OptiPrep™ Application Sheet V30

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae*: *Betaretrovirus*: Mason-Pfizer Monkey virus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **Optiprep™ Reference List (RV06)** provides a comprehensive list of all published papers reporting the isolation of **Group VI viruses** on iodixanol gradients. **To access RV06** return to the initial list of Folders and select "**Reference Lists**". This **Application Sheet** summarizes the published methods for purification of viruses of the *Retroviridae* family.
- This **Application Sheet** describes the use of continuous sedimentation velocity or buoyant density pre-formed gradients for purification of the **Mason-Pfizer monkey virus**, which belongs to the *Betaretrovirus* genus of retroviruses.
- For other retroviral isolation methods see the Virus Index
- To access other Application Sheets **referred to in the text: return to the 2020Virapp file and select the appropriate** V number.

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 (**see Application Sheet V34**). The technique was subsequently extended to the purification of Mason-Pfizer monkey virus by Gottwein et al [2] and the protocol is described in Section 2; an alternative buoyant density method [3,4] is described in Section 3.

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 retroviruses has been noted [5]. 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.

2. Sedimentation velocity method (adapted from ref. 2)

2a. Solutions required

A. OptiPrep™

B. Phosphate-buffered saline

C. Gradient solutions: dilute OptiPrep™ with Solution B to give a series of density solutions from 6 to 18% (w/v) iodixanol in 1.2% steps (i.e. 11 solutions, **see Notes 1 and 2 in Section 2d**)

2b. Rotor requirements

Virus concentration: Swinging-bucket rotor with approx 30-38 ml tubes (e.g. Beckman SW28) or 13-14 ml tubes (e.g. Beckman SW41Ti), **see Note 3 (Section 2d)**

Gradient purification: Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti)

2c-1. Virus concentration

1. Harvest the cell supernatants and filter through a 0.45 μm filter.

2. Concentrate the virus by pelleting it through a density barrier (**see Notes 3 and 4, Section 2d**).

3. Resuspend it in a small volume of Solution B (see Step 2 of the next section).

2c-2. Gradient purification

1. Prepare a discontinuous gradient from approx 1 ml of each density solution. This is best accomplished by overlayering using a peristaltic pump first to draw each 1 ml into a plastic tube and then reversing the flow to expel it gently on top of the denser layer. Alternatively prepare a continuous gradient (approx 12 ml total) from equal volumes of the 6% and 18% iodixanol solutions using a two-chamber gradient maker or a Gradient Master™ (**see Note 5, Section 2d**).

2. Layer the virus suspension (approx 1.0 ml) on top of the gradient and centrifuge at 164,000 g_{av} for 30 min at 4°C (**see Notes 6-9, Section 2d**).

2d. Notes

1. One of the practical alternatives, which might be considered, is the use of a continuous 6-18% iodixanol gradient rather than a multi-step discontinuous gradient (**see Note 5**).
2. For more information on the preparation of density gradient solutions **see Application Sheet V01**.
3. Use a rotor convenient to the virus suspension volume. The tube volume and the relative amount of virus-containing solution and cushion are not critical and can vary with the amount of material.
4. Gottwein et al [2] pelleted the virus through a 20% sucrose cushion (1 h at 53,000g); 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, to avoid loss of infectivity, is sedimentation on to a dense cushion of iodixanol, rather than pelleting. This however may be less convenient for this sedimentation velocity separation as the concentration of iodixanol in the viral suspension needs to be <5% (w/v) to permit loading on to the gradient. For more information on concentration of virus prior to gradient purification **see Application Sheet V06**.
5. A third option is to make an easier 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**.
6. If larger volumes of virus suspension 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. Allow the rotor to decelerate from 2000 rpm without the brake.
8. For more information on harvesting gradients **see Application Sheet V04**.
9. In an alternative 10-55% (w/v) iodixanol gradient centrifuged at 215,000 *g* for 40 min was used to separate unassembled proteins (top of gradient) from denser assembled virus-like particles [6].

3. Buoyant density method (adapted from ref. 3)

3a. Solutions required

A. OptiPrep™
B. Phosphate-buffered saline or any similar medium
C. Gradient solutions: dilute OptiPrep™ with Solution B to give solutions of 10% and 40% (w/v) iodixanol

3b. Rotor requirements

Virus concentration: Swinging-bucket rotor with approx 30-38 ml tubes (e.g. Beckman SW28) or 13-14 ml tubes (e.g. Beckman SW41Ti) (**see Note 1**)
Gradient purification: Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti)

3c. Protocols

3c-1. Virus concentration

1. Harvest the cell supernatants; filter through a 0.45 μm filter; then sediment the virus from the filtered crude suspension on to a 1 ml (Beckman SW41Ti) or 2.5 ml (Beckman SW28) cushion of OptiPrep™ and centrifuge at 35,000 *g* for 1 h. (**see Note 2**).

2. Recover the virus particles from just above the cushion, being very careful to retrieve as little of the cushion as possible (**see Note 2**).

3c-2. Virus purification

1. Prepare approx. 12 ml gradients in tubes for the Beckman SW41Ti rotor from equal volumes of the two iodixanol gradient solutions using a two-chamber gradient maker or a Gradient Master™ or allow a discontinuous gradient of equal volumes of 10%, 20%, 30% and 40% (w/v) iodixanol to diffuse. For more information on making gradients **see Application Sheet V02**.

2. Make sure that the harvested virus suspension from the iodixanol cushion is of a sufficiently low density to allow it to be layered on 10% iodixanol. Dilute with Solution B if necessary.

3. Layer the virus suspension on the gradient and centrifuge at 35,000 *g* for 16 h (**see Note 3**).

4. Collect the gradient in a series of equal volume fractions or harvest the viral particles by aspiration into a syringe and metal cannula (**see Note 4**).

3d. Notes

1. Use a rotor convenient to the virus suspension volume.
2. For more information on concentration of virus on to a density barrier **see Application Sheet V06**.
3. There is no doubt that relatively low *g*-forces, as used in this protocol, are beneficial to any viral particle, but banding times could be reduced to 3-4 h by increasing the *g*-force to say 120,000 *g*, what affect this might have on the infectivity of the virus particles is not known.
4. The wild-type virus particles band at approx 1.125 g/ml (equivalent to 23% (w/v) iodixanol); interestingly Gag protein processing mutants banded at a slightly higher density [3]. For more information on harvesting gradients **see Application Sheet V04**.

4. 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. Gottwein, E., Bodem, J., Müller, B., Schmechel, A., Zentgraf, H. and Kräusslich, H-G. (2003) *The Mason-Pfizer monkey virus PPPY and PSAP motifs both contribute to virus release* J. Virol., **77**, 9474-9485
3. Wildová, M., Hadravová, R. Štokrová, J., Křížová, I., Ruml, T., Hunter, E., Pichová, I. and Rumlová, M. (2008) *The effect of point mutations within the N-terminal domain of Mason-Pfizer monkey virus capsid protein on virus core assembly and infectivity* Virology, **380**, 157-163
4. Voráčková, I., Ulbrich, P., Diehl, W.E. and Ruml, T. (2014) *Engineered retroviral virus-like particles for receptor targeting* Arch.Virol., **159**, 677–688
5. 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 435-445
6. Fuzik, T., Pichalova, R., Schur, F.K.M., Strohalmova, K., Križova, I., Hadravova, R., Rumlova, M., Briggs, J.A.G., Ulbrich, P. and Ruml, T. (2016) *Nucleic acid binding by Mason-Pfizer monkey virus CA promotes virus assembly and genome packaging* J. Virol., **90**, 4593-4603

serumwerk bernburg

Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwwerk.com • serumwerk.de