OptiPrep™ Application Sheet V31

Purification of Group VI (ss)RNA-RT viruses: *Retroviridae*: *Deltaretrovirus*, human T-cell lymphotropic virus (HTLV-1) and human endogenous retrovirus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Reference List (RV06)** provides a full bibliography of all published papers reporting the use of iodixanol gradients for **Group VI** viruses; to access return to the initial list of Folders and select **"Reference Lists".**
- This Application Sheet principally describes the use of a self-generated gradient for purification of members of the *Deltaretrovirus* genus. Section 2 briefly describes use of pre-formed gradients.
- The retrovirus group is extremely diverse; whether the methods described in this Application Sheet can be applied to another retrovirus can only be determined experimentally.
- To access other Application Sheets **referred to in the text: return to the 2020Virapp file and select the appropriate** V number.

1. Self-generated gradients

1-1. Background

In the following protocol [1,2], the viral particles are first concentrated on top of a dense cushion of iodixanol, instead of pelleting the virus. Subsequently, after removal of most of the supernatant, the contents of the tube are simply mixed so that the virus is suspended in 20% (w/v) iodixanol. This suspension is then centrifuged in a tube for a vertical or near-vertical rotor. The self-generated gradient that is formed is designed to band virus particles towards the bottom of gradient while allowing any contaminating membrane material to band at lower densities. Self-generated gradients have the merit of high reproducibility and ease of execution. This strategy was first worked out for Herpes virus; **see Application Sheet V08.**

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 retroviruses has been noted [3]. This may be related to its viscosity, which is much higher than 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.

1-2. Solutions required

A. OptiPrep™

B. Diluent: 0.85% (w/v) NaCl, 60 mM Hepes-NaOH, pH 7.4

C. Working solution of 50% iodixanol (ρ = 1.272 g/ml): mix 5 vol of solution A with 1 vol of solution B (**see Section 1-5, Note 1**).

D. HEPES buffered saline: 0.85% NaCl (w/v), 10 mM HEPES-NaOH, pH 7.4.

Keep Hepes (free acid) as a 200 mM stock solution at 4°C; 4.76g per 100 ml water.

Solutions B and D: Dissolve 0.85g NaCl in 50 ml water; add 30 ml or 5 ml of buffer stock solution respectively; adjust to pH 7.4 with 1 M NaOH and make up to 100 ml.

1-3. Ultracentrifuge rotor requirements

For concentration of the virus (if required): a swinging-bucket rotor of suitable volume to accommodate the volume of crude virus suspension and capable of 100,00-200,000 g_{av} (e.g. Beckman SW28 or Beckman SW28.1 or equivalent rotors).

For gradient purification: any vertical or near-vertical rotor with tube capacity of approx 12 ml and capable of approx 350,000g. The sedimentation path length of the rotor should be 17-25 mm. Separations described in this Application Sheet were obtained with a Beckman VTi65.1 vertical rotor, NVT65 near-vertical rotor or NVT65.2 near-vertical rotor (**see Section 1-5, Note 2**). High performance fixed-angle rotors may only be used for the rapid formation of self-generated gradients if the tube volume is relatively small (less than 6 ml). **For more information on self-generated gradients see Application Sheet V03**.

1-4. Protocol

1. Clarify the virus suspension by centrifugation at 1000 *g* for 10 min.

2. Transfer a known volume of the supernatant to suitable tubes for a swinging-bucket rotor and underlay with a small volume (2-4 ml) of Solution C (**see Section 1-5, Note 3**).

3. Centrifuge at 160,000 g_{av} for 1 h to band the virus sharply at the working solution interface.

4. Remove all of the supernatant except for an amount equal to the 1.5x the volume of cushion.

5. Mix the residual contents of the tubes. This will produce a concentrated virus suspension in 20% (w/v) iodixanol.

6. Transfer the suspension to tubes suitable for a vertical, near-vertical or low-angle fixed-angle rotor to band the virus in a self-generated gradient of iodixanol.

7. Any tubes that are not filled should be topped up and mixed with 20% (w/v) iodixanol (mix Solutions C and D in the volume ratio 1:1.5).

8. Centrifuge at 350,000 g_{av} for 3.5h and at the end of the centrifugation use either a controlled deceleration programme or turn off the brake below 2000 rpm (**see Section 1-5, Note 2**).

9. Either harvest the virus band with a syringe and metal cannula or unload the entire gradient by tube puncture, or other suitable method. Under the centrifugation conditions described the retrovirus will band close to the bottom of the tube (**see Section 1-5, Notes 4-6**).

1-5. Notes

1. **Strategies for preparing working solutions are given in Application Sheet V01**.
2. The method can be scaled up for the use of larger vertical rotors such as the Beckman VTi50, but the longer sedimentation path length and lower maximum RCF means that longer centrifugation times will be necessary. Work with Ebola virus suggests that 6 h at 45,000 rpm is satisfactory. **See Application Sheet V25 for more details**.
3. The actual volumes will depend on the total volume of virus fluid and the volume of the tubes used. For example: for approx. 15 ml supernatant per tube use 1-2 ml cushion solution, for approx. 35 ml use 2-4 ml.
4. Since the virus bands close to the bottom of the gradient and contaminating membranes are lighter, collection from the bottom is the method of choice. **For more information on harvesting gradients see Application Sheet V04**.

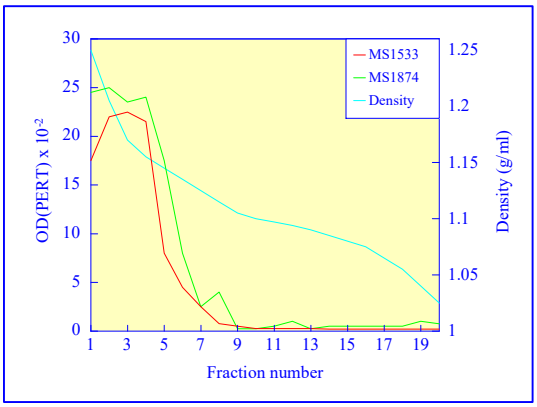

Figure 1: Purification of retrovirus from two lines of MS cells (ex-multiple sclerosis patients) in self-generated iodixanol gradients. Infectivity (PERT) and density profiles in 20% iodixanol after 350,000g for 3.5 h. Adapted from ref 2 with kind permission of the authors and Elsevier Science BV

5. The separations shown in Figure 1 were obtained with a Beckman NVT65 near-vertical rotor. After approx 3.5 h the gradient that is generated is more or less linear, but does become progressively steeper in the densest regions. It is very effective for banding the virus sharply near the bottom of the tube (see Figure 1) while any membranous contamination bands at lower densities. If it is deemed advantageous to band the virus further from the bottom of the tube, increase the starting iodixanol concentration to 25% (w/v).
6. Møller-Larsen and Christensen [1] observed that iodixanol retrovirus particles isolated from iodixanol gradients showed much less damage than those isolated from other gradient media. These authors also noted that the method could be used to purify HTLV-1. The method has been subsequently used in investigations into the relation of retrovirus to multiple sclerosis [4-7]. Retroviral particles (HERV-H) have also been isolated from the supernatants from human melanoma cells on a continuous iodixanol gradient in a swinging-bucket rotor centrifuged at 287,000 *g* for 18 h [8]. The virus particles, as measured by reverse transcriptase assay, had a low density of approx 1.10 g/ml.

2. Other gradient systems

Hirschl et al [9] showed that melanoma cells produced viral particles that were homologous to human endogenous retrovirus. In their short communication there was no detailed description of the continuous iodixanol gradient, which was centrifuged at 287,000 *g* for 18 h.. However the endogenous retrovirus clearly banded within the density range of 1.06-1.11 g/ml (equivalent to approx. 10.5-20% (w/v) iodixanol. Contreras-Galindo et al [10] analyzed human endogenous retrovirus K from patients with lymphoma and breast cancer. The HERV-K was first concentrated by pelleting at 45,000 *g* for 2 h through a 20% (w/v) iodixanol cushion., prior to gradient centrifugation. In a later publication the same group [11] studied the endogenous retrovirus particles from patients infected with HIV-1. The authors used the same 20% iodixanol cushion to concentrate the virus and then loaded the virus suspension on a 10-50% (w/v) iodixanol gradient , centrifuged at 350,000 *g* for 6 h. The particles had a density of approx 1.16 g/ml [11].

HTLV-1 has been isolated using a velocity gradient similar to that that is widely used for HIV (**see Application Sheet V34**); the top-loaded 6-20% (w/v) iodixanol centrifuged at 80,000 *g* for 3h [12]. More recently Cao ey al [13] first pelleted HTLV-1 through an 8% (w/v) iodixanol cushion and then purified the virus through a 10-50% iodixanol gradient – no other details were given.

7. References

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