DGM
Density Gradient Media

OptiPrep™

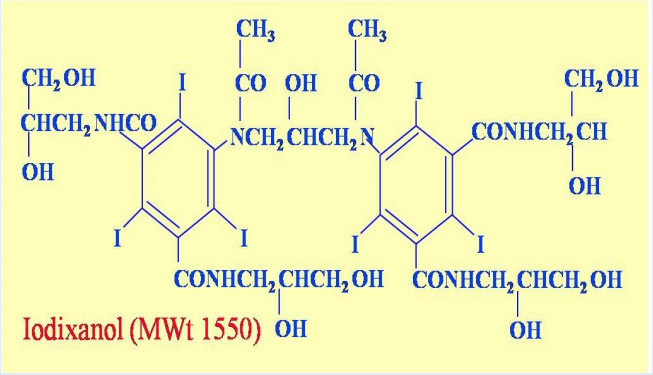
Analysis of membrane trafficking and cell signalling in density gradients

serumwerk bernburg

OptiPrep™, non-ionic, iso-osmotic gradients for high resolution

OptiPrep™

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 and has therefore been subjected to rigorous clinical testing.

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

Iodixanol solutions can be made iso-osmotic at all useful densities.

Iodixanol solutions have low viscosity and osmolarity

OptiPrep™ is manufactured, packed and released by a GMP compliant and ISO 13485 certified manufacturer.

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

In analysis of membrane trafficking and cell signalling, confocal microscopical visualization of fluorescently labelled antibodies is often complimented by electroblotting of SDS-PAGE gels of fractions containing subcellular particles harvested from density gradients. No single density gradient is ever going to be able to resolve the myriad of different membrane compartments present in a post-nuclear supernatant from a tissue culture cell homogenate. Nevertheless, the banding patterns of many membrane compartments are sufficiently distinctive in iodixanol gradients, that they can provide information about the localization of specific components and functions and the shift in location during cellular activity.

Iodixanol gradients preserve vesicle structure and function, which allows them to be used in functional studies after isolation.

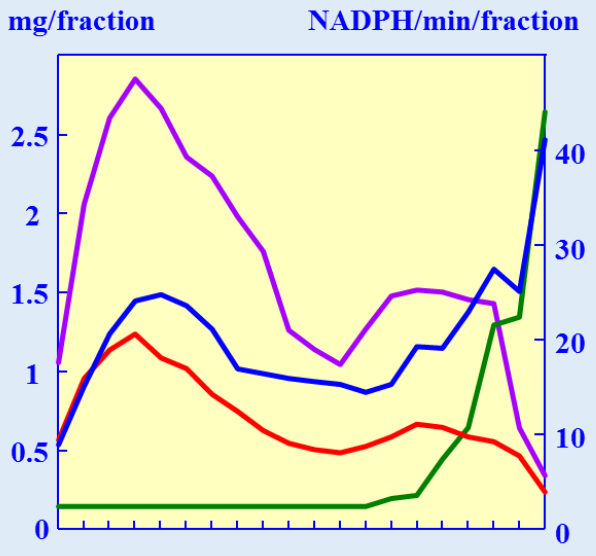
The buoyant density, in iodixanol gradients, of subcellular membranes increases in the following order: PM <early endosomes <Golgi< ERGIC< endoplasmic reticulum (ER)

From some cells the plasma membrane is denser than early endosomes.

Fractionation of rough and smooth ER in self-generated gradients

A simple and effective method for resolving the SER and RER in order to detect specific subfractions of each membrane compartment is to use a self-generated gradient of OptiPrep™. Centrifugation of a microsomal fraction containing 20% iodixanol in a vertical rotor at 353,000g_{av} for 2 h produces two broad bands of material in the gradient as shown by the protein, phospholipid and NADPH cytochrome c reductase profiles (see figure). RNA is only detected in the densest part of the gradient and its concentration increases sharply towards the wall of the tube. The gradient thus achieves a broad distribution of both SER and RER which is ideal for investigating vesicle heterogeneity within each compartment. This system has been used for microsomes from hamster liver, human hepatoma, human plasmacytoma, rabbit enterocytes, rabbit liver and T cells.

The use of these self-generated gradients of iodixanol in the study of the secretory process is extremely valuable because of their high reproducibility and resolution. This self-generated system can also be tailored more specifically to an analysis of the Golgi and SER by layering the microsomal fraction in 20% iodixanol under an equal volume of 15% iodixanol. In this system the Golgi, SER and RER are resolved into three well-defined broad peaks (see next page).



Gradient run in a Beckman VTi65.1 rotor and collected low density end first. NADPH-cytochrome c reductase– Protein– RNA– Phospholipid -

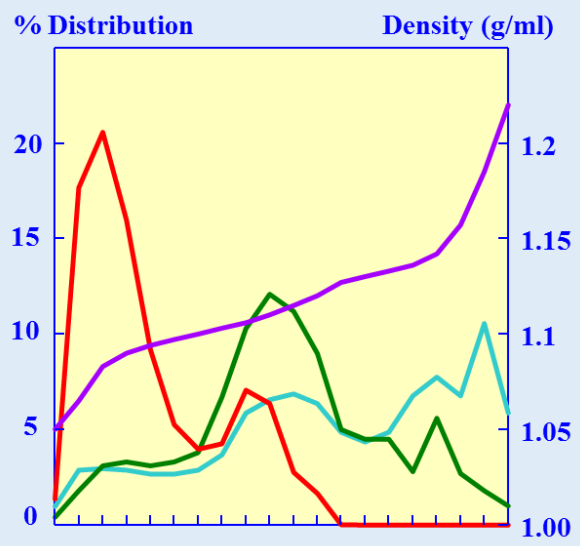
For a detailed protocol and references see Application Sheet S18 at: www.diagnostic.serumwerk.com

Fractionation of Golgi, rough and smooth ER in self-generated gradients

The self-generated gradient system described above provided excellent resolution of the smooth and rough endoplasmic reticulum (SER and RER), but there was no clear separation of Golgi membranes from the lighter SER vesicles. In the method described here, the gradient has been modified to take account of the requirement for a gradient that can achieve simultaneous resolution of Golgi, SER and RER.

Such a gradient needs to be reasonably shallow over its entire density range. Although it is possible to achieve such a gradient profile by increasing the centrifugation time (to approx. 3 h) using a 20% iodixanol starting concentration. Plonné et al preferred an alternative approach of using a biphasic iodixanol starting concentration (equal volumes of 15% and 20% iodixanol). Such a technique has been previously shown to provide shallow gradients over a quite wide density range while maintaining either relatively short centrifugation times or low RCFs. It is particularly important in the use of density gradients to analyze secretion and endocytosis to keep the centrifugation time and any concomitant proteolysis to a minimum.

If the microsomal fraction is only included in the high density layer (20% iodixanol), the Golgi and smooth ER will float out of the load zone into the gradient formed within the 15% iodixanol layer. Any soluble proteins in the crude microsomes on the other hand will tend to sediment through the 20% layer. If it is important to resolve soluble and membrane-bound proteins, such a system might be preferable to one in which the crude microsomes are distributed throughout the starting solution. The protocol described here was designed for rat liver or for isolated rat hepatocytes, but might be extended (with or without modification) to other tissue or cell types.



Density — Galactosyl transferase — NADPH-cytochrome c reductase — Protein —

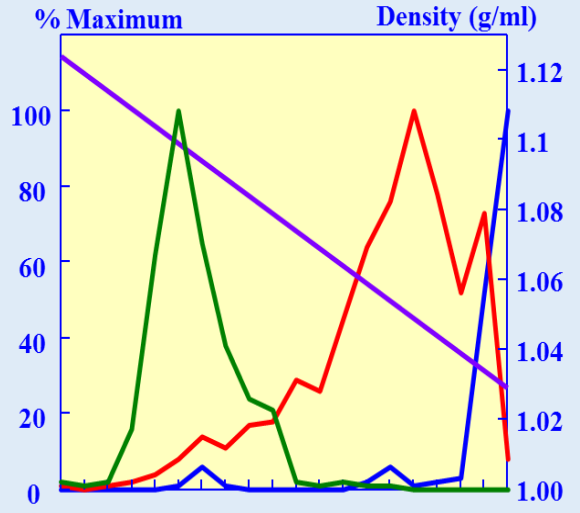
A typical distribution of Golgi (galactosyl transferase) and ER (NADPH cytochrome c reductase) markers is shown in the figure. The top six fractions contain almost exclusively Golgi membranes, while the smooth ER bands in the mid-region of the gradient. RNA (not shown) increases from tube 16 indicating that the rough ER is located towards the bottom of the gradient.

For a detailed protocol and references see Application Sheet S20 at www.diagnostic.serumwerk.com

Analysis of ER, Golgi and plasma membrane in linear iodixanol gradients

The protocol described in this application is based on methods first published by Yang et al and Zhang et al. Yang et al used a linear 0-26% (w/v) iodixanol gradient to study the localization of UBC6 ubiquitin-containing protein in COS-7 cells. By using the gradient to separate endoplasmic reticulum (ER) and Golgi, they established that the transmembrane domain of a carboxyl-terminal anchored protein predisposes it to locate to the ER, while modulation of this domain resulted in re-targeting of the protein to the Golgi. Zhang et al used a 1-20% (w/v) iodixanol gradient also to separate the ER and Golgi from transfected CHO and human embryonic kidney (HEK293) cells. The authors showed that the full-length presenilins (PS1 and PS2) were located in the ER while N- and C-terminal fragments were distributed to the Golgi membranes.

Although the ER and Golgi were the membranes of interest in these studies, the gradient may provide simultaneous purification of the plasma membrane (see figure). The density of the three types of membrane generally increases in the order PM<Golgi<ER; some exceptions to this have however been observed.



Density — Galactosyl transferase — NADPH-cytochrome c reductase — Biotinylated surface protein —

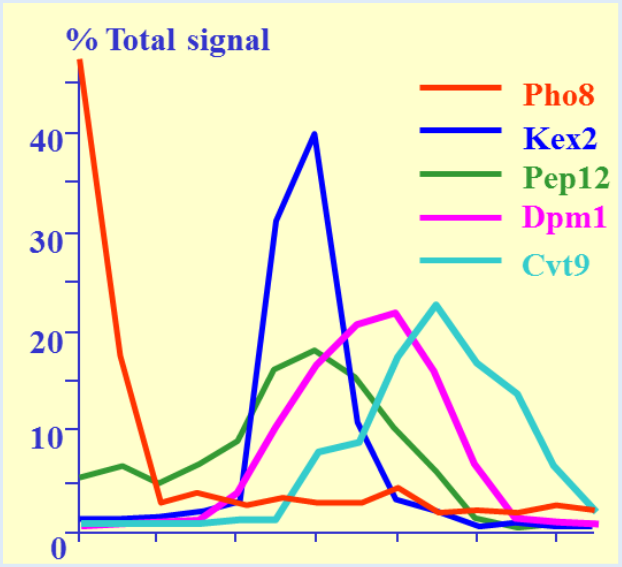
For a detailed protocol and references see Application Sheet S21 at: www.diagnostic.serumwerk.com

Fractionation of membranes from yeast spheroplasts in pre-formed iodixanol gradients

Iodixanol gradients can provide an efficient means of fractionating many of the subcellular compartments from yeast spheroplasts. A continuous gradient covering a range of densities, 0-40%, 0-45%, 10-50% or 10-55% (w/v) iodixanol being the most common, centrifuged at 100-180,000g for 12-16 h is routinely able to separated the vacuole, ER and TGN from denser cytoplasm-to-vacuole transport (Cvt) vesicles.

The figure illustrates an example of this fractionation. Cvt vesicles can alternatively be banded at 30%/37% iodixanol interface of a 19%, 25%, 30%, 37% and 50% (w/v) discontinuous gradient centrifuged at 80,000g for 4 h. Vacuoles and sub-vacuolar vesicles may also be separated in discontinuous gradients.

For detailed protocol and references see Application Sheet S52 and S53 at: www.diagnostic.serumwerk.com



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