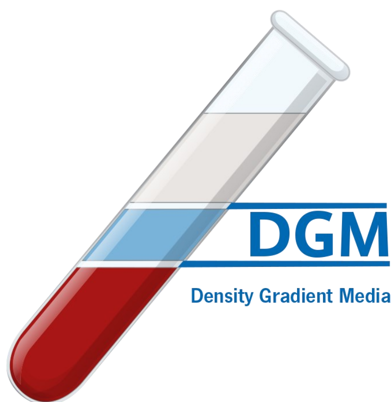
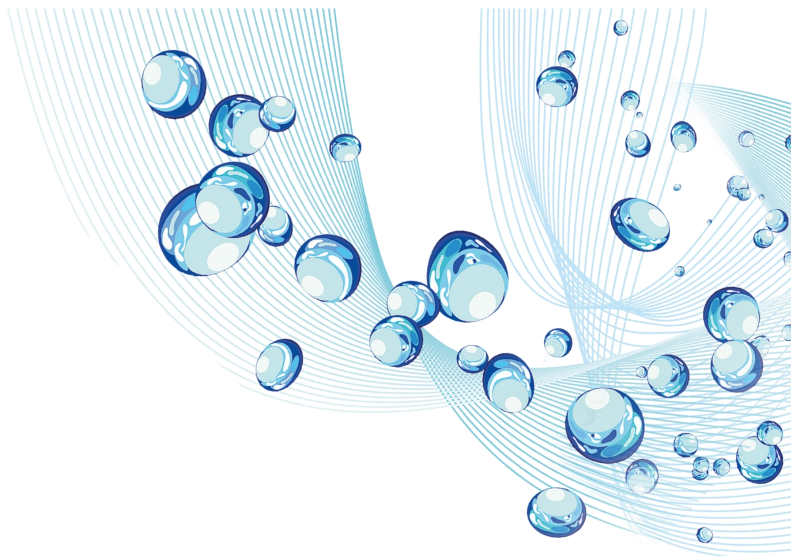




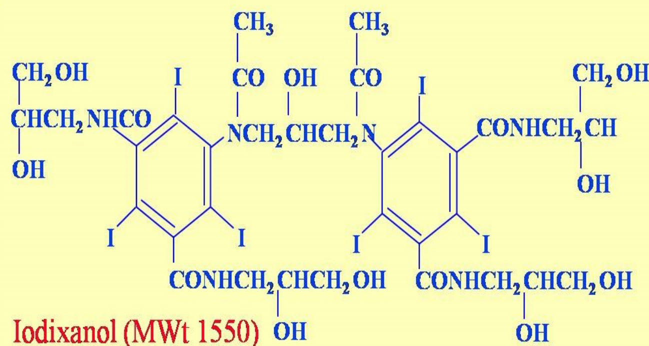
OptiPrep™
The ideal density gradient medium

serumwerk
bernburg

OptiPrep™, non-ionic, iso-osmotic gradients

OptiPrep™

OptiPrep™ is the flagship of Serumwerk's range of density gradient media. Brought to the market by Nycomed in 1994, it has gained a great amount of popularity in cells, cell organelle, membrane vesicles, virus and plasma lipoprotein isolation using centrifugation techniques. OptiPrep™ is a sterile solution of 60% (w/v) iodixanol in water. It has a density of 1.32 g/ml. Iodixanol is non-ionic, non-toxic to cells and metabolically inert. Iodixanol solutions are spectrophotometrically transparent above 340 nm.



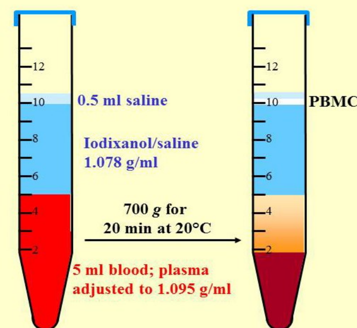
The vast majority of biological particles, mammalian and non-mammalian cells, subcellular organelles, membrane vesicles, viruses, plasma lipoproteins and many macromolecules and macromolecular complexes can be isolated under iso-osmotic conditions.

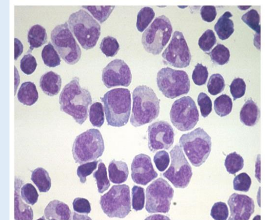
- OptiPrep™ avoids the high viscosity of sucrose and polysaccharides: centrifugation time are often shorter
- OptiPrep™ avoids the high osmolality of sucrose and inorganic salts; particles are recovered as close to their native state as possible.
- OptiPrep™ often avoids the requirement to remove ionic media and Percoll® prior to many common analytical techniques- notably electrophoresis. Only for electron microscopy is removal of iodixanol a requirement.

A new approach to cell purification

In the traditional method of layering the sample on top of a discontinuous gradient or density barrier all the particles (including any partially damaged cells) are sedimenting and aggregation can occur at the first interface.

The problems can be demonstrated by the requirement to isolate platelet-free human blood mononuclear cells. When diluted blood is centrifuged over a 1.077 g/ml barrier, cells and platelets band at the interface. Platelet removal requires one or more additional steps. If the plasma or whole blood is adjusted to 1.095 g/ml by mixing with OptiPrep™ and overlaid by an iodixanol solution of 1.077 g/ml and a small layer of saline, the PBMCs float to the top interface and all other components remain in the sample layer.





The flotation technology has been applied to a variety of low density cells, including:

- Huma blood monocytes
- Dendritic cells (from a variety of tissues)
- Pancreatic islets of Langerhans
- Hepatic and pancreatic stellate cells
- Removal of non-viable cells

Percoll® is a trademark of GE Healthcare

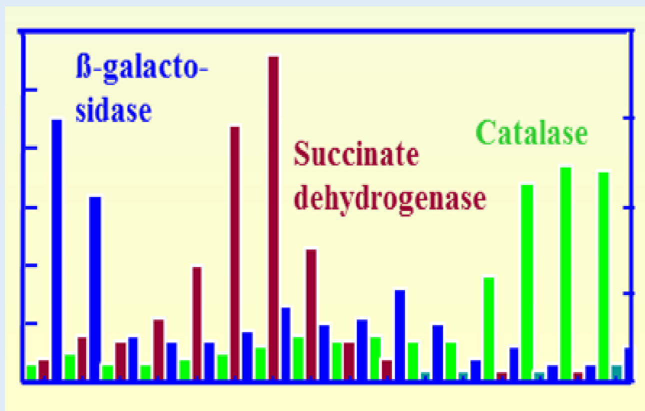
serumwerk bernburg
Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwerk.com • serumwerk.de

for high resolution

Analysis of subcellular membranes

Sucrose gradients were used in the study of nuclei, mitochondria, lysosomes, peroxisomes, endoplasmic reticulum and Golgi membranes. Although relatively "membrane-friendly" the high osmolality and viscosity of denser solutions limit its resolving power. Polysaccharides solved the osmolality problem only at low concentrations and made the viscosity problem far worse. Percoll® (a colloidal silica medium) solved both problems; it is principally used for cells and the larger subcellular organelles.

Iodixanol gradients also solved both problems. Aside from the purification of all the larger subcellular organelles, analysis of the smaller membrane vesicles derived from the endoplasmic reticulum, endocytic compartment, Golgi membranes, trans-Golgi network, plasma membrane etc are major applications. Recently the purification of exosomes and extracellular vesicles (from both mammalian and non-mammalian cells) have become very important. The figure below gives and example of the resolution of lysosomes, mitochondria and peroxisomes (left to right) by flotation through a 20-27% (w/v) iodixanol gradient centrifuged at 70 000xg for 1.5 h.

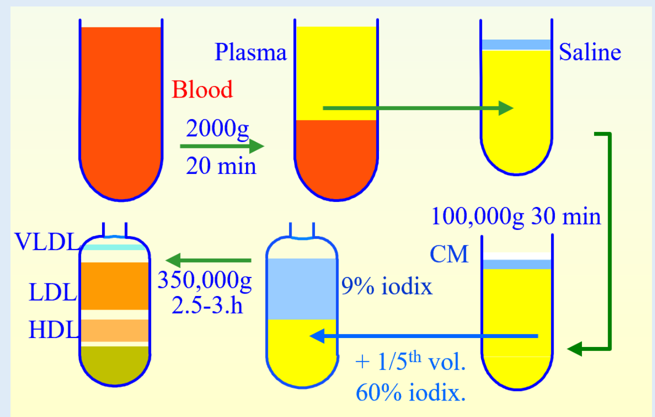


Fractionation of plasma lipoproteins

Although ultracentrifugation is regarded as the "gold standard" method for the fractionation of plasma lipo-proteins, the traditional method of sequential flotation by incrementally increasing the density of the plasma with KBr to provide sequentially VLDL, LDL and HDL is technically simple but excessively tedious (requiring approx. 3 days).

The alternative: discontinuous or continuous KBr/NaCl gradients are technically difficult to produce and handle. Moreover use of some add-on analytical techniques often necessitates removal of the salt by dialysis, adding up to 12 h to the procedure and use of high salt concentrations may cause the loss of certain surface apoproteins from lipoproteins. The introduction of self-generated gradients of iodixanol in 1996 solved many of these problems.

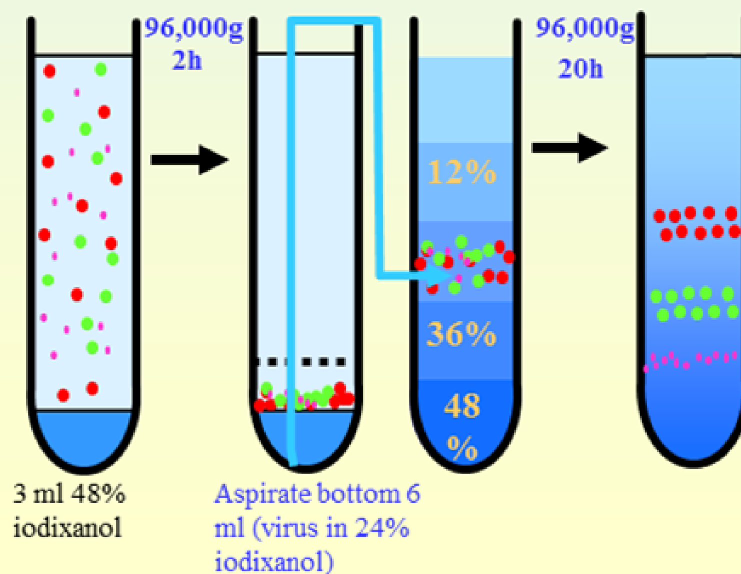
A simple one step centrifugation for 3 h that resolves VLDL, LDL and HDL, avoiding the use of technically-difficult salt gradients. Chylomicron-free plasma is simply mixed with OptiPrep™, transferred to tube for a near-vertical rotor; overlayered with 9% iodixanol and a little saline to fill the tube and centrifuged for 3 h.



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

A new approach to virus purification

Density gradient ultracentrifugation has been widely used as a means of purifying viruses and the principal choice of medium has been either sucrose or CsCl, neither of which are ideal. Both sucrose and CsCl gradients are significantly hyperosmotic, leading to a loss of water from the virus and consequent increase in density. Except for the densest viruses, gradient solutions prepared from iodixanol are usually close to being iso-osmotic; as a result the density of viruses in iodixanol gradients is much lower than in CsCl gradients and usually lower than in sucrose gradients. CsCl solutions are highly toxic and lead to a major reduction of infectivity. Sucrose gradients are less toxic but their viscosity may cause enveloped viruses to lose surface proteins due to hydrodynamic shear. Over ninety different viruses and phages have been purified in iodixanol gradients. In J. Biol. Chem., **286**, 3018-3032 (2011) Merz, A. et al (2011) described a method that avoided pelleting of Hep C virus prior to gradient centrifugation. It is illustrated in a slightly modified version below.



www.diagnostic.serumwerk.com

Distributed in North America by:

COSMO BIO USA
2792 Loker Ave W, Suite 101
Carlsbad, CA 92010

TEL: 760-431-4600
FAX: 760-431-4604
email : info@cosmobiousa.com
web : www.cosmobiousa.com