S20 Purification of lipid rafts from cultured cells

OptiPrep™ is a 60% (w/v) solution of iodoxanol in water; density = 1.32 g/ml.

The importance of lipid-rich microdomains of the plasma membrane in signal-transduction events, in lipid transport and in various internalization processes and in the regulation of plasma membrane-cytoskeleton interactions have only become well established over the last five years. A number of important cholesterol and sphingolipid-rich structures have been identified and studied, notably caveolae and lipid rafts. The isolation of caveolae using OptiPrep™ is the subject of Application Sheet S19.

New methods have been recently developed for the isolation of these lipid rafts based on the insolubility of these structures in the non-ionic detergent TritonX-100. Either the intact cells are treated with a detergent-containing solution or a post-nuclear supernatant is prepared from a cell homogenate and then Triton X-100 is added to this supernatant. The former approach was adopted by Oliferenko et al [1] for Eph4 cells (a spontaneously immortalized mouse mammary epithelial cell line); the latter by Lafont et al [2] for fowl-plague infected MDCK cells. The detergent-treated material is then adjusted to a high density and layered under a discontinuous iodoxanol gradient. The lipid rafts, which have a relatively low density, float away from soluble proteins and detergent-insoluble cytoskeleton-associated proteins, which remain in the load zone.

Oliferenko et al [1] used the technique to study the association of hyaluronic acid cell surface receptor CD44 and annexin II with lipid rafts and their interaction with the cytoskeleton. Lafont et al [2] investigated the role of lipid rafts in apical trafficking and in particular their association with SNAP receptors.

The following protocol is based on both of these published methods.

Solutions required
A OptiPrep™
B Isolation medium: 150 mM NaCl, 5 mM dithiothreitol (DTT), 5 mM EDTA, 25 mM Tris-HCl, pH 7.4 supplemented with a cocktail of protease inhibitors (see Note 1).
C Triton X-100
D Phosphate-buffered saline (PBS)

Carry out all operations at 0-4°C

Rotor requirements
Any small volume (approx 4 ml) swinging bucket rotor for an ultracentrifuge (e.g. Beckman SW60Ti or Sorvall TH660)

Protocol
Isolation from a total cell lysate
Adjust Solution B to 1% Triton X-100

Wash the cell monolayer twice with PBS and scrape into this medium.

Pellet the cells and resuspend in 0.2 ml of Solution B+Triton; then leave on ice for 30 min.

Isolation from a post-nuclear supernatant
Homogenize the cells in Solution B (see Note 2).

Centrifuge the homogenate at 1000g for 10 min.

Adjust the supernatant to 1% Triton X-100 and leave on ice for 30 min.

Add 4 vol of OptiPrep™ to 2 vol of either the homogenate or 1000g supernatant.

Dilute OptiPrep™ with Solution B+Triton to give 35%, 30%, 25% and 20% (w/v) iodoxanol (see Note 3).

In tubes for the swinging-bucket rotor layer 0.6 ml each of the sample, the four gradient solutions and Solution B+Triton to fill the tube.

Centrifuge at 160,000gav for 4 h (see Note 4).
Collect the lipid rafts from the top interface.

**Notes**

1. The isolation media used by both Oliiferenko et al [1] and Lafont et al [2] were similar, although the level of DTT used by Oliiferenko et al was 1 mM rather than 5 mM and EDTA was omitted. Protease inhibitors such as PMSF, leupeptin, antipain, aprotinin etc should be included in all of the media.

2. Use a small Dounce homogenizer or passage through a fine gauge syringe needle or a cell cracker to effect homogenization. For more information on the homogenization of cultured cells see Application Sheet S6.

3. The gradient in the protocol is as described by Oliiferenko et al [1]. Lafont et al [2] used slightly fewer steps of either 30% iodixanol and Solution B+Triton or 30%, 20% and 5% iodixanol. In all cases the lipid rafts band close to the top of the gradient.

4. Oliiferenko et al [1] used a longer centrifugation time of 12 h at a slightly lower RCF (120,000g_{av}). Because of the relatively short sediment path length of the rotor, 4 h at the higher RCF is probably satisfactory, but this time requirement may vary with the mode of preparation and cell type.

**References**


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