OptiPrep™ Application Sheet V23

Purification of Group V ((-)ss) RNA viruses: *Arenaviruses*: Lassa virus, Tacaribe virus, Junin virus, Lymphocytic choriomeningitic virus (LCV)

- 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.
- This OptiPrep™ Application Sheet primarily describes the purification of Lassa virus (the methodology can probably be applied to other arenaviruses), with comments regarding Junin virus (Section 6) and LCV (Section 7)
- Section 8 describes the gradient analysis of Tacaribe virus nucleoproteins.

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 Lassa virus by Lenz et al [2] and Strecker et al [3]. These workers have used the technique to study the processing of Lassa virus glycoproteins grown in Vero-E6 cells. Purification has also been carried out on an 8%/30% iodixanol discontinuous gradient [4].

All comparative studies between CsCl and iodixanol show that 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.

The following protocol is adapted from ref 2.

2. Solutions required

A. OptiPrep™
B. Phosphate-buffered saline
C. Gradient solutions: dilute OptiPrep™ with Solution B to give density solutions of 7.2 and 18% (w/v) iodixanol (**see Notes 1 and 2**)

3. Ultracentrifuge rotor requirements

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

4. Protocol

1. Concentrate the virus suspension by pelleting it, from a clarified cell culture supernatant, through a density barrier (**see Note 3**) and resuspend it in a small volume of Solution B.

2. Using a two-chamber gradient maker or a Gradient Master™ prepare a continuous gradient from approx 6 ml each of the two iodixanol solutions (**see Notes 4-6**).

3. Layer the crude virus suspension (1.0-1.5 ml) on top of the gradient and centrifuge at 200,000 g_{av} for 1.5 h at 4°C (**see Notes 7 and 8**).

4. Collect the gradient by upward displacement, low-density end first in approx 0.8-1.0 ml fractions (**see Note 9**). The virus bands sharply at approx 14% (w/v) iodixanol.

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 7.0%, 10%, 13%, 16% and 19% (w/v) iodixanol.
3. Lenz et al [3] pelleted Lassa virus through a 20% sucrose cushion (2 h at 48,000 g_{av}); 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 7.0%, 10%, 13%, 16% and 19% (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 gradient formation see Application Sheet V02**.
5. Confirm that the gradient is continuous by checking the density of a blank gradient. For more information about density measurement **see Application Sheet V05**.
6. 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**.
7. If larger volumes of crude virus are to be purified then larger volume gradients must be used. As this is a rate-zonal separation the volume of crude virus suspension should not exceed 10-15% of the gradient volume.
8. 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.
9. Collection of the gradient by tube puncture may be a useful alternative. For more information on harvesting gradients **see Application Sheet V04**.

6. Pelleting through a density cushion

A simple cushion of 10% (w/v) iodixanol (150,000 g for 2 h) has been used to separate fluorescent dye-labelled Junin virus from the dye solution in flow cytometry studies [6-8].

7. Lymphocytic choriomeningitis virus (LCV)

LCV was purified in a continuous iodixanol gradient generated by diffusion from 7%, 10%, 13%, 16% and 19% (w/v) iodixanol, centrifuged at 122,000 *g* for 12 h [9]

8. Analysis of Tacaribe virus nucleoproteins

In a study of RNA replication in Tacaribe virus Baird et al [10] developed a very useful iodixanol gradient for the analysis of replication transcription complexes (RTCs). The virus-containing cells are lysed in a medium containing K-aspartate, K-glutamate and K-gluconate (all 38 mM), !0 mM $KHCO_3$, 2 mM $MgCl_2$ (or 5 mM EDTA), 2mM DTT, 10μM $ZnCl_2$ and 20 mM MOPS pH 7.1 (plus protease inhibitors), either by Dounce homogenization or addition of a detergent (2% NP40). A 15-48% (w/v) iodixanol gradient (containing the same reagents as the lysis medium) is formed in 5 ml tubes for a swinging-bucket rotor; either using a gradient former or by allowing a discontinuous gradient (equal volumes of 15%, 26%, 37% and 48% iodixanol) to diffuse. After adjusting the sample to 50% (w/v) iodixanol it is layered beneath the continuous gradient and centrifuged at 100,000 *g* for 20 h. For more information on gradient formation and the underlayering of samples see **Application Sheet V02**.

As with all flotation gradients, soluble proteins remain in the load zone, allowing the other macromolecules and macromolecular complexes to float into the gradient. Most of the Tacaribe virus nucleoprotein banded at a density that confirmed its association with the virus membrane [6]. Importantly the gradient was also able to distinguish the full length RNAs (which co-banded with the

nucleoprotein) and denser mRNA nucleoprotein. Baird et al [6] also observed that the gradient was able to resolve other novel RNA species. For more information on the analysis see ref 6.

9. References

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