DGM
Density Gradient Media

OptiPrep™

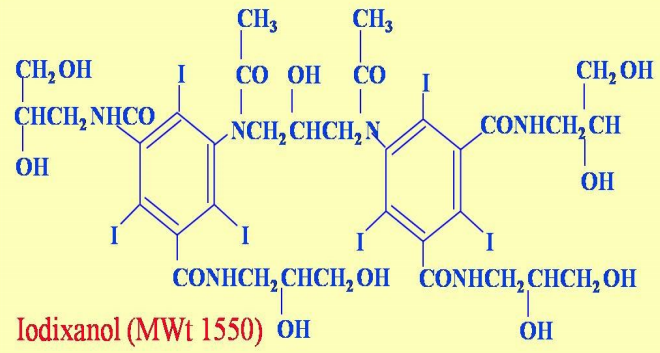
The ideal density gradient medium for isolation of macromolecules and lipoproteins

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.

Plasma lipoproteins are classified and separated by centrifugation according to their density: high-density (HDL), low-density (LDL) and very low density (VLDL).

The flotation of the different classes of human plasma lipoproteins in a centrifugal field by the sequential elevation of the density of the plasma by dissolution of KBr or NaCl/KBr mixtures has been widely used over many years. The operation is routinely carried out in a fixed-angle rotor and takes at least three days. In spite of its cumbersome nature, the technique is still widely used today.

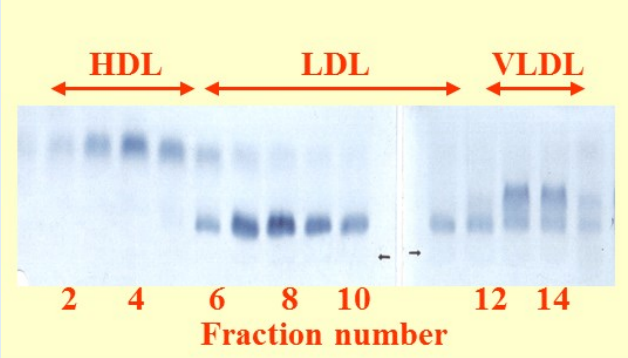
Lipoprotein fractionation in self-generated OptiPrep™ gradients takes less than 3 hours at about 350,000g.

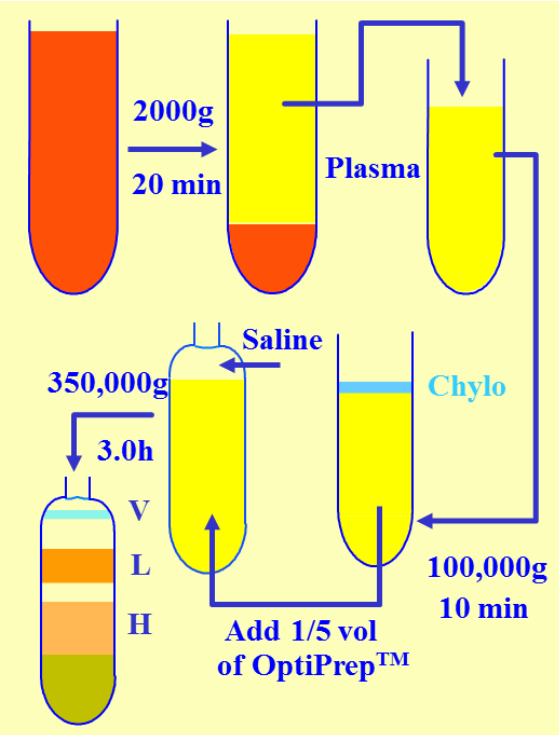
Because iodixanol is non-toxic and, non-ionic, add-on techniques can be carried out on gradient fractions directly without removing the medium.

Fractionation of human plasma lipoproteins

A typical strategy for fractionation of the major classes of human plasma lipoproteins is given in the figure. For a routine analysis the ideal starting concentration of iodixanol is 12% (w/v) and after centrifugation for 2.5 h a shallow gradient is formed in the top two-thirds of the tube and it is in this region that the plasma lipoproteins are normally resolved. Under these conditions the densest HDL will merge with the soluble proteins which band at the bottom of the gradient.

The three major lipoproteins (VLDL, LDL and HDL) are resolved with very little overlap. The agarose gel electrophoresis profile shows a typical agarose gel electrophoresis analysis of the gradient fractions.





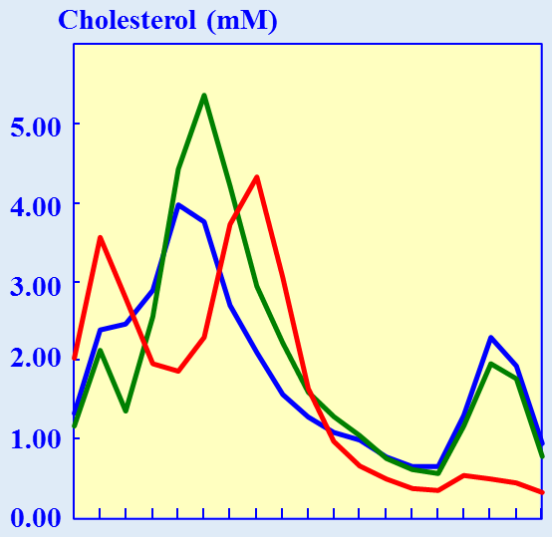
For a detailed protocol and references see Application Sheet M07 at: www.diagnostic.serumwerk.com

Fractionation of lipoprotein subclasses

Changes in the density profile of the gradient, which can be achieved by modulations of the iodixanol starting concentration and/or centrifugation conditions, can spread out either the LDL or the HDL to make sub-fractionation of these particles more easy. For example, by using a two-layer starting configuration of equal volumes of 9% and 12% (w/v) iodixanol, the LDL can be spread out so differences in banding density between individuals can be detected (see figure).

A very effective variation on the basic OptiPrep™ strategy, and one, which simplifies the analytical procedures considerably, involves pre-staining of the plasma with Coomassie Brilliant Blue. After centrifugation in a Beckman NVT65 near-vertical rotor (using the 6%/12% iodixanol gradient format), the banding position of the LDL in the self-generated gradient was determined by scanning an image of the tube (produced by a digital camera) using polyacrylamide gel scanning software. The methodology is able to identify clearly LDL subclasses.

Because of the resolving power of these self-generated gradients, and the considerable time saving over the KBr gradient methods, these strategies have been used to investigate the lipoprotein profiles of clinical specimens. The cholesterol profiles of three specimens are shown in the graph (gradient density increases right to left): red (healthy donor, high HDL, very low VLDL),



green/blue donors (relatively low HDL, large amounts of especially dense LDL, and significant VLDL

For a detailed protocol and references see Application Sheet M08 at: www.diagnostic.serumwerk.com

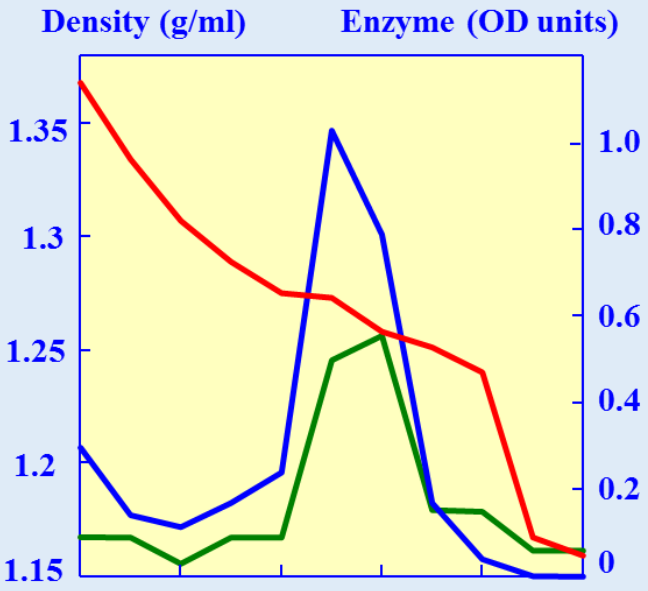
Purification of proteins in self-generated gradients

In OptiPrep™ and Nycodenz®, proteins have a density of approx. 1.26 g/ml; glycoproteins approx. 1.28 g/ml. Self-generated gradients of Nycodenz® have been used to band these macromolecules and also to separate them from nucleoprotein complexes. One general problem regarding the use of iodinated density gradients for this purpose is that because Nycodenz® and iodixanol absorb significantly in the UV, it is not possible to monitor the gradients spectrophotometrically. They also may interfere in protein assays using the Folin reagent. Assays based on Coomassie blue binding are not affected at all and Winterbourne has developed a simple and reliable method.

Most other functional assays however and any form of electrophoresis can be carried out directly on gradient fractions without prior removal of the medium. If the medium must be removed then ultrafiltration using microcentrifuge cone filters is the recommended method.

Because iodixanol forms self-generated gradients more quickly than does Nycodenz®, it is probably more suited to protein banding procedures.

A protein solution is placed on top of 25-30% iodixanol and centrifuged in a vertical or near-vertical rotor at 350,000g for 3-4 h. During this time the gradient is generated and the protein moves to its banding density.



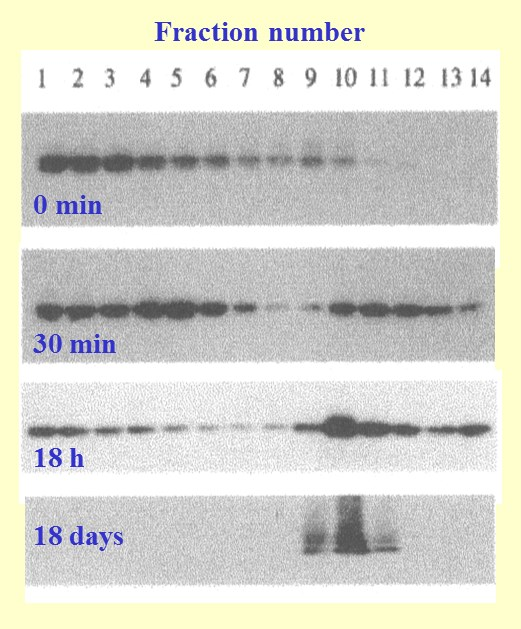
The figure shows the banding of catalase (blue) and ß-galactosidase (green). Density profile in red.

For a detailed protocol and references see Application Sheets M11 and M12 at: www.diagnostic.serumwerk.com

Protein oligomerization and protein complexes

Protein oligomerization and the formation of protein complexes in general is best studied in a non-ionic medium which does not affect the hydration of the molecules, since this is known to influence the propensity of protein molecules to aggregate. Moreover sucrose gradients caused proteolysis of a kinesin-related motor protein, while in iodixanol gradients there was no proteolysis whatsoever. OptiPrep™ therefore provides the ideal medium to study these processes. Discontinuous gradients of iodixanol (5-50%) for example have been used to study the oligomerization of the β-amyloid (Aβ) peptide by Ward et al. Near-vertical or vertical rotors are used to provide high resolution (and in such rotors the gradient will diffuse to become linear quite rapidly) but the method may be adapted to a swinging-bucket rotor. The figure shows the change in the banding profile of the peptide with time of incubation. In principle the protocol can be extrapolated to study any protein complex formation. The method has been adapted to slightly larger tubes (6ml) of the Sorvall TV865 by Khlistunova et al using more or less the same centrifugation conditions. Its has also been adapted to small volume swinging-bucket rotors: Rzepeck et al used a similar gradient, which was scaled down to use in a Beckman TLS55 (2.2 ml tubes) with centrifugation at 259,000g for 4 h and Lockhart et al used a Beckman MLS50 (5 ml tubes) for studying the formation of β-amyloid fibrils at 268,000g for 3 h.

Aggregates of α-synuclein can be fractionated on a discontinuous iodixanol gradient of 2.5%,25% and 35% (w/v) at 50,000g for 30 min.

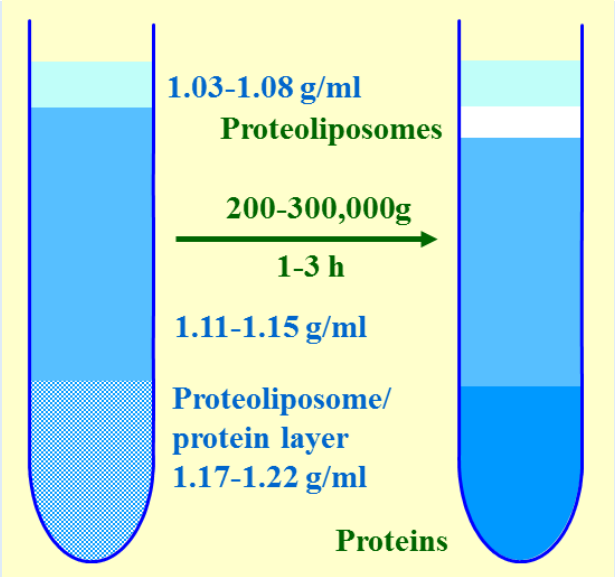


For a detailed protocol and references see Application Sheet M09 at: www.diagnostic.serumwerk.com

Purification of proteoliposomes; separation from component molecules

After protein has been incorporated into some form of liposome, it is usually necessary to resolve the newly formed proteoliposomes from any unincorporated protein. Most of the published papers reporting the use of a discontinuous gradient of iodixanol to achieve this separation adjust the sample to a high density and allow the proteoliposomes to float up through a low-density barrier, layered on top of the sample. The big advantage of this strategy is that the unincorporated protein remains in the sample zone and actually will tend to sediment in the opposite direction (see figure). There are instances where the sample has been top-loaded on to a density barrier, but in this case both the proteoliposomes and the free proteins will sediment in the same direction and the separation is based on the more rapid movement of the larger proteoliposomes.

For a detailed protocol and references see Application Sheet M10 at: www.diagnostic.serumwerk.com



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