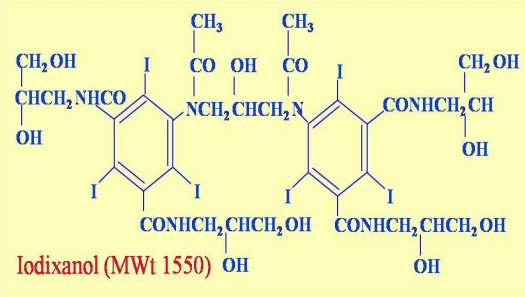
*Excellence in Separations*

# DENSITY GRADIENT MEDIA

## OptiPrep™
## The ideal density gradient medium for the purification of plasma membrane and domains

**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™** is manufactured in compliance with current EU guide to cGMP.

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

**OptiPrep™** offers three gradient formats for the isolation of the plasma membrane (PM).

Continuous or discontinuous gradients (1-3h)

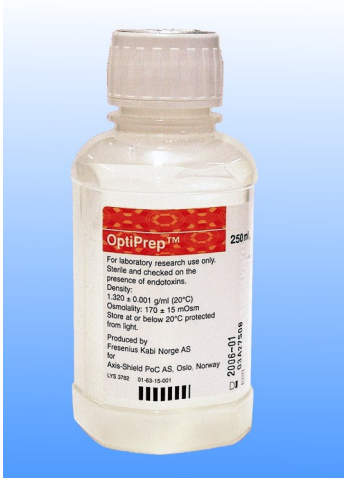
Continuous gradients (12-16h)

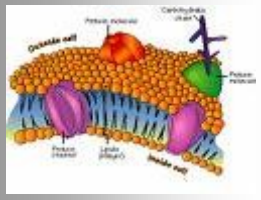
Flotation through discontinuous gradients

**OptiPrep™** offers two approaches to the purification of lipid-rich micro domains.

The first to be developed involves the isolation of caveolae from a sonicated total plasma membrane fraction. This strategy has also been used for the isolation of lipid rafts.

The lipid-rich nature of these microdomains renders them relatively insensitive to detergent solubilisation, compared to the rest of the plasma membrane and this forms the basis of their separation in detergent containing iodixanol gradients.



# Isolation of plasma membrane form cardiac muscle by flotation

Using iodixanol gradients, isolation of a plasma membrane (PM) fraction is often achieved simultaneously with the purification of other subcellular membranes. Most of these methods are suitable for work with cultured cells and soft tissues such as liver but tissues such as muscle often require a customized method. Nevertheless some of the other iodixanol gradients may be adaptable to muscle tissue.

Research into cardiac muscle function often involves the complex intracellular processes by which the GLUT4 glucose transport protein is translocated to and from the plasma membrane (sarcolemma). Thus in fractionations of cardiac muscle, a prime requirement is to be able resolve the plasma membrane from GLUT-4 transport vesicles and other membrane compartments. The overall strategy of this flotation gradient protocol is thus similar to that using a cultured cell line but the details of the technique are rather different.

The PM is usually the least dense of all the subcellular membranes and our application highlights a novel exploitation of this property. In this protocol, a crude plasma membrane suspension is adjusted to a density (1.049 g/ml, equivalent to 5% w/v iodixanol) just greater than that of the plasma membrane of cardiac muscle, thus during the centrifugation the plasma membrane will float to the top the liquid. By layering the sample upon a small cushion of 15% (w/v) iodixanol (1.103 g/ml) and centrifuging for 16 h, a shallow gradient will form due to diffusion of iodixanol and also to some sedimentation of iodixanol molecules close to the bottom of the tube. This will therefore allow not only isolation of the plasma membrane, but also partial fractionation of some of the denser membrane compartments.

Flotation often provides better recovery and/or resolution of the least dense components of a crude fraction than does sedimentation. Overloading the gradient (instability of the sample/gradient interface) cannot occur in a flotation format. The particular format described in this protocol also allows the sample to be contained within a relatively large volume; this also minimizes interactions between particles in the gradient.

**For detailed protocols and references see S27 on the OptiPrep™ Application CD or online at: www.axis-shield-density-gradient-media.com**

# Isolation of plasma membrane using a continuous gradient

Although both continuous and discontinuous iodixanol gradients, centrifuged for 1-3 h have been used for the isolation of a plasma membrane (PM) fraction, often simultaneously with the purification of other subcellular membranes, there is evidence that resolution is often improved by centrifugation for longer periods, since it is only under these conditions that true equilibrium density banding is achieved. In this method two strategies, employing the longer centrifugation time format are presented. Dunphy et al and Woods et al applied the post-nuclear fraction to a continuous iodixanol gradient, while Bivona et al initially used a discontinuous sucrose gradient to provide a crude plasma membrane before loading on to the continuous iodixanol gradient.

Generally the most successful gradients for purification of the PM cover a lower density range than do those primarily aimed at resolving the Golgi and endoplasmic reticulum (ER) but because the density of the plasma membrane (and other compartments) varies with the cell or tissue source, customized gradients may cover either a broader and/or higher density range. There is however another consideration in selection of the density range of the iodixanol gradient. Because Bivona et al used a fraction containing principally PM and ER, they were able to use a shallow low-density gradient (5-20% (w/v) iodixanol. A total post-nuclear fraction however also contains organelles such as mitochondria and peroxisomes which are much more rapidly sedimenting than the small vesicles of the PM and ER and may disturb such shallow low-density gradients. Thus

Dunphy et al and Woods et al chose steeper gradients covering a wider density range.

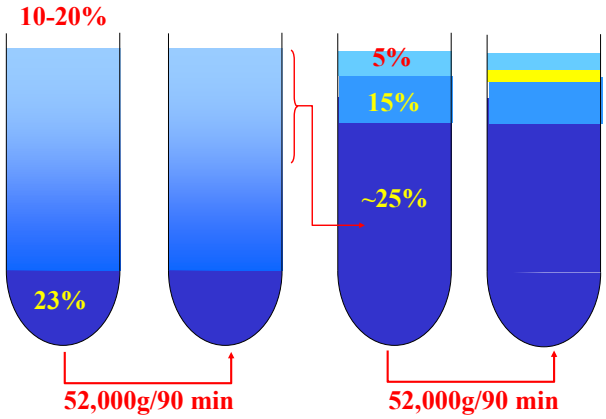
**For a detailed protocol and references see S25 on the OptiPrep™ Application CD or online at www.axis-shield-density-gradient-media.com**

## Detergent free purification of caveolae by sonication of a PM fraction

This is a detergent-free strategy and was first described by Smart et al for the isolation of a caveolae from human fibroblasts. It was later modified by Babitt et al and subsequently it has been applied either in the original or the revised form (without any further adaptation) to a wide range of other cell types and to membrane fractions from brain tissue, the parathyroid gland and chicken intestinal mucosal epithelium.

According to all published papers the sonicated plasma membrane suspension is adjusted to 23% iodixanol and layered beneath a continuous 10-20% iodixanol gradient and centrifuged at 52,000g for 90 min. After harvesting the gradient (from the top) in 15 fractions more than 95% of the protein was detected in fractions 9-15 while the caveolin was more or less confined to fractions 3-12. In the top 7 or 8 fractions therefore the specific activity of the caveolin is very high and it is this cut of the gradient which is processed further in the concentration phase.

Approximately the top half of the gradient is readjusted to 23% iodixanol; in the original method this was then overlaid with a solution of 5% iodixanol and the centrifugation repeated to allow the caveolae to band sharply at the interface. Subsequently a layer of 15% iodixanol was interposed to remove any denser protein-rich material harvested from the first gradient (see figure). Some of the published papers report the use of the original second gradient, some use the modification.




**For a detailed protocol and references see S35 on the OptiPrep™ Application CD or online at www.axis-shield-density-gradient-media.com**

## Purification of lipid rafts as detergent-resistant membranes /DRMs)

In one of the most widely used strategies, lipid raft isolation is based on the insolubility of these domains in a non-ionic detergent such as Triton X100. Detergent is added either to intact cells or a postnuclear supernatant, which is then normally adjusted to a high density and layered under a discontinuous gradient. Lipid rafts, which have a relatively low density, float away from soluble proteins and dense cytoskeleton- associated proteins, which remain in the load zone. Originally sucrose gradients were used for the separation but when ycodenz® flotation gradients were introduced Naslavsky et al observed that these gradients were far superior to those of sucrose.

Routinely cell lysis or homogenisation is performed in a buffered saline containing a chelating agent, a detergent,protease inhibitors and/or other additives such as DTT or $CaCl_2$. Whether the light mitochondrial fraction is used to prepare Golgi membranes depends on their response to homogenization; if they vesiculate they will be recovered in the microsomal fraction; if they retain a tubular structure they will be recovered in the light mitochondrial fraction. They are the least dense membrane in the light mitochondrial fraction and can be effectively

Web:

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

**AXIS-SHIELD**

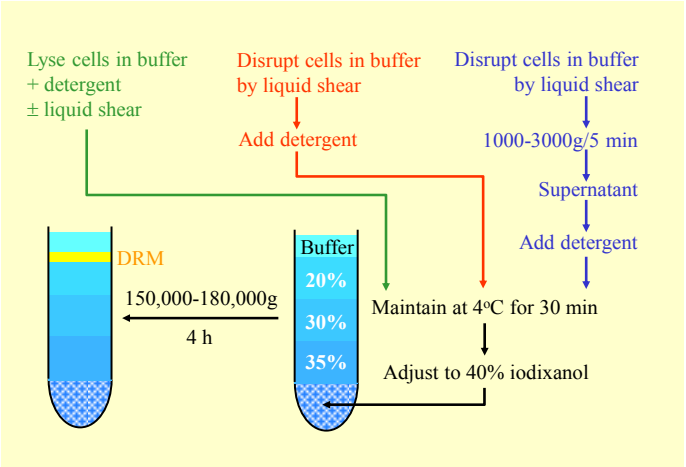
PO Box 6863 Rodelokka
N-0504 Oslo
Norway
Phone: +47 24 05 60 00
Fax: +47 24 05 60 10
Email: bjh@no.axis-shield.com or
john@jgrescon.fsbusiness.co.uk

In this leaflet we have presented some of the applications available for the isolation of plasma membrane and domains using OptiPrep™. More information can be found at: **www.axis-shield-density-gradient-media.com.** On the front page click on "Subcellular membranes and cell organelles".


isolated in a self-generated OptiPrep™ gradient (see figure).

Pre-formed gradient may also be used. Note that the isolation of Golgi membrane vesicles and other components of the secretory endocytic and synthetic systems are described elsewhere.

**For a detailed protocol and references see S33 on the OptiPrep™ Application CD or online at: www.axis-shield-density-gradient-media.com**




AXIS-SHIELD
Innovation for Life