OptiPrep™ Application Sheet V32

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae: Gammaretrovirus*: murine oncornavirus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- To access other Application Sheets referred to in the text return to the Virus Index; key Ctrl "F" and type the V-Number in the Find Box.

1. Background

Dettenhoffer and Yu [1] were the first to report the use of discontinuous 6-18% (w/v) iodixanol gradient in a sedimentation velocity mode to purify HIV-1 virions without affecting the infectivity of the virus. **This is described in Application Sheet V34**. The technique was subsequently extended to the purification of murine oncornavirus by Fujisawa et al [2].

In all comparative studies between CsCl and iodixanol, it has been shown that 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 [3]. This may be related to its viscosity, which is much higher than that of iodixanol. Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast, many add-on techniques can be performed and cells infected with virus, without dialysis of iodixanol.

The following protocol is adapted from ref 2.

2. Solutions required

A. OptiPrep™

B. Suspension Buffer: 0.15 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.2

C. Gradient solutions: dilute OptiPrep™ with Solution B to give two solutions of 6 and 18% (w/v) iodixanol (**see Notes 1 and 2**)

Keep the following stock solutions at 4°C:

1 M Tris (free base),	12.1 g per 100 ml
100 mM $EDTANa_2 \bullet 2H_2O$	3.72 g per 100 ml
1 M NaCl	5.84 g per 100 ml

Solution B: To 50 ml water, add 1.0 ml each of Tris and EDTA, and 15 ml of NaCl stock solutions; adjust to pH 7.2 with 1 M HCl; make up to 100 ml.

3. Ultracentrifuge rotor requirements

Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti or Sorvall TH641).

4. Protocol

1. Clarify the supernatant from infected cells by centrifugation at 1500 *g* for 20 min and pass through a 0.22 μm filter.

2. Concentrate the virus suspension by pelleting it through a density barrier at 100,000 *g* for 2 h (**see Note 3**) and resuspend it in a small volume of Solution B.

3. Using a two-chamber gradient maker or a Gradient Master™ prepare a continuous gradient from approx 6 ml each of the two iodixanol solutions in the 13-14 ml tubes (**see Notes 4 and 5**).

4. Layer the crude virus suspension (1.0-1.5 ml) on top of the gradient and centrifuge at 187,000 g_{av} for 1 h 20 min at 4°C (**see Notes 6 and 7**).

5. Collect the gradient by upward displacement, low-density end first in approx 0.8-1.0 ml fractions (**see Note 8**). The virus bands sharply, 1-2 ml from the bottom of the gradient.

5. Notes

1. For more information on the preparation of density gradient solutions **see Application Sheet V01**.
2. If a gradient making device is unavailable, then make up solutions of 6.0%, 9.0%, 12.0, 15.0% and 18.0% (w/v) iodixanol.
3. Fujisawa et al [2] pelleted the virus through a 20% sucrose cushion; to maintain an isoosmotic environment for the virus, the 20% sucrose might be replaced by 15% (w/v) iodixanol. The ideal way of concentrating the virus is sedimentation on to a dense cushion of iodixanol, rather than pelleting. This however may be less convenient when, as in this case, the concentration of iodixanol in the viral suspension needs to be <5% (w/v) to permit loading on to the gradient. When recovering the band of virus as little as possible of the cushion must be aspirated. For more information on concentration of virus prior to gradient purification **see Application Sheet V06**.
4. Alternatively make a discontinuous gradient from equal volumes of 6.0%, 9.0%, 12.0, 15.0% and 18.0% (w/v) iodixanol and allow the formation of a continuous gradient by diffusion (approx. 5 h at room temperature, or overnight at 4°C). For more information on making gradients **see Application Sheet V02**.
5. Dettenhoffer and Yu [1], who introduced the sedimentation velocity strategy for HIV-1, prepared gradients that were "essentially continuous" by layering solutions with a 1.2% iodixanol concentration interval. It takes considerable practice to be able to form discontinuous gradients from numerous small volume steps, irrespective of whether a pipette or a syringe is used and whether an overlayering or underlayering technique is used; **see Application Sheet V02**.
6. If larger volumes of crude virus are to be purified then larger volume gradients must be used. As this is a sedimentation-velocity separation the volume of crude virus suspension should not exceed 10-15% of the gradient volume.
7. If the separation is to be carried out at higher temperatures then it may be necessary to reduce the centrifugation time to take account of the reduced viscosity of the gradient.
8. Collection of the gradient by tube puncture may be a useful alternative. **For more information on harvesting gradients see Application Sheet V04**.

6. References

1. Dettenhoffer, M. and Yu, X-F. (1999) *Highly purified human immunodeficiency virus type 1 reveals a virtual absence of Vif virions* J. Virol., **73**, 1460-1467
2. Fujisawa, R., McAtee, F.J., Favara, C., Hayes, S.F. and Portis, J.L. (2001) *N-terminal cleavage fragment of glycosylated Gag is incorporated into murine oncornavirus particles* J. Virol., **75**, 11239-11243
3. Palker, T.J. (1990) *Mapping of epitopes on human T-cell leukemia virus type 1 envelope glycoprotein* In: Human Retrovirology: HTLV (ed. Blattner, W.A.) Raven Press, NY, pp 435-445Dettenhoffer, M. and Yu, X-F. (1999) *J. Virol.*, **73**, 1460-1467

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