OptiPrep™ Application Sheet V35

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae: Spumaretrovirinae:* Foamy viruses

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review (MV05)** "Purification of retroviruses" summarizes the published methods for purification of viruses of the *Retroviridae* family (*Orthoretrovirinae* and *Spumaretrovirinae* subfamilies) and provides a full bibliography of all published papers reporting the use of iodixanol gradients for their purification; to access return to the initial list of Folders and select "**Mini-Reviews**".
- This Application Sheet describes the use of continuous and discontinuous buoyant density gradients for purification of human and feline foamy virus (*Spumaretrovirinae*).
- The retrovirus group is extremely diverse; whether the methods described in this Application Sheet can be applied to another retrovirus, of the same or different genus can only be determined experimentally. For other retroviral isolation methods see the Virus Index
- 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

There are now many published papers that report the use of iodixanol gradients not only to purify viruses but also to investigate their assembly. 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 [1]. This may be related to its viscosity, which, in solutions of the same density, is much higher than iodixanol.

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 analysis much more convenient than that of CsCl or sucrose.

Foamy virus can be banded according to its buoyant density in either a discontinuous or continuous gradient; both types of gradient will be described, see Step 6 or Step 7 respectively. Some of the variations in methodology are presented in Table 1 at the end of this Application Sheet. The protocols are adapted principally from refs 2 and 3.

2. Solutions required (see Section 5, Note 1)

A. OptiPrep™

B. OptiPrep™ diluent: 6 mM EDTA, 300 mM Tris-HCl, pH 7.5

C. 50% (w/v) Iodixanol Working Solution: mix 5 vol. Solution A with 1 vol. of Solution B

D. Diluent: 0.14 M NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 7.5

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 30 ml and 6 ml of Tris and EDTA stock solutions respectively; adjust to pH 7.5 with 1 M HCl; make up to 100 ml.

Solution D: To 50 ml water, add the following:
14 ml of NaCl stock solution
5 ml of Tris stock solution
1 ml of EDTA stock solution; adjust to pH 7.5 with 1 M HCl; make up to 100 ml.

3. Rotor requirements

For concentrating the virus from large volumes of culture fluid: swinging-bucket rotor with 36-39 ml tubes (e.g. Beckman SW28 or Sorvall AH629)

For the iodixanol gradient: swinging-bucket rotor with either 5 ml (e.g. Beckman SW55Ti or Sorvall AH650) 14 ml tubes (e.g. Beckman SW41Ti) or 17 ml tubes (e.g. Beckman SW28.1 or Sorvall AH-629). **See Section 5, Note 2 for more information**.

4. Protocol

1. Once the virus has been released from the cells, clarify the suspension by low speed centrifugation (approx 2000 *g* for 15 min) to remove cellular debris.

2. If required filter the supernatant through a 0.45 μm filter.

3. Prepare a 12% (w/v) iodixanol solution (1.2 vol. of Solution C with 3.8 vol. of Solution D).

4. To concentrate the virus, use the tubes for the 36-39 ml swinging-bucket rotor and underlay the suspension with 5 ml of the 12% iodixanol using a syringe and metal cannula (**see Section 5, Notes 3 and 4**).

5. Centrifuge at 80,000 g_{av} for 2.5 h

6. **For a discontinuous gradient:** During Step 5 prepare from Solutions C and D: 10%, 20%, 30% and 40% (w/v) iodixanol, and layer 1 ml or 2.5 ml of each in 5 or 14 ml tubes, respectively (**see Section 5, Notes 5 and 6**).

7. **For a continuous gradient:** During Step 5 prepare from Solutions C and D two solutions of 10% and 32% (w/v) iodixanol and using a two-chamber gradient or a Gradient Master™ make a 4 ml or 10 ml gradient (5 ml or 15 ml tubes respectively) from equal volumes of the two solutions (see Section 5, Note 5).

8. Resuspend the virus pellet in 5% (w/v) iodixanol (1 vol. of Solution C + 9 vol. of Solution D) and layer over the chosen gradient (**see Section 5, Note 4**).

9. Centrifuge at 122,000 g_{av} for 4-16 h at 4°C

10. Unload the gradient either by upward displacement, aspiration from the meniscus or by tube puncture in 0.2-0.5 ml fractions. Once the position of the foamy virus in the tube has been established, it can alternatively be recovered using a syringe (**see Section 5, Note 7**).

- **For a brief summary of some of the variations in gradient and centrifugation conditions see Section 5, Notes 8-11)**

5. Notes

1. The mode of preparing the solutions described in this OptiPrep™ Application Sheet ensures that the concentrations of buffer and EDTA are constant throughout the gradient. If this is not considered important, the OptiPrep™ may simply be diluted with the virus suspension solution. Any suitable buffer can be used for suspending the virus and for making the gradient solutions. It may be customized to the operator's own requirements, as long as the buffer has a low density (approx 1.006 g/ml) the density of the gradients will not be compromised. Some of the variations are given in Table 1. More details on the making up of gradient solutions are given in **Application Sheet V01**.
2. Larger volume gradients are permissible (e.g. in the Beckman SW41) but the time will need increasing to compensate for the lower RCF. If a vertical rotor is substituted for the swinging-bucket rotor (e.g. Beckman VTi90 or VTi65.1), the shorter sedimentation path length will permit shorter centrifugation times.

3. Baldwin and Linial [2] sedimented the foamy virus through a 20% sucrose layer, this has been substituted with the more virus-friendly iodixanol in this protocol.
4. Virus concentration by pelleting, either directly or through a low-density layer may be undesirable. This procedure can result in some loss of infectivity either because of the physical aggregation of particles, high hydrostatic pressure at the bottom of the tube or the dispersal forces used to resuspend the pellet (or a combination of all of these problems). A useful alternative is to sediment the virus on to a small cushion (2-3 ml) of 45% w/v iodixanol or even pure OptiPrep™. However, unless the virus band is harvested with the minimum amount of cushion, it may have to be diluted to an unacceptable volume for loading on top of the subsequent density gradient. The use of Beckman "*k*onical" tubes overcomes this problem to some extent. Moreover, as long as the purification is based on buoyant density rather than sedimentation velocity then the sample volume is not really important. For buoyant density banding, the mode of banding in this protocol, the sample may alternatively be layered beneath the gradient, in which case the contamination from the cushion is irrelevant. **For more information on concentrating virus see Application Sheet V06**. Occasionally the iodixanol is only used as a cushion for the concentration step [4].

Table 1: Human foamy virus (HFV) and feline foamy virus (FFV) protocols

Virus suspension in:			Gradient (% iodixanol)	RCF; time	Virus	Ref. #
[NaCl]	[EDTA]	[Tris]; pH				
140 mM	1 mM	50 mM; 7.5	10; 20; 30; 40	120,000g; 4-12 h	HFV	2
140 mM	1 mM	50 mM; 7.5	8.5; 20; 30; 40	120,000g; 4-12 h	HFV	5
150 mM	1 mM	10 mM; 8.0	10-32	124,000g; 16 h	FFV/HFV	3,6,7
136 mM		10 mM; 7.4[1]	10; 20; 30; 40; 50	130,000g; 12-18 h	HFV	8
150 mM	1 mM	20 mM; 7.5	10-40	152,000g; 4 h[2]	PFV[3]	9

1. HEPES used rather than Tris
2. 2 ml Beckman TLS55 rotor
3. PFV = prototypic FV

5. A number of gradient variations are summarized in Table 1. Most methods use either 14 ml or 5 ml tubes, but smaller volume tubes have been used. For more information on making continuous and discontinuous gradients **see Application Sheet V02**.
6. More recently the range of the discontinuous gradient has been changed to 20-55% iodixanol, this may allow loading of the sample on top of the gradient more easy [10,11].
7. For more information on harvesting gradients **see Application Sheet V04**.
9. The banding density of foamy virus is in the range 1.12-1.15 g/ml [2], but the precise value may vary with centrifugation conditions. Some of the variations in centrifugation conditions are given in Table 1. Recently Spannaus and Bodem [12] used a 6-35% (w/v) iodixanol gradient centrifuged at approx. 172,000 *g* for 1.5 h, within which the virus banded in the 19-25% (w/v) iodixanol zone.
10. In the 20-55% (w/v) iodixanol reported in ref 11, the peak density may be slightly higher at approx. 1.17 g/ml. This particular paper reported the use of a 5 ml swinging-bucket rotor centrifuged at approx 100,000 *g* for 18 h; it was used to show the similar banding of wt, ΔGR1 and ΔGR1Ala mutants. The 15-40% (w/v) iodixanol gradient, centrifuged at 197,000 *g* for 3 h in the short sedimentation path-length Beckman TLS55 rotor used by Swiersy et al [13], was described as a sedimentation velocity gradient. It was used to analyze Pol processing products: $p85^{PR-RT}$ and $p40^{IN}$ coincided with the Gag and env proteins; the resolving power of the gradient allowed the authors to identify a shift to higher densities of the $p127^{Pol}$ protein. In a recent paper by Lee et al [14], the 20-55% (w/v) iodixanol gradient was changed to 20-50%. It clearly demonstrated that the wt virus-like particles (VLPs) peaked at approx 1.12-1.14 g/ml, while VLPs bearing Gag-Pol fusions banded at a slightly lower density. Hamann et al [15] used a small-volume gradient (15-40% w/v iodixanol) constructed from nine equivolume steps of 0.22 ml, centrifuged at 197,000 *g* for 3 h to investigate particle morphology of mutant and wild-type particles. The gradient was able to distinguish wild-type and mutant forms indicating a variation of morphology induced Gag-nucleic acid interactions.

11. Spannaus et al [16] have recently published a paper documenting the methodology for purification of foamy viruses.

6. References

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