OptiPrep™ Application Sheet C33

Purification of *Toxoplasma gondii* from cell cultures (Part A)
Separation of sporocysts and oocyst walls (Part B)

- 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

Part A

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

1. Coppens, I., Sinai, A.P. and Joiner, K.A. (2000) *Toxoplasma gondii exploits low-density lipoprotein receptor-mediated endocytosis for cholesterol acquisition* J. Cell Biol., **149**, 167-180E
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
3. Quittnat, F., Nishikawa, Y., Stedman, T.T., Voelker, D.R., Choi, J-Y., Zahn, M.M., Murphy, R.C., Barkley, R.M., Pypaert, M., Joiner, K.A. and Coppens, I. (2004) *On the biogenesis of lipid bodies in ancient eukaryotes: synthesis of triacylglycerols by a Toxoplasma DGAT1-related enzyme* Mol. Biochem. Parasitol., **138**, 107-122
4. Nishikawa, Y., Quittnat, F., Stedman, T.T., Voelker, D.R., Choi, J-Y., Zahn, M.., Yang, M., Pypaert, M., Joiner, K.A. and Coppens, I.et al (2005) *Host cell lipids control cholesteryl ester synthesis and storage in intracellular Toxoplasma* Cell. Microbiol., **7**, 849-867
5. Massimine, K.M., Doan, L.T., Atreya, C.A., Stedman, T.T., Anderson, K.S., Joiner, K.A. and Coppens, I et al (2005) *Toxoplasma gondii is capable of exogenous folate transport a likely expansion of the BT1 family of transmembrane proteins* Mol. Biochem. Parasitol., **144**, 44-54
6. Sehgal, A., Bettiol, S., Pypaert, M., Wenk, M.R., Kaasch, A., Blader, I.J., Joiner, K.A. and Coppens, I. (2005) *Peculiarities of host cholesterol transport to the unique intracellular vacuole containing Toxoplasma* Traffic, **6**, 1125-1141

Part B

1. Background

The resistance of *Toxoplasma gondii* is thought to be related to the oocyst wall that surrounds the sporocysts [1]. To investigate the nature and functional properties of the oocyst wall, divorced from the sporocysts could be an important step in understanding the infectious properties of this organism.

Although Percoll® gradients were able to provide a purified sporocyst fraction, because these particles do not all band in a discrete manner in such gradients, they were unable to provide a simultaneous isolation of a pure oocyst wall fraction [1]. Gradients formed from OptiPrep™ on the other hand are able to provide purified sporocysts and oocyst walls in the same gradient.

The following protocol is adapted from ref 1. Everson et al investigated top-loaded and bottom-loaded discontinuous iodixanol gradients and both alternatives are presented in this Application Sheet.

It describes only the gradient separation and not the method for mechanical fragmentation of the oocysts –see ref 1 for this information. See ref 2 for a review of *Toxoplasma* methodology.

2. Solutions required

E. OptiPrep™ (shake gently before use)

F. Diluent: 0.25 M sucrose, 15 mM Tris-HCl, pH 7.5

Keep the following stock solution at 4°C:
1 M Tris (free base), 12.1 g per 100 ml water

Solution F: Dissolve 8.5 g sucrose in 50 ml water; add 1.5 ml of Tris stock; adjust to pH 7.5 with 1 M HCl and make up to 100 ml.

3. Protocol

1. Fragment the oocysts using glass beads and vortexing according to ref 1.

2. Prepare the following density gradient solutions by diluting OptiPrep™ with Solution B: 2.5%, 5%, 10%, 15%, 20%, 25% and 30% (v/v) OptiPrep™. For bottom loading omit the 30% OptiPrep™ (see Note 1 **and Important Note at the end of Section 4**).

3. For bottom loading only: mix 7 vol. of the fragmented oocyst suspension with 3 vol. of OptiPrep™ (i.e. adjust the suspension to 30% v/v OptiPrep™) and use this in place of the 30% OptiPrep™ (step 1).

4. Prepare a discontinuous gradient from equal volumes (1-2 ml) of each of the iodixanol solutions; Underlayering is probably the easiest way of creating the gradient (see Notes 2-4).

5. For top-loading only, load the gradient with the fragmented oocysts in 2.5% v/v OptiPrep™ (see Note 5).

6. Centrifuge at 1000 *g* for approx. 1 h (see Note 6). Do not use the brake for deceleration.

7. Harvest the intact sporocysts which band between 5% and 15% iodixanol and the oocyst walls which band at the 25%/30% interface (see Figure 1 and Notes 7 and 8).

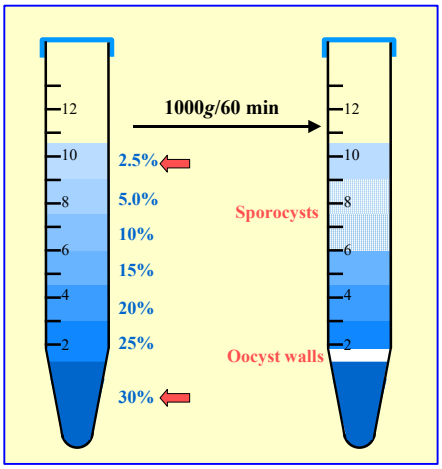

Figure 1: Separation of sporocysts and oocyst walls from fragmented *Toxoplasma gondii*, in a discontinuous iodixanol gradient. Sample may be loaded at either of the two arrowed positions.

4. Notes

1. Everson et al [1] investigated gradients with step intervals of both 2.5% and 5% OptiPrep™.
2. Underlayering using a metal cannula attached to a 1-2 ml syringe is the best way of creating multiple step gradients. Alternatively a small volume ("low-pulse") peristaltic pump might be used to introduce each layer, dense end first. Use the pump to take up the aliquot of solution and then reverse the flow to expel into the centrifuge tube. For more information **see Application Sheet C02**.
3. The gradient will become more or less continuous (particularly if 2.5% steps are used) due to the mixing that is bound to occur and diffusion during the setting up and centrifugation.
4. Dumètre and Dardè [3] used bottom loading with slightly different discontinuous iodixanol gradient of 30%, 25%, 20%, 15% and 5% (v/v). This was also used by Fritz et al [4].
5. Top-loaded gradients tend to give a lower yield of oocyst walls than do bottom-loaded gradients. Although the yield of sporocysts was greater with top-loaded gradients, contamination by some non-sporulated oocysts was greater.
6. Everson et al [1] investigated centrifugation times from 20-100 min. One hour is probably optimal, although if flotation is used some of the non-sporulated oocysts, which are found below the 15% iodixanol, have probably not had time to reach their banding density.

7. 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**.
8. Other groups have also reported the use of these OptiPrep™ techniques for the studies of oocysts and sporocysts (e.g. see ref 5 and 6).

IMPORTANT NOTE: published papers below often describe gradient solutions as "% v/v iodixanol". Since OptiPrep™ is the commercial name for a 60% (w/v) iodixanol solution it has been assumed that the solutions are actually % v/v OptiPrep™.

5. References

1. Everson, W. V., Ware, M. W., Dubey, J. P. and Lindquist, H. D. L. (2002) *Isolation of purified oocyst walls and sporocysts from Toxoplasma gondii* J. Eukaryot. Microbiol., **49**, 344-349
2. Dumètre, A. and Dardé, M-L. (2003) *How to detect Toxoplasma gondii oocysts in environmental samples* FEMS Microbiol. Rev., **27**, 651-661
3. Dumètre, A. and Dardé, M-L. (2005) *Immunomagnetic separation of Toxoplasma gondii oocysts using a monoclonal antibody directed against the oocyst wall* J. Microbiol. Meth., **61**, 209-217
4. Fritz, H.M., Bowyer, P.W., Bogyo, M., Conrad, P.A. and Boothroyd, J.C. (2012) *Proteomic analysis of fractionated toxoplasma oocysts reveals clues to their environmental resistance* PLoS One **7**: e29955
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6. Escotte-Binet, S., Da Silva, A.M., Cancès, B., Aubert, D., Dubey, J., La Carbona, S., Villena, I. and Poulle, M-L. (2019) *A rapid and sensitive method to detect Toxoplasma gondii oocysts in soil samples* Vet. Parasitol., **274**: 108904

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