DGM

Density Gradient Media

OptiPrep™

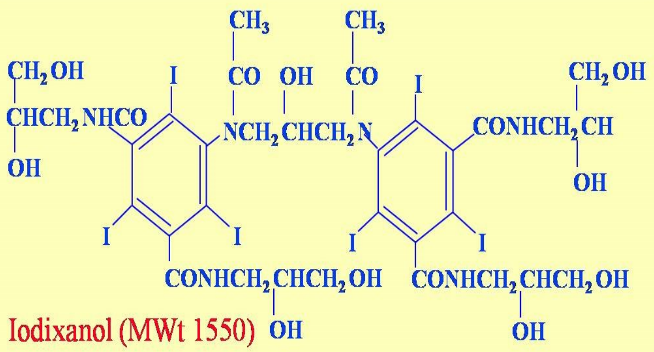
The ideal density gradient medium for purification and analysis of viruses

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 (MWt 1550)

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.

Advantages of OptiPrep™ based methods

Traditionally CsCl or sucrose has been used for the purification of viruses, but iodixanol is now replacing these media in many virus purification and analytical studies.

CsCl and sucrose solution preparation is time-consuming and may require sterilisation before use. Sucrose and CsCl solutions are hyper-osmotic at the densities used to band viruses.

The buoyant density of viruses is always much lower in iodixanol than in CsCl.

CsCl in particular leads to major reductions in infectivity of most viruses. Virus purified using **OptiPrep™** gradients shows infectivity: particle number ratios at least 100x those from CsCl gradients.

CsCl and sucrose must be removed prior to infection of cells.

Purification of viruses in a self-generated gradient

The ability of iodixanol to form self-generated gradients (SGG) considerably simplifies the process of purifying viruses. Herpes simplex virus (HSV) was the first virus to be purified in such SGG and it is now a very widely used method for HSV vectors and other viruses.

Self-generated gradients make sample handling very easy (see figure 1 next page). The virus is first sedimented on to a 50% (w/v) iodixanol cushion (1-2). All of the supernatant is discarded (3) except for a volume equal to that of the cushion. After mixing the remaining liquids, the virus suspension (now in 25% iodixanol) is transferred to a tube for a vertical or near-vertical rotor and centrifuged at approx. 350,000*g* for approx. 3 h (4-5). During this time the gradient forms and the virus moves to its banding density.

There are no interfaces to produce particulate aggregation. The shape of the density profile changes gradually during the centrifugation after which time the profile is more or less stable.

This strategy for virus concentration and purification has also been used by Møller-Larsen and Christensen for the purification of retrovirus from multiple-sclerosis patients.

Comparisons of infectivity and density profiles after centrifugation of a suspension of retrovirus in 25% (w/v) iodixanol in a Beckman VTi65.1 vertical rotor after 350,000 *g* for 1.5 and 2.5h, shows the shallower S-shaped gradient formed after 1.5 h allows a better separation of the lower density immature virus (see figure 2 next page).

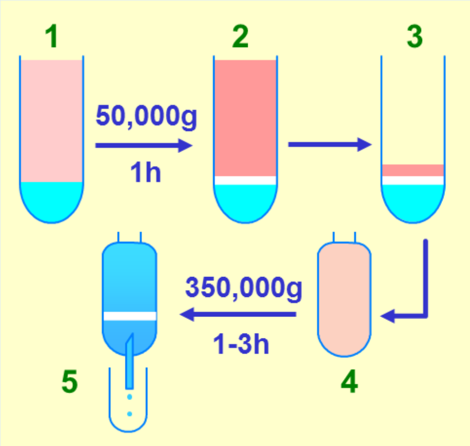


Figure 1

For a detailed protocol and references see Application Sheet V06 at: www.diagnostic.serumwerk.com

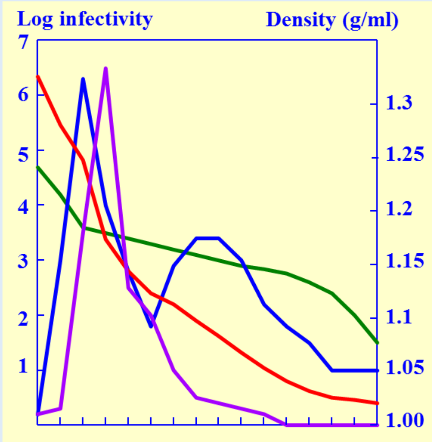


Figure 2

For a detailed protocol and references see Application Sheet V08 at: www.diagnostic.serumwerk.com

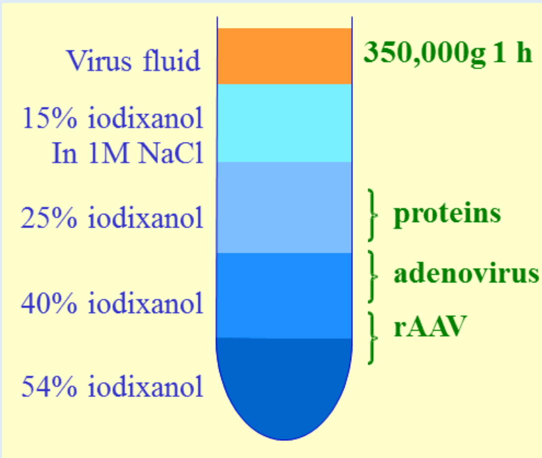
Purification of rAAV and parvovirus in pre-formed discontinuous gradients

Zolotukhin et al used a four layer discontinuous iodixanol gradient (see figure) to purify rAAV from crude cell lysates. Aggregation of rAAV with proteins in the cell lysate can pose a serious problem to its isolation as the aggregates are heterogeneous and consequently exhibit a broad range of densities. Inclusion of 1M NaCl in the 15% iodixanol prevents this aggregation and allows the rAAV to be isolated as a single band in the 40% iodixanol layer after centrifugation at 350,000g in a fixed angle rotor for 1 h. The density of the rAAV in this system banded at a density >1.23 g/ml. All of the contaminating proteins in the lysate banded at the 25%/40% iodixanol interface and more than 99% of the adenovirus contaminant banded at a density of <1.22 g/ml.

Recovery of infectivity is significantly higher than with CsCl gradients.

A similar method can also be used for parvovirus purification.

The strategy has been widely adopted by research workers in gene therapy.



For detailed protocols and references see Application Sheet V14 at: www.diagnostic.serumwerk.com

Purification of HIV-1 virions using a sedimentation velocity gradient

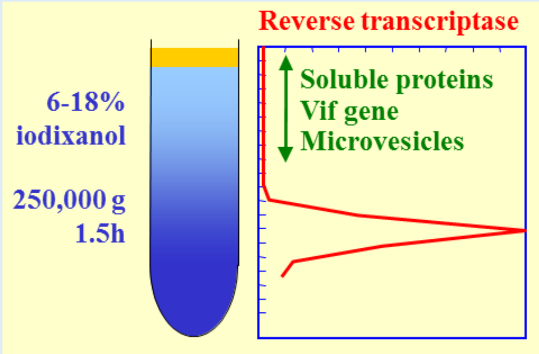
Dettenhoffer and Yu developed a sedimentation velocity iodixanol gradient to purify HIV-1 virions without affecting the infectivity of the virus. The crude virus suspension is layered over a 6-18% gradient and centrifuged either 250,000g for 1.5 h or lower RCFs for longer times (see figure). The authors constructed the gradient from a multistep discontinuous gradient but the gradient is effectively continuous and may therefore be formed using a Gradient Master™ or two-chamber gradient maker.

In buoyant density sucrose gradients the extracellular Vif gene always co-purifies with the virus.

In sucrose gradients the HIV is contaminated with cell-derived micro vesicles.

In iodixanol gradients the HIV-1 is effectively separated both from Vif and from the micro vesicles.

Similar gradients have been used to purify other viruses and the gradients are widely used to study the assembly of these viruses



For detailed protocol and references see Application Sheet V34 at: diagnostic.serumwerk.com

Purification of human papillomavirus using a continuous gradient

Cervical cancer is one of the most common cancers in women worldwide. Persistent infection with human papillomavirus (HPV) is considered to be the etiological factor for cervical cancer. Therefore, an effective vaccine against HPV infections may lead to the control of cervical cancer. An ideal HPV vaccine should aim to generate both humoral immune response to prevent new infections as well as cell-mediated immunity to eliminate established infection or HPV-related disease.

There are now many published papers that report the use of iodixanol gradients not only to purify viruses but also to investigate their assembly. In all comparative studies between CsCl and iodixanol, the recovery of virus infectivity is much higher and the particle: infectivity ratio much lower when viruses are purified in iodixanol. Although sucrose is generally less deleterious to viral infectivity than CsCl, it can nevertheless also have serious effects on certain important aspects of viral function; in particular the loss of surface glycoproteins from retroviruses has been noted. This may be related to its viscosity, which, in solutions of the same density, is much higher than that of iodixanol.

Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast both infectivity measurements using cultured cells and many add-on techniques can be performed without dialysis of iodixanol. Combined with the availability of OptiPrep™ as a sterile solution, this makes the use of OptiPrep™ for virus purification and assembly analysis much more convenient than the use of either CsCl or sucrose.

The protocol described for papillomavirus vector purification has also been used for the purification of pseudovirus carrying a secreted alkaline phosphatase (SEAP) reporter gene. The iodixanol solutions are prepared in PBS supplemented with additional NaCl, KCl and divalent cations.

HPV16 pseudovirions with encapsulated secreted alkaline phosphatase (SEAP) is generated by co-transfection of 293TT cells with plasmids encoding HPV16 L2 and a SEAP reporter plasmid as described by Buck et al.

Cells collected after transfection is treated with Brij 58 and Benzonase and purified by centrifugation on an OptiPrep™ step gradient (27, 33, and 39%) at 40,000 rpm for 4.5 h. Pseudovirus neutralization assays were carried out as outlined previously. Briefly, the pseudovirus and the pooled mouse immune sera were incubated for 1 h and the mixture was used to infect 293TT cells. 68-72 h post-infection, the supernatants were collected and SEAP activity in the supernatants was measured by colorimetric assay. Serum neutralization titers were defined as the highest dilution that caused at least a 50% reduction in SEAP activity, compared to control pre-immune serum samples.

For a detailed protocol and references see Application Sheet V10 at: www.diagnostic.serumwerk.com

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