DGM

Density Gradient Media

OptiPrep™

The ideal solution for isolation of exosomes

serumwerk bernburg

OptiPrep™ - non-ionic, iso-osmotic gradients

Introduction

Exosomes are membrane-enclosed vesicles that are released by different cell types into the extracellular space. They play an important role as mediators of cell-to-cell communication and are thus involved in a variety of physiological processes. These vesicles are also associated with the pathogenesis of various human malignancies (e.g. brain tumours, colorectal cancers and leukaemia). Specific diagnostic biomarkers (e.g. miRNA) in the exosomes from neoplastic cells may aid the prediction of a particular cancerous condition. Exosomes may also assist in the targeted delivery of vaccines or drugs. It is therefore not surprising that the purification of these membrane vesicles from body fluids has become one of the most rapidly expanding applications of OptiPrep.

Pre-gradient methodology

Since the source material for exosomes is commonly the liquid medium from a cell culture, it is important to ensure that this medium is devoid of particles that may contaminate the exosomes. This is achieved by ultra-centrifugation and/or filtration of the medium prior to introduction to the cell monolayer.

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* or 300*g*, 1000*g* and 3000*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 commonly used in combination with differential centrifugation, although it may be the sole pre-gradient treatment (continued on next panel).

Virtually all of the published methods involve a final pelleting of the exosomes at 100-150,000 *g* for 1-2 h before resuspending in a suitable buffered medium. Duelli, D.M. et al (2005) J. Cell Biol., **171**, 493-503 described a method in which the CM was initially centrifuged at 500 *g* and filtered, before being centrifuged sequentially at 12,000 *g*, 70,000 *g* and 110,000 *g*. In the differential centrifugation fractions, exosomal fusogenic activity was heavily concentrated in the 70,000 *g* pellet, with rather little in the 110,000 *g* pellet and it was the former that was further purified in the iodixanol density gradient. Some protocols, particularly those that are applied to large volumes of CM, include a preliminary concentration using centrifugal ultrafiltration, to reduce the total volume prior to this final pelleting at 100-150,000 *g*. This conveniently avoids the use of large volume ultracentrifuge rotors. These ultrafilters have cut-offs from 5 kDa to 100 kDa.

Iodixanol gradient formats

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 or top-loaded discontinuous gradients with centrifugation times of 2-3 h.
3. 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 in (2) and (3).
4. 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.

All the options are illustrated on the next panel.

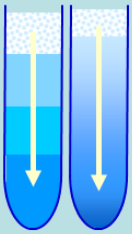
serumwerk bernburg

Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwerk.com • serumwerk.de

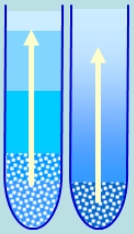
for high resolution

Iodixanol gradient options for exosome purification

Exosomes generally have a low banding density but this parameter depends on the nature of their cargo. An important point is that because iodixanol gradients can be made iso-osmotic with the cytoplasm and plasma, the type of gradient should have little effect on the exosome banding density. This contrasts with all sucrose gradients, which are also gradients of osmotic pressure. Exosomes top-loaded on a sucrose gradient will have a much lower starting density than ones loaded in a denser medium.

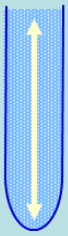


Top-loaded discontinuous or continuous gradient (left). Disadvantages: all particles move in the same direction; possible particle aggregation at any interface (particularly the sample/ gradient interface). Continuous gradient provides higher resolving power, but particle banding is generally less clear. The continuous gradient may be used for sedimentation-velocity separations



Bottom-loaded discontinuous or continuous gradient (right). Ideally the densest particles will remain in the load zone (or sediment). Least dense and largest particles will float most rapidly. Resolution often better than in top-loaded format

Median-loaded discontinuous gradient (left). This is the ideal buoyant density separation format. Particles will either remain in the load zone or float or sediment towards their banding density.



Self-generated gradients: In a final variant (right) the sample is simply adjusted to a median density and a continuous gradient generated at approx 300,000*g* in a vertical or near-vertical rotor

An alphabetical list of cells, tissues and viruses analysed for exosome function using iodixanol gradients

AAV-associated, Amoeboid cancer cells, Arabidopsis, Astrocytes, Astrocytoma cells, Atlantic salmon kidney
Bone marrow mast cells, Bone marrow mesenchymal stromal, Brain cortex, Brain tumour, Breast cancer cells
Carcinoma cells (colorectal, prostate), Cardiac progenitor cells, Cerebrospinal fluid, Chicken blood, Chinook salmon embryo, Cytomegalovirus
Daudi cells, Dendritic cells, Dengue virus transmission, Dermal fibroblasts, *Drosophila*
Ebola virus VP40 infected cells, Embryonic stem cells, Endometrial stromal cells, Endothelial cells, Endothelial progenitor cells, Epididyosomes, Erythrocytes (*Plasmodium*-infected) Erythroleukaemic cells, Erythrocytes (equine anaemia), Extravillous trophoblast cells, Eye diseases
Fibrosarcoma cells, Flavivirus transmission
Gastrointestinal cancers, Ginger root, Glioblastoma cells, Glomerular mesenchymal stromal cells
HEK cells, HeLa cells, HEp-2 cells, Hepatocytes, Hepatoma cells, Hepatic cells, Hepatitis A, B and C virus-associated cells, Herpes simplex virus infected cells, HIV infected cells, HTLV infected cells, Human CD4+ T cells, Human carcinoma cells,Human kidney epithelial, Human liver stem cells, Human lung carcinoma cells Human lung fibroblast (MRC-5), Human mast cells, Human myelomonocytic cells, Human saliva, Human sperm, Human trophoblast, HUVEC derived, Hypothalamus cells
IL-15 cell priming, Induced pluripotent stem cells, Insects, Intestinal epithelial
Jurkat cells
Keratinocytes, Kidney (primary embryonic and proximal tubule cells), Kirsten rat sarcoma cells
Leukaemia derived, Liver fibrosis, Lung cancer, Lymphoblastoid cells, Lymphocytic/leukaemia cells

serumwerk bernburg

Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwerk.com • serumwerk.de

Alphabetical list (contd)

Macrophages, Mammary epithelial cells, Mast cells, MCF-7 cells, MDCK cells, Medulloblastoma, Melanocytic cells, Melanoma cells, Mesenchymal stem cells, Mesenchymal stromal cells, Metastatic cancer, Milk, MIN6 (insulinoma) cells, Monocytes, Macrophages; Multiple myeloma bone disease, Murine lung, Mouse embryo fibroblasts, Myeloma cells, Multivesicular bodies

NCI60 cancer cells, NK cells, Neuroblastoma cells, Neural tumours, Neuronal cells, Neutrophils (human)

Oncosomes. Osteoclasts, Osteosarcoma cells

Pancreatic carcinoma cells, Pancreatic beta cells, Papilloma virus, Pheochromocytoma cells, Placenta-derived, Plasma, Platelets, Prostate cancer cells

Rat1 cells, Renal carcinoma, Renal tubule cell, Retinal pigmented epithelial

Salivary. *Schistosoma mansoni*, Seminal fluid, Sheep hyatid cysts, Skeletal muscle cells, Spinal cord, Suprachiasmatic nuclei cells, Synovial fibroblasts and fluid

T cells, Thyroid cancer cells, Tissue factor-containing, Trabecular meshwork cells, Trophoblasts, Tumour epithelial cells

U937 cells, **U**rinary, **V**SV infected cells, **Z**ebra-fish, **Z**ika virus

www.diagnostic.serumwerk.com

serumwerk bernburg

Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwerk.com • serumwerk.de