 g_{av}. The transitional zones banded at the 60%/30% iodixanol interface, diluted. For more details see ref 12. Secretory vesicles from *Candida albicans* [13] were fractionated according to a method first described by Chang et al (**see Application Sheet S50**).

- Note the other **Application Sheets** devoted to algae and fungi:
- Algae: Acidocalcisomes (**S48**)
- Fungi: Extracellular vesicles (**S60**)

8. References

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Application Sheet S57; 5th edition, January 2018

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