DGM
Density Gradient Media

OptiPrep™

The ideal density gradient medium for isolation of cells

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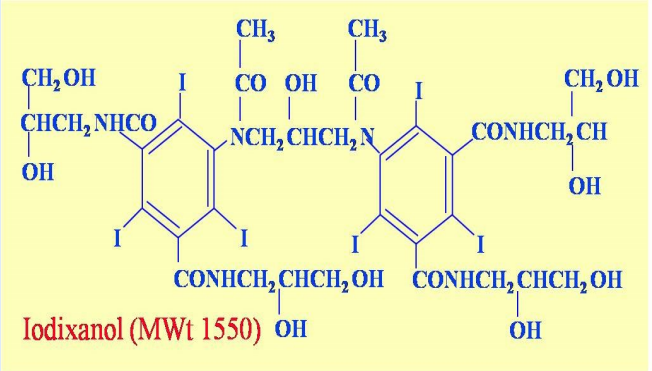
OptiPrep™, non-ionic, iso-osmotic gradients for high resolution

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.

Unlike manufacturers of other density gradient media, Serumwerk Bernburg do not have to offer special, more expensive, low endotoxin-containing products; all their density gradient media have always been, and continue to be, produced to the same high rigorous standards.

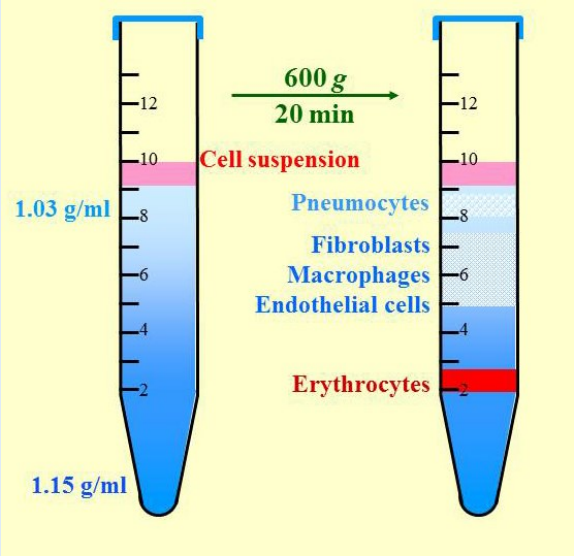
For every batch produced, a Certificate of Analysis is available.

The high density of **OptiPrep™** facilitates the fractionation of cells by flotation from a dense load zone through either a continuous or discontinuous gradient or through a simple density barrier.

Isolation of alveolar cells from lung tissue

Alveolar (pneumocyte) type II cells are widely studied because they synthesize and secrete the phospholipid-rich lung surfactant, which lines the air-alveolar interface and prevents alveolar collapse by lowering surface tension at low lung volumes. Isolation of these cells from both adult and foetal lung is an important prerequisite for their culture and study. Viscardi et al, who successfully developed a Nycodenz® gradient technique, emphasised the importance of the non-toxic, non-invasive properties of this gradient medium and pointed out that although Percoll® gradients had been used previously for purifying these cells, the potentially toxic nature of a polyvinyl-pyrollidone-coated silica colloid was of considerable concern in studies of their function.

The technique for isolation of pneumocytes is a multi-step one; after tracheostomy of the anaesthetized animal, the alveolar vasculature is perfused to remove blood cells and the lungs lavaged to remove alveolar macrophages. Lung tissue cells are then dispersed by enzymic disaggregation and mincing. Following filtration, the mixed cell suspension is loaded on to a continuous OptiPrep™ gradient (1.03-1.15 g/ml) and centrifuged at 600g for 20 min at 15°C. The pneumocytes band at approx. 1.056 g/ml just below the sample/gradient interface, while the broad band at approx. 1.086 g/ml contains fibroblasts, endothelial cells and macrophages and any erythrocytes band close to the bottom of the gradient.



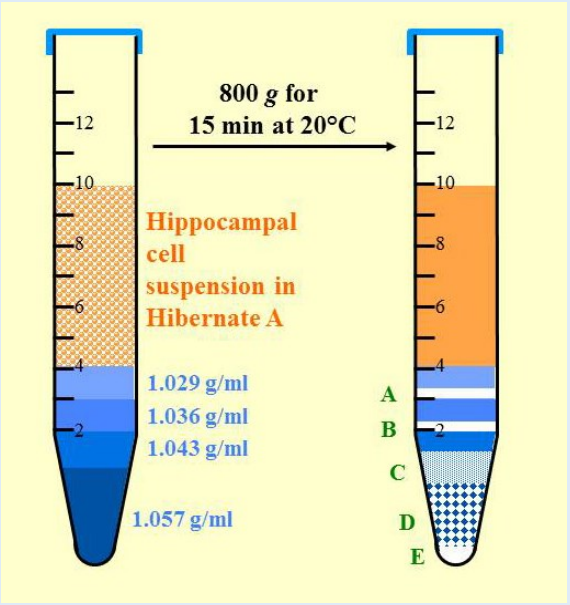
For a detailed protocol and references see Application Sheet C29 and C30 at: www.diagnostic.serumwerk.com

Isolation of rat and human hippocampal neuron fractions

As with any tissue, the first step in fractionating the cells from neural tissue is to release the cells by enzymic disaggregation and (in the case of the hippocampus) trituration. Both OptiPrep™ and Nycodenz® gradients have been used, primarily to isolate a motoneuron-rich fraction from both spinal cord and from hippocampus, using either a simple density barrier or a discontinuous gradient. The discontinuous gradients may additionally provide a partial purification of some other neural cells.

Generally the gradients for rat hippocampal tissue are three or four-step discontinuous ones and not simple density barriers. In the method devised by Brewer et al the cell suspension is layered over a discontinuous gradient consisting of 1.029, 1.036, 1.043 and 1.057 g/ml Nycodenz® in Hibernate A/B27 medium.

Brewer et al was one of the first groups to shift from Nycodenz® to OptiPrep™ for hippocampal neuron purification using a four-layer gradient covering a similar density range. Marks et al and Kretz et al reported the use of an almost identical gradient. Neurons from cortical tissue, hypothalamus and brain stem have been isolated in similar gradients. In all cases the motor neurons band towards the bottom of the gradient (fraction D).

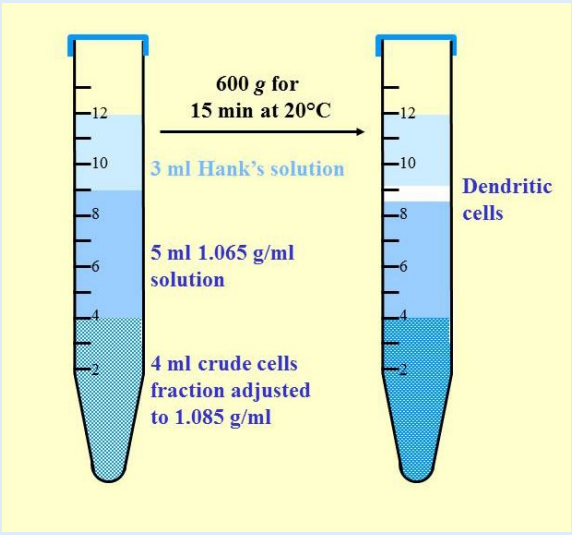


For a detailed protocol and references see Application Sheet C36 at: www.diagnostic.serumwerk.com

Isolation of dendritic cells from blood and tissue

More recently OptiPrep™ has been used for the isolation of DCs from a variety of tissue types and blood. As with many low density cells, flotation through a "low density barrier" has been found to give improved results. Once the tissue has been disaggregated enzymically, the cells are suspended in a relatively dense medium and layered beneath the low density medium as described originally by Ruedl et al. The low density dendritic cells float to the top of the low density solution to band at the interface between this solution and a small volume of culture medium (or balanced salt solution), which is always layered on top (see figure). This methodology is widely used and has been applied to the following sources: blood, bone marrow, inflammatory cells, Langerhans cells, liver, lung, lymph, lymph nodes, Peyer's patch tissue, spleen and thymus. In early publications the density of the barrier was 1.068 g/ml but more recently this has been reduced to 1.065 g/ml.

There are also a few examples of sedimentation on to a density barrier.



For a detailed protocol and references see Application Sheet C21, C22 and C41 at: www.diagnostic.serumwerk.com

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Isolation of stellate and other cells from liver and pancreas

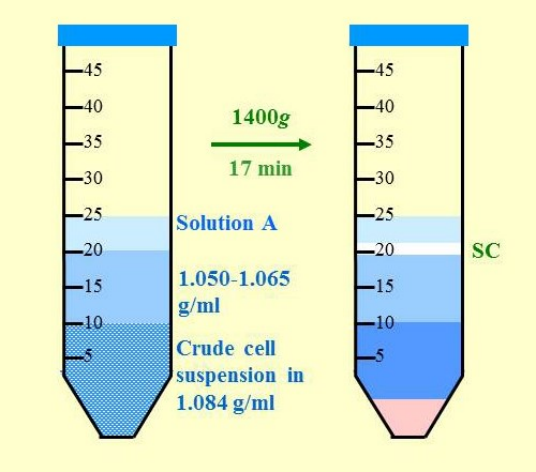
Stellate cells are present in both the liver and pancreas and represent a major and clinically important cell population in both tissues. In liver the cells are important in the development of hepatic fibrosis and in the pancreas they mediate the fibrosis associated with chronic pancreatitis.

In the case of liver, the purification of other cell types is also of major consideration. Stellate cells are just one type of hepatic non-parenchymal cells (the others include sinusoidal cells, Kupffer cells, endothelial cells etc) and the separation is carried out in at least two stages. The first stage is the removal of the parenchymal cells, followed by the fractionation of the non-parenchymal cells.

Stellate cells are the least dense of the different cell types in both tissues and they can be purified either by sedimentation on to, or flotation through, a suitable density barrier. Both strategies have been used effectively but flotation is often regarded as superior to sedimentation as stellate cells tend to adhere to other cells when sedimenting across a sample/gradient interface. The method has been performed with both Nycodenz® and OptiPrep™.

Barriers prepared from OptiPrep™ have densities ranging from 1.053 to 1.067 g/ml and it is generally accepted that the lower the density, the purer the stellate cell preparation. As with Nycodenz® gradients the crude cell suspension may be layered on top of the density barrier or adjusted to a higher density and layered beneath the barrier.

Iodixanol barriers have been used for the preparation of stellate cells from the following sources: human liver, human pancreas, mouse liver, rat liver and rat pancreas.



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

Non-parenchymal cells C25 and C26
Stellate cells C27, Kupffer cells C28

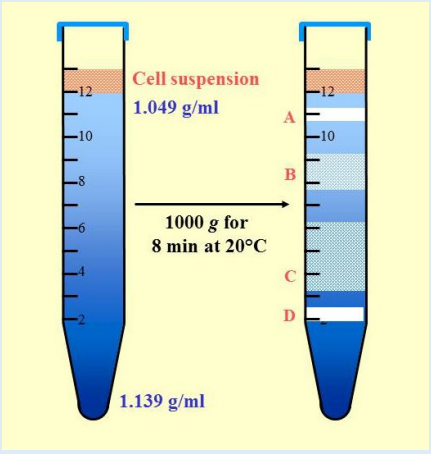
Isolation of gastric mucosal cells using continuous or discontinuous gradients

Probably the most intensively studied cell present in the gastric mucosa is the acid-secreting parietal cell as this provides a very useful model for studying both the regulation of ion-transport and intracellular signalling pathways. ECL cells are the main endocrine/paracrine cell type and they play an important role in controlling acid-secretion. The other major cell type is the chief cell, which secretes pepsin.

Parietal cells

Since the late eighties the most widely used strategy is to purify parietal cells from pronase/ collagenase-disaggregated gastric mucosa (from both rats and rabbits) on either continuous or discontinuous Nycodenz® gradients, followed by centrifugal elutriation. More recently Nycodenz® has been replaced by OptiPrep™ as the latter gave improved purities (80-90%) and for some studies this may be a sufficient degree of purification without the need to carry out a subsequent elutriation step.

In a continuous iodixanol gradient covering the range 1.049-1.139 g/ml; the parietal cells band at approx. 1.052 g/ml (A), while the chief cells have a much higher density (D) (see figure).



ECL cells

Rat ECL cells are routinely purified by centrifugal elutriation, but are contaminated by largely denser particles which can be removed in a discontinuous Nycodenz® gradient of 1.046 and 1.058 g/ml. The corresponding iodixanol gradients are slightly denser, ρ = 1.061 and 1.084 g/ml, the cells banding above the lower density layer. A simple iodixanol density barrier ρ = 1.061 g/ml can be used as an alternative.

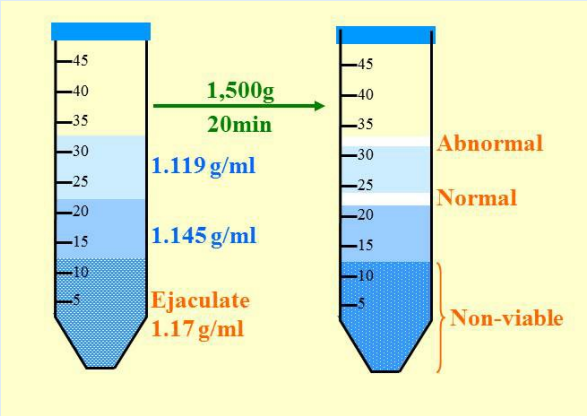
For a detailed protocol see Application Sheets C47 and C48 at: www.diagnostic.serumwerk.com

Isolation of spermatozoa from experimental animals

Ejaculates contain variable proportions of viable spermatozoa of normal morphology, sometimes a very low percentage of the total cell population. OptiPrep™ gradients can be used for the recovery of highly viable fractions from bovine ejaculates. Previously, a method for the separation of the viable fraction from bovine ejaculates using Nycodenz® gradients was developed. In field tests, using a split ejaculate, one part separated on a Nycodenz® gradient and the other processed normally; the Nycodenz® purified cells fertilized as efficiently as those treated by routine methods.

OptiPrep™ has certain significant advantages over its predecessor, Nycodenz®, for the separation of sperm cells in particular, because of their relatively high density Adjustment of the raw ejaculate to a sufficiently high density (1.17 g/ml) and maintenance of isoosmotic conditions can only be achieved with OptiPrep™. OptiPrep™ thus offers convenience in sample handling.

Bovine semen in a sorbitol-containing diluent (density 1.018 g/ml) is mixed with an equal volume of OptiPrep™ so that the density of the suspension is approx. 1.17 g/ml. This is then overlayered with two density barriers of density 1.145 and 1.119 g/ml (see figure). After centrifugation at 1500g for 20 min at 20°C the deformed cells and cytoplasmic droplets will have floated to the meniscus, non-viable cells will remain in the load zone and the viable motile sperm cells of normal morphology will band at the 1.119/1.154 g/ml interface. The medium used for washing and storing sperm cells varies from laboratory to laboratory and if, for example, it is based on a simple culture medium containing 10% serum rather than a sorbitol-containing medium, then it will have a density of approx. 1.009 g/ml. The amount of OptiPrep™ required to raise the density of the semen to 1.17 g/ml and for ratios of OptiPrep™ and medium to make the low density layers will vary.



The quality of the semen has been assessed by using membrane integrity as an indicator of general cell function and viability (The Osmotic Resistance test). The motile band from the ρ = 1.12/1.14 g/ml interface shows over 95% viability by this test, while the pelleted material and particulate material remaining in the loading layer are found to be 99% non-viable cells by this method.

OptiPrep™ has also been used as a dense cushion to concentrate spermatozoa and remove contaminating debris with equine, boar, gazelle and mouse semen.

A huge advantage of using OptiPrep™ for human semen fractionation is that iodixanol has been clinically tested and screened as an X-ray imaging agent and it is the only gradient medium that is routinely tested for endotoxin, levels of which are < 1 EU/ml (well within the limits for human applications).

For a detailed protocol see Application Sheets C17 and C18 at: www.diagnostic.serumwerk.com

Removal of non-viable cells from tissue and cultured cells

Cell suspensions prepared from a lung or peritoneal lavage, or from enzymically dissociated tissue, will comprise live and dead cells together with cell debris. The cells are often contained in a comparatively large volume at low concentration and need to be concentrated prior to further processing in smaller volumes. Pelleting and resuspending the cells causes further damage and attempts to separate the various cell-types in the suspension by density gradient centrifugation are usually unsuccessful because the DNA, which is released by broken cells, causes aggregation of cells throughout the gradient.

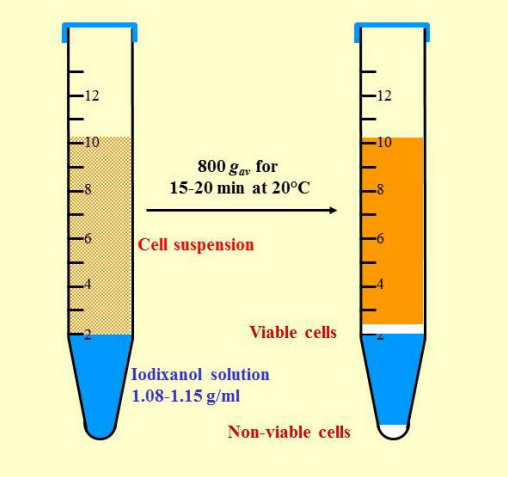
There is also a need to remove large numbers of nonviable cells from cultured cell lines after some operational failure has caused a major loss of cell viability.

Simple density barrier techniques can provide a means of eliminating non-viable cells and removing contamination by released intracellular contents, thereby facilitating the success of subsequent separation techniques or cell culture.

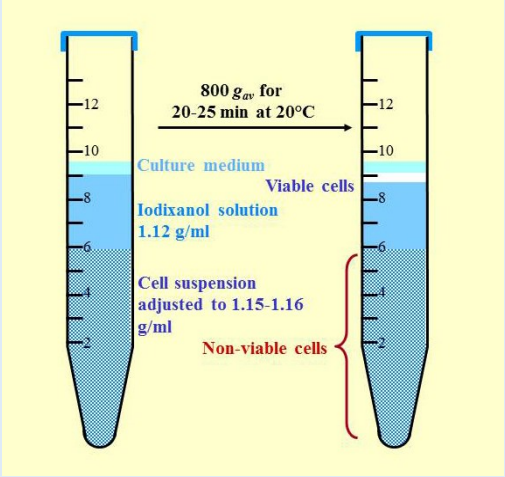
Non-viable cells tend to lose their osmotic integrity, thus their aqueous cytosolic compartment becomes accessible to solute molecules in the surrounding medium. When this occurs the density of the cell increases, usually to a value above 1.15 g/ml; such non-viable cells can therefore be easily separated from the viable ones.

A simple approach to separate viable and non-viable cells is to centrifuge the cell suspension over a barrier solution of density approx. 1.15 g/ml (see figure). The barrier can be prepared from OptiPrep™ or Nycodenz®.

OptiPrep™ will provide a slightly higher density and there is some evidence that the smaller molecular weight Nycodenz® may be more accessible to the cytosolic compartment of nonviable cells. The viable cells will form a band at the interface, while the non-viable cells will form a pellet.



An alternative approach, which may be more efficient with OptiPrep™ is to adjust the density of the cell suspension to 1.15-1.16 g/ml and to overlay this with a solution of 1.12 g/ml. After centrifugation the viable cells band at the top of the upper layers while non-viable cells and contaminants remain in the load zone (see figure next page). This has been used very successfully for retrieving a "damaged" cultured cell line.



Both methods are extremely gentle as the viable cells are concentrated without pelleting.

Iodixanol barriers have been used for the removal of non viable cells from: amphibian cells, cultured cells, hepatocytes, muscle cells and progenitor cells.

For detailed protocols and references see Application Sheet C14 at: www.diagnostic.serumwerk.com

Isolation of Islets of Langerhans from pancreatic tissue

The development of a routine method for the isolation of Islets of Langerhans from pancreas will have important clinical and research applications. Islet transplants are used in the treatment of diabetes and a simple procedure for the preparation of functional islets in bulk would be a significant improvement on present methods.

Islets are first prepared by disaggregation of the pancreas by enzymic digestion in order to release the islets from the acinar cells. The optimal methods of disaggregation of tissue and choice of medium will vary with the source of tissue and from laboratory to laboratory.

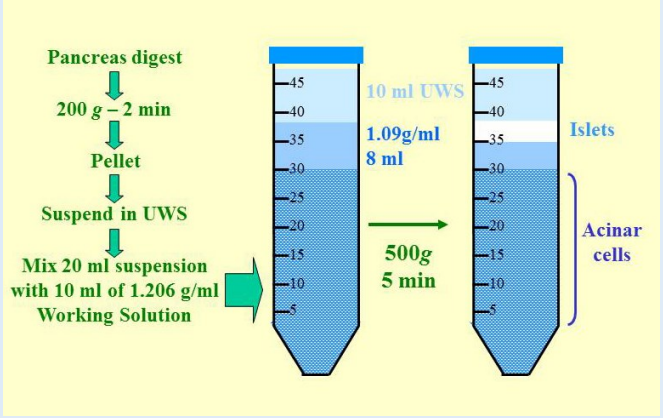
In OptiPrep™ gradients it has been demonstrated that islets have a lower buoyant density than acinar cells. This allows them to be separated by flotation through a low density barrier. Dr van der Burg, University Hospital, Leiden, Netherlands has developed a method using OptiPrep™ which produces islets from porcine pancreas with a high viability. Note that the method uses the University of Wisconsin Solution (UWS) as the suspending medium, other customized media which are used to retain high islet viability may have different densities and the method may consequently require adaption.

Tissues from other species may also require some protocol optimization.

Cells from the pancreatic digest are sedimented and suspended in a solution of density 1.10 g/ml and then overlayered with a low-density barrier (ρ = 1.09 g/ml) and a layer of UWS (see figure). After centrifugation the islets band at the interface below the UWS. The load zone not only retains the acinar cells but also any residual digestive enzymes used for the disaggregation of the tissue. The Working Solution is simply produced from equal volumes of OptiPrep™ and double-strength UWS - the slightly raised osmolality of the Working Solution is beneficial to the separation.

The use of OptiPrep™ has been reported to contribute significantly to improved recovery of islets from pancreas digests and the retention of islet integrity and viability. Diabetic single-donor pig allograft recipients became normoglycemic within 24 h following transplantation; similar results were obtained with transplantation into primates. More recently Shibata et al have proposed reducing the RCF used to band the islets in the iodixanol gradient to 100g. At the lower RCF, recovery, purity, resistance to fragmentation and insulin response to glucose, were all improved compared to that at the higher RCF.

Although the method as described originally and illustrated in the figure was designed for use with tubes in a low-speed centrifuge, it has been scaled up to use with the Cobe 2991 centrifuge.



For detailed protocol and references see Application Sheet C16 at: www.diagnostic.serumwerk.com

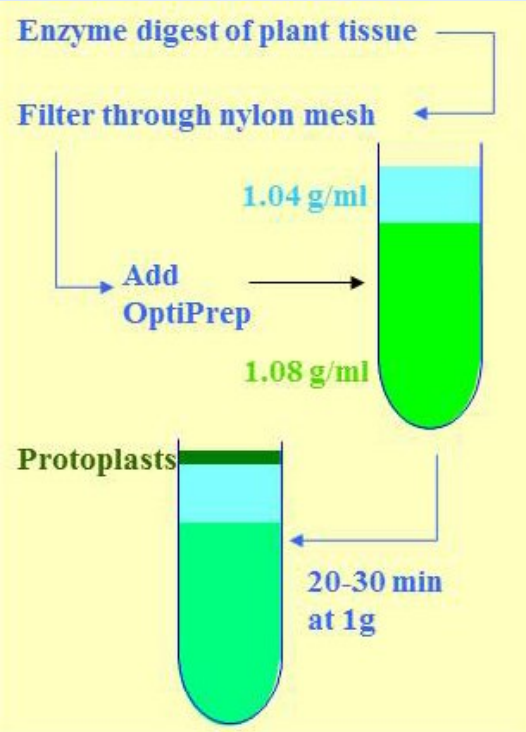
Isolation of plant protoplasts from wheat or barley leaves

Once the cellulose walls are removed from plant cells, they become sensitive to osmotic changes in the environment, shrinking and swelling in response to the environment, with consequent changes to their buoyant densities. In the preparation of plant protoplasts the osmolality of the medium used to digest the plant wall is important: use of a digest mixture whose osmolality is 1.8x that of the living plant tissue is a widely used guideline. A raised osmolality promotes shrinkage of the protoplast away from the cell wall and facilitates its isolation.

Using wheat or barley leaves as starting material, excellent populations of intact protoplasts can be prepared using a simplified modification of the Bethke et al method which omits the densest layer and also modulates the density of the lighter layers. OptiPrep™ is added directly to the plant digest to make a suspension with a final density of 1.08 g/ml. The separation procedure is outlined in the figure.

Centrifugation is actually not necessary to band the protoplasts: their size means that if the tube is allowed to stand on the bench (1g), they will float to the upper interface in about 20-30 min.

The top band contains over 95% intact protoplasts. The number of intact protoplasts remaining in the ρ = 1.08 g/ml layer is insignificant.



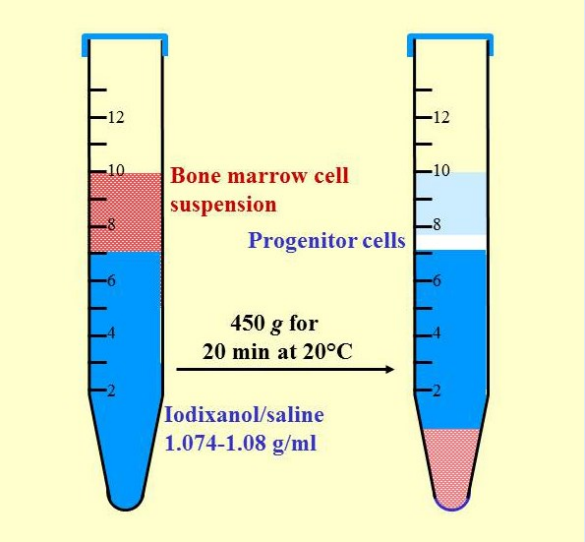
For detailed protocol and references see Application Sheet C19 at: www.diagnostic.serumwek.com

Isolation of a progenitor enriched fraction from bone marrow

Although the resolving power of density gradients is insufficient to allow megakaryocytic progenitor cells to be isolated in a sufficiently pure form for further analysis and culture; they do provide a useful preliminary purification step, which can usually achieve a two-threefold enrichment of these cells from crude bone marrow samples. This allows a more economical and effective use of expensive immuno-magnetic beads to remove lineage cells with a cocktail of lineage specific monoclonal antibodies.

OptiPrep™ has been used as a simple density barrier of density 1.080 g/ml or 1.077 g/ml for enrichment of progenitor cells from bone marrow; the method is summarized in the figure. Interfacial cells are harvested for subsequent depletion of lineage-committed cells using antibody-beads. A discontinuous gradient of 1.050, 1.080 and 1.090 g/ml is another option.

Iodixanol gradients have also been used to enrich for progenitor cells from blood and thymus. In the case of hippocampal progenitor cells, the denser fractions from a 1.029, 1.036, 1.043 and 1.057 g/ml discontinuous gradient were harvested (Porritt, H.E. et al, Tabrizifard, S. et al).



For detailed protocol and references see Application Sheet C24 at: www.diagnostic.serumwerk.com

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