OptiPrep™ Reference List RS10

Mammalian (including all chordata) cell exosomes and other microvesicles from, tissues, cells and conditioned medium

Introduction

There are three areas of investigation where iodixanol gradients have been widely used in studies of exocytosis and exocytotic vesicles.

1. The control and organization of membrane trafficking within the cells that permits the movement of vesicles to, and ultimately their fusion with, the plasma membrane or a specific plasma membrane domain. This is covered in **OptiPrep™ Application Sheet S47**
2. The analysis of microvesicles that are expressed from the surface of cells is very widely researched and often involves separation from virus particles in the conditioned medium from virus-expressing cells. This Reference List provides a full bibliography of papers reporting these fractionations. **A detailed methodology is provided in Application Sheet S63**
3. A third associated area of investigation is the isolation and study of extracellular vesicles (EVs) from Gram-positive bacteria and fungi, and of outer membrane vesicles (OMVs) from Gram-negative bacteria. The latter in particular are widely researched and have been shown to be important in the transfer of virulence factors and the initiation of immune and inflammatory responses in host cells. This is covered in **Reference List RS11 and Application Sheet S62**

- These and other **Reference Lists** or **OptiPrep™ Application Sheets** can be accessed from the website: www.Optiprep.com; click on "**Reference Lists**" or "**Methodology**".

Methodological summary

Various forms of pre-gradient processing are employed, during which intact cells and aggregated material in the culture medium are mostly removed and the exosomes or microvesicles concentrated. This is covered in much greater detail in **Application Sheet S63**

To minimize contamination of exosomes from the culture medium and or serum these solutions are either ultracentrifuged or filtered prior to contact with the cell monolayer. Occasionally serum-free medium is used.

Post-culture, cells and other large particles are first removed from the conditioned medium (CM) by differential centrifugation (**clarification step**). Sometimes a single low-speed centrifugation is used, more often two or three steps (e.g. 300 *g* and 5,000 *g*), usually for 10-15 min. Omission of the first step may lead to entrapment and loss of small vesicles into aggregates of rapidly-sedimenting larger particles at the higher *g*-force. Filtration is also used to remove larger contaminants: this is commonly performed using a 0.20 or 0.22 µm syringe filter, occasionally a smaller (0.1µm) or larger-pore (0.45µm) may be used. Filtration is usually used in combination with differential centrifugation, although it may be the only pre-gradient treatment.

Concentration of exosomes and other vesicles from the clarified CM usually involves pelleting 100-150,000 *g* for 1-2 h before resuspending in a suitable buffered medium for application to the iodixanol gradient; although there are variations to this strategy (**see Application Sheet S63**). Particularly large volumes of CM may be treated to a preliminary concentration using centrifugal ultrafiltration (5 kDa-100 kDa cut-off), to reduce the total volume prior to ultracentrifugation. Occasionally a discontinuous sucrose gradient may be used as part of the concentration process.

Purification of exoxomes has been successfully executed in the following types of iodixanol gradient:

1. Top-loaded sedimentation velocity iodixanol gradients, normally centrifuged for 1.5-2 h. The gradients, although often constructed from multiple layers (i.e. discontinuous) the density interval of only 1.2% (w/v) iodixanol is so small that the 6-18% (w/v) iodixanol gradient is essentially continuous.
2. Bottom-loaded discontinuous gradients with centrifugation times of 2-3 h.
   Bottom-loaded or top-loaded continuous gradients; centrifuged usually at 100-200,000 *g* for 16-21 h; the vesicles are banded according to their buoyant density.
3. Self-generated gradients: usually run in vertical or near-vertical rotors at approx. 350,000 *g* for 2-3 h. The method, as with any self-generated gradient separation, has the advantage of being both simple to set up and capable of producing a very reproducible density profile.
4. **For full details of methodology see Application Sheet S63**

Bibliography

- **The published papers on mammalian exosomes have been divided into the following sections:**
- **1. Isolation, characterization and markers of exosomes**
- **2. Clinical and biological relevance of exosomes**
- **3. Cancer detection, growth and therapy – general principles**
- **4. Electroporation of exosomes**
- **5. All other papers listed according to cell or tissue type and/or clinical study**

In **Sections 1-4** references are listed alphabetically according to **first author.**
In all references research topic key words are highlighted in blue.

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4. Electroporation of exosomes

The use of exosomes to introduce into target cells, molecules that have been artificially inserted by electroporation, is being investigated as potential treatment for a number of diseases. The methodology involves the selection of cells, such as dendritic cells, that are engineered to express an exosomal protein linked an organ-specific peptide (e.g. the neuron-specific RVG peptide). siRNA drugs are then introduced into the exosomes by electroporation, which can thus be targeted to a specific organ (the brain in the case of the RVG peptide). This technology may be viewed as a potential means of controlling, for example in the case of the RVG peptide, Alzheimer's disease.

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5. Analytical studies on exosomes purified in iodixanol gradients

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HeLa cells

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Macrophages (see "Monocytes")

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Osteoblasts/osteoclasts – see Myeloma cells

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Plasma

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Saliva, see "Human saliva"

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