DGM

Density Gradient Media

OptiPrep™

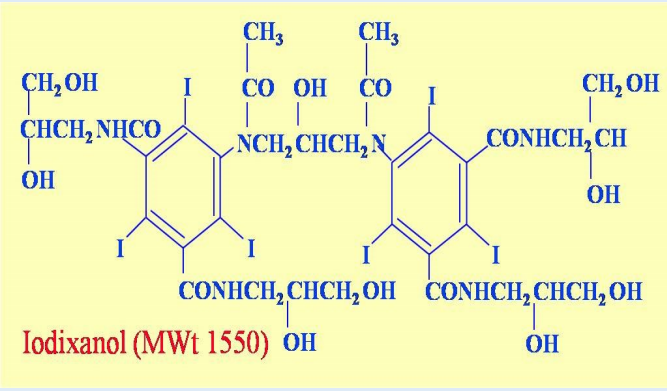
The ideal density gradient medium for purification of subcellular organelles

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 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 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.

Improved resolution of cell organelles

Low viscosity, iso-osmotic gradients provide rapid and efficient separation of the major organelles in preformed gradients.

OptiPrep™ avoids the high viscosity of sucrose and Ficoll®.

OptiPrep™ avoids the inconvenience of removing Percoll® from subcellular organelles.

Iodixanol can be removed efficiently and rapidly from all particle suspensions if required.

In **OptiPrep™**, organelles have more distinctively different densities than in sucrose or Percoll®. Preformed gradients in fixed-angle or swinging-bucket rotors can be used preparatively or analytically.

Ficoll and Percoll are trademarks of GE Healthcare companies.

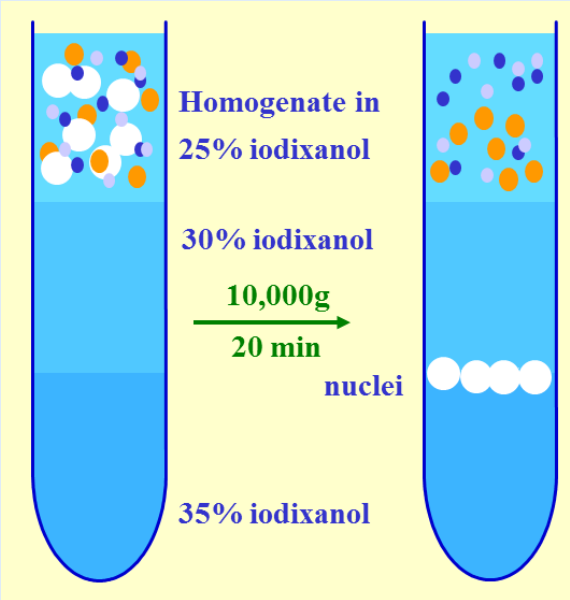
Purification of nuclei from mammalian tissue or a cell homogenate

The preparation of intact nuclei must be carried out in an environment which causes the least possible disruption of the nucleoprotein complexes within them. Sucrose solutions are not dense enough to band nuclei and although both CsCl and diatrizoate can provide solutions of sufficient density, their ionic strength causes disruption of the nucleoprotein structure unless the chromatin is first fixed using a cross-linking agent, such as formaldehyde, to prevent dissociation of the protein from the DNA. Thus the routine method for purifying nuclei involves pelleting them through 60% sucrose at 100,000g for 1-2 h.

Using OptiPrep™ it is now possible to band nuclei isopycnically in an environment which optimizes the retention of the native state of the organelle.

Because of the mush lower viscosity of the OptiPrep™ solution required, the purification process can be carried out in much lower g-force for shorter time; 10,000g for 20 min.

Because of the much lower osmolality of the iodixanol solutions used to isolate nuclei (compared to those of sucrose) the density of the nuclei is also much lower (approx. 1.20 g/ml versus >1.32 g/ml). The figure shows the separation of nuclei from mammalian liver using OptiPrep™.



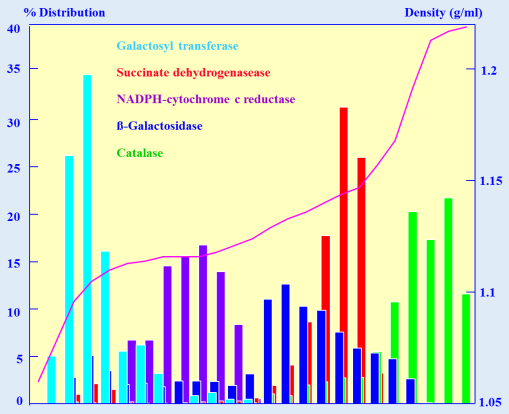
For detailed protocols and references, see Application Sheets S10 and S10a at:
www.diagnostic.serumwerk.com

Fractionation of a light mitochondrial fraction in a pre-formed gradient

OptiPrep™ gradients can be used to resolve the sub-cellular organelles (Golgi membranes, lysosomes, mitochondria, peroxisomes) from a light mitochondrial fraction. Because of their lower osmolality and viscosity, the iodinated density gradient media provide improved resolution and, using preformed gradients, reduced centrifugation times, compared to standard sucrose gradients.

Unlike separations in Percoll®, it is not necessary to remove the medium to be able to perform standard spectrophotometric enzyme assays or SDS-PAGE on gradient fractions.

The figure shows the fractionation of a light mitochondrial fraction, from mammalian liver, in a pre-formed 10 -30% (w/v) iodixanol gradient in a swinging-bucket rotor. The sample was bottom loaded .

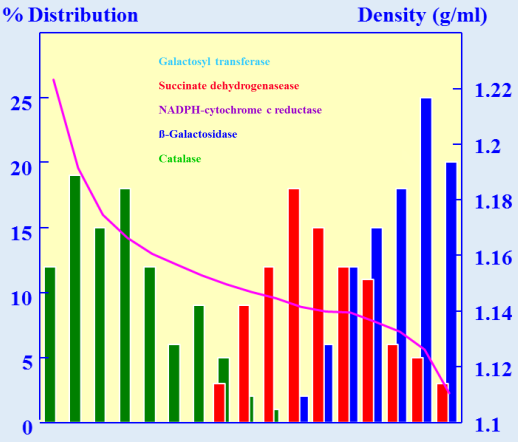


For detailed protocols and references, see Application Sheet S14a at: www.diagnostic.serumwerk.com

Purification of mammalian peroxisomes in a self-generated gradient

An option for the purification of peroxisomes is the use of a self-generated gradient. The optimal manner of producing useful self-generated gradients is to use approx. 365,000gav for approx. 3 h in a near-vertical or vertical rotor. Under these conditions gradients that are more or less linear can be produced. Such a high g-force may be considered unattractive for the recovery of good organelle function and structure. However the gradient shape that is required for the separation of peroxisomes from the less dense organelles of a light mitochondrial fraction is an S-shaped one (see Figure), which is shallow in the middle. To achieve such a density profile, the g-force required can be reduced to 180,000g and there is a less strict requirement for a near vertical or vertical rotor, so many fixed-angle rotors are suitable.

Self-generated gradients have been used for rat liver and human hepatoblastoma cells ; He et al were able to use a Beckman 50.2 Ti fixed-angle rotor – a rotor that would be totally unsuitable for creation of linear gradients.

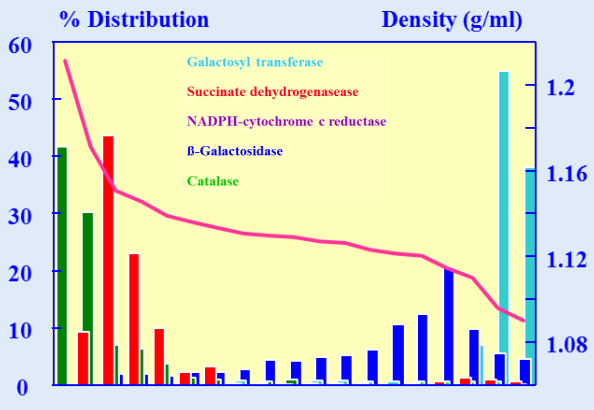


For detailed protocols and references, see Application Sheet S13 at: www.diagnostic.serumwerk.com

Purification of mammalian lysosomes in a self-generated gradient

Mammalian lysosomes have generally been isolated in continuous pre-formed gradients of iodixanol and the basic technique of underlayering a 10-30% or 19-27% (w/v) iodixanol gradient with the light mitochondrial fraction is a good starting point from which many protocols have been developed.

Another popular strategy is to use a self-generated gradient; this was first developed for mouse liver. The crude mitochondrial fraction is adjusted to 20% (w/v) iodixanol and centrifuged at 180,000g for 3 h ; a typical result is shown in the Figure.



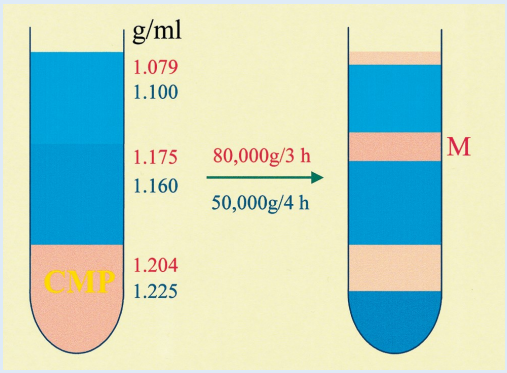
For detailed protocols and references, see Application Sheet S16 at: www.diagnostic.serumwerk.com

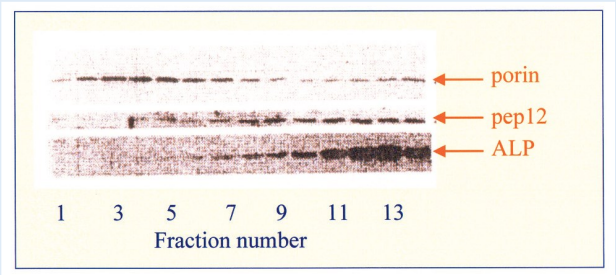
Purification of mitochondria using a discontinuous gradient

Nycodenz® has been widely used as a density gradient medium for the isolation of pure mitochondria from yeast since methods were first published by Glick and Pon. Commonly a crude mitochondrial pellet is layered on top of 14.5% and 18% (w/v) Nycodenz® in buffered 0.6 M sorbitol; after 30 min at approx. 120,000g, the mitochondria band at the 14.5%/18% Nycodenz® interface.

With OptiPrep™ a flotation strategy through a discontinuous gradient has been used (see figure). Yeast mitochondria have also been purified (from a light mitochondrial pellet) by sedimentation through a preformed continuous 0-25% (w/v) iodixanol gradient at 10-12,000g for 2 h (17-22). (In this protocol, which has been used to study iron metabolism in yeast, the mitochondria band in the bottom third of the gradient, separated from the lighter prevacuole and vacuole (see figure below).

For detailed protocols and references, see Application Sheets S14 and S14a at:
www.diagnostic.serumwerk.com

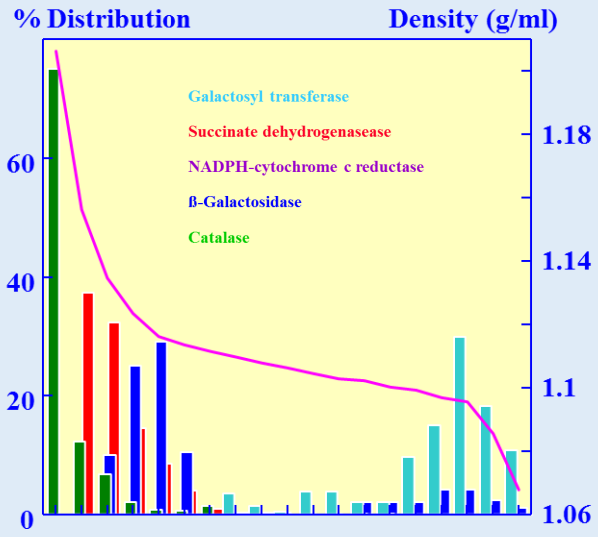




Fractionation of a light mitochondrial fraction in a self-generated gradient

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 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 detailed protocols and references, see Application Sheet S16 at: www.diagnostic.serumwerk.com

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