OptiPrep™ Mini-Review MV03

Purification and analysis of *Herpesviridae* viruses and herpes virus vectors (and including Epstein-Barr virus)

- OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Mini-Review principally provides (in Section 2) a bibliography of all those papers reporting the use of OptiPrep™ in the purification and analysis of herpes simplex virus. Section 1 briefly summarizes the advantages of using OptiPrep™; the gradient strategies and the technical data that is available from the Axis-Shield website.

1. Technical background to the use of OptiPrep™

1a. Background

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 enveloped viruses has been noted [1] and with HIV-1 the disorganization of the Gag sub-membrane layer [2]. The loss of surface glycoprotein may be related to its viscosity, which, in solutions of the same density, is much higher than that of iodixanol. Most iodixanol gradients can also be made isoosmotic over the entire density range.

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 only analytical technique for which removal of the iodixanol is essential is electron microscopy. Consequently iodixanol is being increasingly used for the purification of hepatitis C virus particles from lysed cultured cells, from conditioned culture medium or from plasma samples from infected patients.

1b. Solution preparation

Isoosmotic solutions for making gradients may be prepared simply by diluting OptiPrep™ with a HEPES- or Tris-buffered saline solution; sometimes 1 mM EDTA is included. The ability to produce sterile solutions that are isoosmotic by this procedure, greatly facilitates the procedure, Nycodenz® solutions must be produced by dissolution of a powder and then sterilized.

1c. Gradient strategy

A wide range of iodixanol density gradient strategies is available. It can be used in the standard manner as a pre-formed discontinuous or continuous gradient or as self-generated gradient. The preformed gradients, broadly speaking, cover the range 10-40% (w/v) iodixanol. Most of them are top-loaded with the crude virus preparation. If however the virus has been concentrated by sedimenting onto a cushion of dense iodixanol, it may be prudent consider bottom loading the virus. It may be difficult to harvest the virus from the top of a dense cushion and maintain a virus suspension that has a sufficiently low density to allow its easy layering on top of a gradient. With bottom loading (flotation gradient) this is never a problem.

Herpes simplex virus particles have also been analyzed in self-generated gradients of iodixanol. This very simple technique, which has been used for a number of other viruses, requires only adjustment of the crude virus suspension to 25% (w/v) iodixanol. The formation of a self-generated gradient is most efficiently carried out in a vertical or near-vertical rotor at approx 350,000 *g* for 2-3 h but a fixed-angle rotor (approx. 10 ml tube size) may be substituted for longer times. The low virus concentration at the start of the centrifugation and the lack of any liquid/liquid interfaces may optimize resolution of virus particles from contaminants.

1d. References

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1e. OptiPrep™ Application Sheets

Detailed protocols for the isolation of the herpes virus may be accessed from the Axis-Shield OptiPrep™ Applications flash-drive or from the following website: www.axis-shield-density-gradient-media.com, click on "Methodology" then "Viruses" to open up the Virus Index. Other relevant OptiPrep™ Application Sheets may also be accessed from the top of the Index.

- Herpes virus purification in pre-formed gradients — OptiPrep™ Application Sheet V09
- Herpes virus purification in self-generated gradients — OptiPrep™ Application Sheet V08
- Preparation of density gradient solutions — OptiPrep™ Application Sheet V01
- Preparation of continuous and discontinuous gradients — OptiPrep™ Application Sheet V02
- Preparation of self-generated gradients — OptiPrep™ Application Sheet V03
- Harvesting gradients — OptiPrep™ Application Sheet V04
- Analysis of gradients — OptiPrep™ Application Sheet V05
- Concentration of virus samples — OptiPrep™ Application Sheet V06

2. Bibliography

- The references have been divided into research topic and within each section they are listed alphabetically by first author; multiple entries on the same first author are listed chronologically. Papers reporting the study of Epstein-Barr virus are given in separate section, see pp 6-7. To aid selection key words in the titles are highlighted in light blue.

Angiogenesis

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Gene expression

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Glycoprotein K

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Interferon regulatory factor

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Publications on Epstein-Barr virus

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