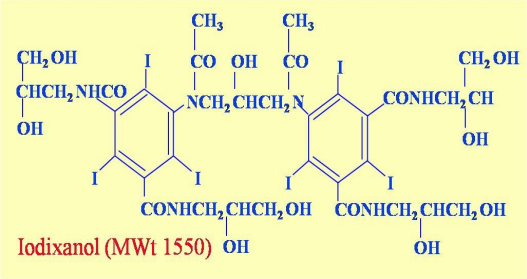
Excellence in Separations

DENSITY GRADIENT MEDIA

OptiPrep™
Purification of microorganisms and their subcellular membranes

OptiPrep™ is a sterile endotoxin tested solution of 60% iodixanol in water with a density of 1.32 g/ml.

Iodixanol was developed as an X-ray contrast medium an has therefore been subjected to rigorous clinical testing.



Iodixanol is non-ionic, non-toxic to cells and metabolically inert.

Iodixanol solutions can be made isoosmotic at all useful densities.

Iodixanol solutions have low viscosity and osmolarity.

OptiPrep™ has been manufactured in compliance with current EU guide to cGMP.

Actual endotoxin levels in each batch are usually measured at <0.13 EU/ml.

The high density of **OptiPrep™** facilitates the fractionation of cells by flotation from a dense load zone through either a continuous or discontinuous gradient or through a simple density barrier.

Improved resolution of cell organelles

Low viscosity, isoosmotic gradients provide rapid and efficient separation of the major organelles in preformed gradients.

OptiPrep™ avoids the high viscosity of sucrose and Ficoll®.

OptiPrep™ avoids the inconvenience of removing Percoll® from subcellular organelles.

Iodixanol can be removed efficiently and rapidly from all particle suspensions if required.

Organelles have more distinctively different densities in **OptiPrep™** than in sucrose or Percoll®.

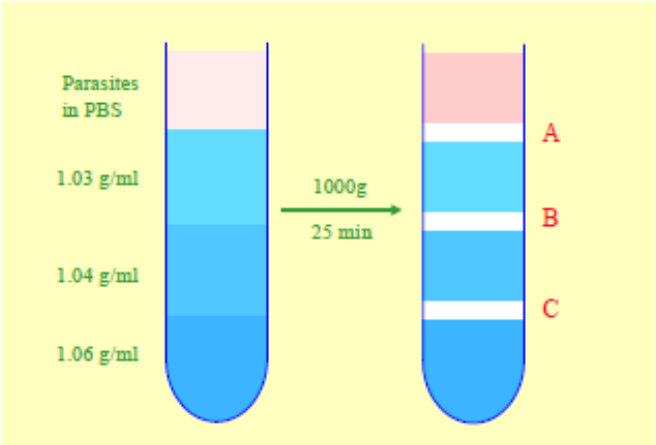
Fractionation of *Sarcocystis neurona* in a discontinuous gradient

To be able to study the cell and molecular biological characteristics of the parasites that cause debilitating diseases in a variety of livestock requires an effective system for their growth in, and release from, cultured cells. Subsequent density gradient centrifugation is often an important add-on technique for the separation of the parasites from cell debris and for resolving morphologically distinct forms of the parasite.

Sarcocystis neurona grows relatively slowly in host cells and the merozoites are also released from the cells rather slowly. Ellison et al have described a procedure that uses the calcium ionophore A23187 to cause rapid and synchronous release of the merozoites. A discontinuous iodixanol gradient was also developed by Ellison et al to resolve the different morphological forms of the parasite.

Discontinuous iodixanol (OptiPrep™) gradients of 1.03,1.04 and 1.06 g/ml can resolve different morphological forms of the merozoite of *Sarcocystis neurona*.

The protocol is summarized in the figure. The morphology and the nucleus:cytoplasm ratio of the three forms of merozoites that banded in the gradient were distinctively different (see figure). Parasites banded at interface A were tear-shaped to oblong, while those at interface B were more rounded with a lower nucleus: cytoplasm ratio. The early merozoites banded at interface C along with host cells.



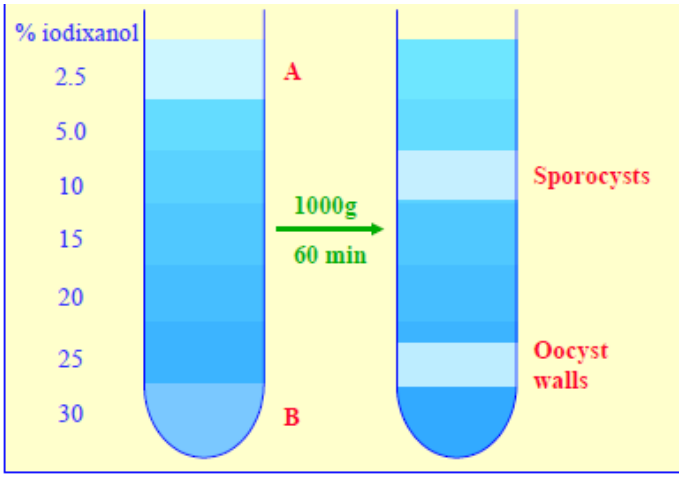
For a detailed protocol and references see C27 on the OptiPrep™ Application CD or online at: www.axis-shield-density-gradient-media.com

Separation of sporocysts and oocyst walls in a discontinuous gradient

Toxoplasmosis has been recognized in both humans and livestock and it is a serious disease in pregnancy and in immunologically-compromised individuals. The resistance of the infective agent, the parasite *Toxoplasma gondii* is thought to be related to the oocyst wall which surrounds the sporocysts. To be able 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. Gradients formed from OptiPrep™ on the other hand are able to provide purified sporocysts and oocyst walls in the same gradient.

The sporocysts are considerable less dense than the oocyst walls and band around 10% iodixanol (approx. 1.058 g/ml). Top-loaded gradients give a higher yield of sporocysts but contamination by some sporulated oocysts was greater.

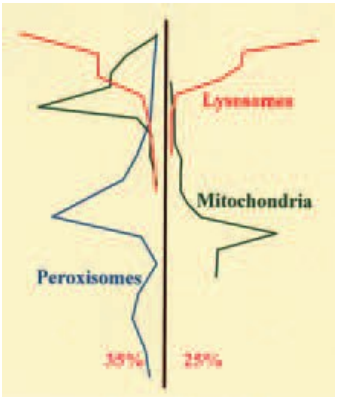


See Application sheet C34 at www.axis-shield-density-gradient-media.com

Fractionation of a light mitochondrial fraction from *Cladosporium resinae*

Self-generated iodixanol gradients have successfully fractionated the major organelles from *Cladosporium resinae*. Homogenates of *Cladosporium resinae* spheroplasts were centrifuged at 2000g for 10 min and then a light mitochondrial fraction (LMF) prepared from the post-2000g supernatant. The LMF was suspended in a buffer containing 0.25 M sucrose and after adjusting to the appropriate iodixanol concentration centrifuged in a near-vertical rotor (Beckman NVT65) for 4h at 202,000g.

At 35% (w/v) iodixanol, the gradient completely resolves peroxisomes from mitochondria and partially resolves mitochondria and lysosomes (see figure).



At 25% (w/v) mitochondria and lysosomes can be resolved completely.

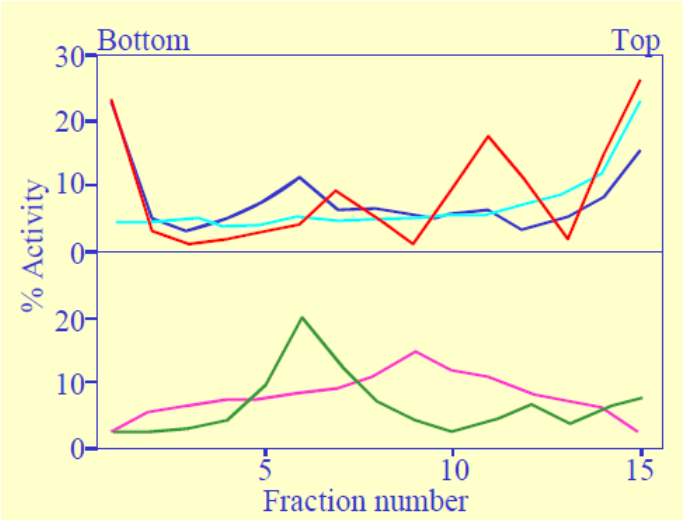
For more information about self-generated gradients see OptiPrep™ Application Sheet S4.

Goswami, P. and Cooney, J.J.
Appl. Microbiol. Biotechnol., ***51***, *860-864 (1999)*

Fractionation of acidocalsisomes from *Trypanosoma cruzi*

Acidocalcisomes, the dense acidic calcium-storing organelles, which were originally identified in *Trypanosoma cruzi* by Docampo et al in 1995, have no parallels in mammalian cells. They thus represent a unique functional characteristic, not shared by the host and hence offer an important potential target for chemotherapy of Chagas disease. Scott and Docampo developed a discontinuous iodixanol gradient for purifying acidocalcisomes that effectively replaces the previous Percoll® method and overcomes many of the disadvantages associated with the use of Percoll®.

A 325g supernatant from an homogenate is centrifuged at 10,500g for 10 min and the resuspended pellet loaded on to a 24%, 28%. 34%, 37% and 40% (w/v) iodixanol gradient. After centrifugation at 50,000g for 1 h, the acidocalcisomes form a pellet (see figure).



pyrophosphatase (dark blue); V-H^+ ATPase (red), alkaline phosphodiesterase (blue), acid phosphatase (green), succinate-cyt c reductase (pink).

The original methodology of Scott and Docampo was aimed primarily at purification of acidocalcisomes from *T. cruzi*; the same gradient was later extended to the isolation of similar dense particles from *Leishmania major*, *Chlamydomonas reinhardtii* and *Dictyostelium discoideum*.

For a detailed protocol and references see S48 on the OptiPrep™ Application CD or online at: www.axis-shield-density-gradient-media.com

Web:

www.axis-shield-density-gradient-media.com

AXIS-SHIELD

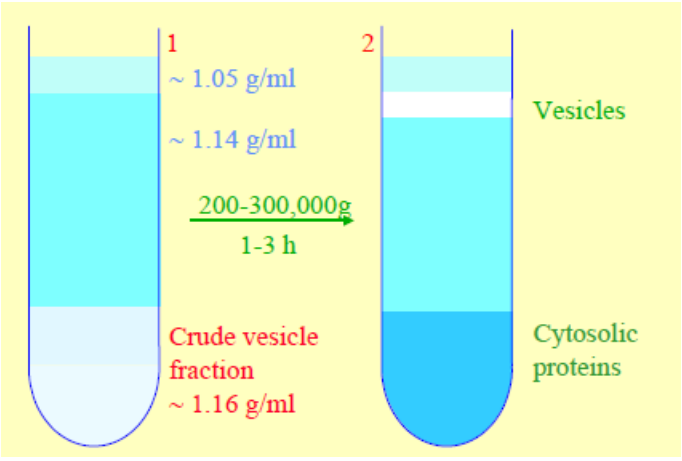
PO Box 6863 Rodelokka
N-0504 Oslo
Norway
Phone: +47 24 05 60 00
Fax: +47 24 05 60 10
Email: bjh@no.axis-shield.com or
john@jgrescon.fsbusiness.co.uk

In this leaflet we have presented some of the applications available for the purification of microorganisms and their subcellular organelles using OptiPrep™. More information can be found on: **www.axis-shield-density-gradient-media.com.** On the front page click on "Mammalian and non-mammalian cells" or "Subcellular membranes and cell organelles".

Ficoll and Percoll are trademarks of GE Healthcare companies

Separation of bacterial membrane vesicles from cytosol using flotation

To determine the location of a particular protein, a simple and unambiguous separation of the membrane and cytosolic fractions is essential. A flotation strategy first established for mammalian cells has been extended to bacteria. The much lower osmolality of iodixanol gradients, compared to those of sucrose, means that the resolution of soluble proteins and membranes is correspondingly greater in iodixanol gradients than in sucrose gradients. This is particularly important with bacterial cytoplasmic membrane vesicles, because their high protein/lipid ratio means that their density is closer to that of free proteins. The method, described in the figure, was first published by De Leeuw et al to study the localization of FtsY in *Escherichia coli*.



For a detailed protocol and references see S36 on the OptiPrep™ Application CD or online at: www.axis-shield-density-gradient-media.com

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