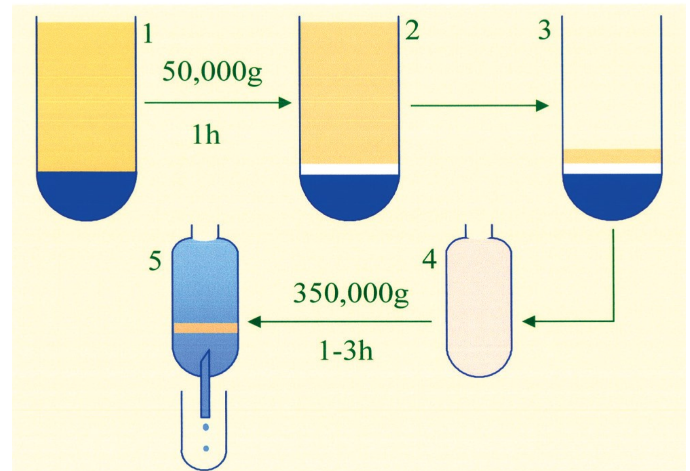
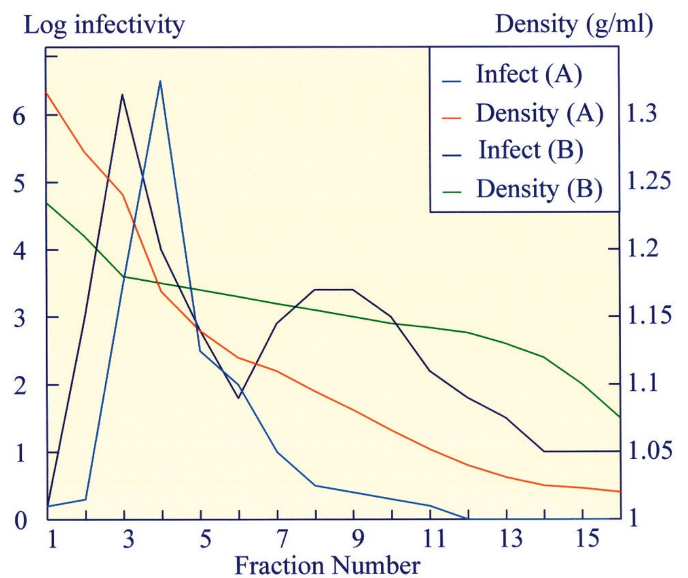
Purification of viruses in a self-generated gradient

A protocol for virus purification has been designed to avoid pelleting the virus and to band the virus particles towards the bottom of a self-generated iodixanol gradient while allowing any contaminating membrane material to band at lower densities. The process is simple and requires fewer manipulations than a pre-formed gradient. The figure (upper right) illustrates the method as applied to the purification of Herpes virus. By centrifuging at 350,000g for 2.5 h a steep density gradient profile is generated towards the bottom of the tube and in this part of the gradient the virus bands quite sharply. Any contaminating membranes from the host cells band in the lighter region of the gradient. The small shoulder of infectivity that is observed on the low density side of the virus peak may represent a minor subpopulation of particles and if the intent is to investigate virus morphology and growth rather than simple purification the gradient density profile can be modulated by changing the centrifugation time. By decreasing the centrifugation time a shallower region spanning a relatively small density range can be used to advantage to resolve the lighter virus particles from the denser ones (figure lower right).

This strategy for virus concentration and purification has also been used successfully by Møller-Larsen and Christensen for the purification of retrovirus from multiple sclerosis patients. The authors found that the "particle-friendly" iodixanol allowed the recovery of virus which was more functionally competent compared to isolation in sucrose gradients.





For detailed protocol and references see Application Sheet V08 at: www.axis-shield-density-gradient-media.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.

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In this leaflet we have presented some of the applications available for the purification of viruses using OptiPrep. More information can be found on: **www.axis-shield-density-gradient-media.com/methodology**

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.axis-shield-density-gradient-media.com