OptiPrep™ Application Sheet C34

Purification of oocyst walls and sporocysts from *Toxoplasma gondii*

- 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 purification of parasites from culture see **Application Sheet C51**

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 [1] 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 (see Note 1)

A. OptiPrep™ (shake gently before use)

B. OptiPrep™ Diluent: 0.25 M sucrose, 90 mM Tris-HCl, pH 7.5

C. Working Solution (50% w/v iodixanol): Mix 5 vol. of Solution A with 1 vol. of Solution B

D. Suspension Solution: 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 B: Dissolve 8.5 g sucrose in 50 ml water; add 9 ml of Tris stock; adjust to pH 7.5 with 1 M HCl and make up to 100 ml.

Solution D: 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 Solution C with Solution D: 2.5%, 5%, 10%, 15%, 20%, 25% and 30% (w/v) iodixanol; for bottom loading omit the 30% iodixanol (see Note 2).

3. For bottom loading only: mix 2 vol. of the fragmented oocyst suspension with 3 vol. of Solution C (i.e. adjust the suspension to 30% iodixanol) and use this in place of the 30% iodixanol (step 2).

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 3-5).

5. For top-loading only, load the gradient with the fragmented oocysts in 2.5% iodixanol (see Note 6).

6. Centrifuge at 1000 *g* for approx. 1 h (see Note 7). 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 Note 8).

4. Notes

1. The preparation of a Working Solution (Solution C) from which the density solutions are produced by dilution with Solution D enables the concentration of buffer to be constant throughout the gradient. Other additives at concentrations <10 mM in the gradient may be included at 6x the required concentration in Solution B, as long as the osmolality of the gradient is not compromised **(see Application Sheet C01)**.
2. Everson et al [1] investigated gradients with step intervals of both 2.5% and 5% iodixanol.
3. 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**.
4. 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.

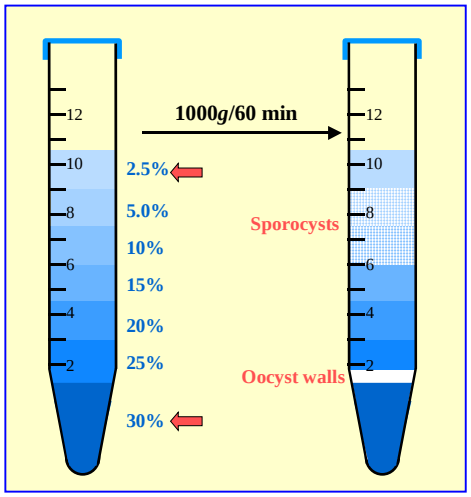

Figure 1: Separation of sporocysts and oocyst walls from fragmented *Toxoplasma gondii*, in a discontinuous iodixanol gradient. Sample may be loaded at either of the two arrowed positions.

5. Dumètre and Dardè [3] used bottom loading with slightly different discontinuous iodixanol gradient of 30%, 25%, 20%, 15% and 5% (w/v). This was also used by Fritz et al [4].
6. 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.
7. 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.
8. 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**.
9. Other groups have also reported the use of these OptiPrep™ techniques for the studies of oocysts and sporocysts (e.g. see ref 5)

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
5. Gondim, L.F.P., Wolf, A., Vrhovec, M.G., Pantchev, N., Bauer, C., Langenmayer, M.C., Bohne, W. Teifke, J. P. et al (2016) *Characterization of an IgG monoclonal antibody targeted to both tissue cyst and sporocyst walls of Toxoplasma gondii* Exp. Parasitol., **163**, 46-56

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