OptiPrep™ Application Sheet C48

Purification of *Toxoplasma gondii* from cell cultures

- 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 the separation of sporocysts and oocyst walls **see Application sheet C34**

1. Background

Toxoplasma gondii can be maintained in cell culture using Vero, Chinese hamster ovary (CHO) cells or human foreskin fibroblasts (HFF). Coppens et al [1] developed a simple continuous 10-30% Nycodenz® gradient to purify the parasite cells away from the host cell material; it has been used in many later studies [2-6]. Although iodixanol gradients have been used separating oocyst walls and sporocysts, they have not been used in this particular *Toxoplasma* application. Since Nycodenz® is only available as a powder, while iodixanol solutions are prepared by simple dilution of OptiPrep™, this alternative is given in the methodology below. Although it is highly likely that this modification would be effective in the purification of the organism, it has not been validated.

The following methodology is adapted from ref 1.

2. Solution preparation

A. Nycodenz® powder **OR**
B. OptiPrep™ (shake gently before use)
C. Phosphate-buffered saline (PBS)
D. For Nycodenz® solutions only: Phosphate buffer: 100 vol. of 1.78% (w/v) $Na_2HPO_4.2H_2O$ + 25 vol. 1.38% (w/v) $NaH_2PO_4.H_2O$

To make up a 30% (w/v) Nycodenz® stock solution place 50 ml of water in a 150 ml beaker on a heated magnetic stirrer set at approx. 50°C and add 30 g of Nycodenz® powder in small amounts until dissolved. Allow the solution to cool to room temperature; add 5 ml of Solution D and then make up to 100 ml with water. Filter sterilize if required (see Note 1). For the continuous gradient prepare also a solution of 10% (w/v) Nycodenz® by diluting the 30% stock solution with Solution C. For a discontinuous gradient alternative also make up similarly a 20% Nycodenz® solution (see Section 3, Step 2).

For the iodixanol option simply dilute OptiPrep™ (60% w/v iodixanol) with Solution C to make a 10% and 30% **OR** 10%, 20% and 30% (w/v) iodixanol solutions (see Note 1 and Section 3, Step 2).

3. Protocol

1. Culture the *Toxoplasma* in Vero cells, CHO cells or HFF (see ref 1).

2. Preparation prepare continuous gradients (total volume approx 8 ml in a 15 ml tube or approx 30 ml in a 50 ml tube) of Nycodenz® or iodixanol from equal volumes of 10% and 30% Nycodenz® or iodixanol using a two-chamber gradient maker or Gradient Master™. If neither of these devices is available prepare discontinuous gradients from equal volumes of 10%, 20% and 30% Nycodenz® or iodixanol; carefully rotate the tubes to a horizontal position and allow the gradient to form by diffusion (see Note 2).

3. During gradient production harvest parasites from the cell culture supernatants and pass the suspensions twice through a 27-gauge syringe needle to disrupt any contaminating cells.

4. Wash the *Toxoplasma* three times in Solution C; centrifuging the suspension each time at 1000 *g* for 10 min.

5. Finally suspend the pellet in 10% Nycodenz® or iodixanol and layer on top of the continuous gradient (see Note 3).

6. Centrifuge at 2000 *g* for approx. 30 min (see Note 4).

7. Harvest the *Toxoplasma* that bands around 1.09-1.11 g/ml (just above half way down the gradient).

4. Notes

1. The 30% (w/v) Nycodenz® solution will be slightly hyperosmotic (approx. 315 mOsm); all the iodixanol solutions will be isoosmotic with mammalian cells.
2. Diffusion of the discontinuous gradient should take no more than about 1 h at room temperature. If the tubes are kept vertical, the process will take several hours. For more information **see Application Sheet C02**. Because the *Toxoplasma* bands at a density just below that of 20% (w/v) Nycodenz® or iodixanol, a discontinuous gradient may be effective in the purification process but this has not been tested (as far as we know).
3. The residual buffer on and in the pellet will dilute the gradient medium to allow layering on the 10-30% gradient. If difficulty is encountered in layering the sample, dilute it with about 0.2 ml of Solution C.
4. Do not use the brake for deceleration of the rotor.

5. References

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2. Coppens, I. and Joiner, K.A. (2003) *Host but not parasite cholesterol controls Toxoplasma cell entry by modulating organelle discharge* Mol. Biol. Cell, **14**, 3804-3820
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