OptiPrep™ Application Sheet V34

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae: Lentivirus*: Human immunodeficiency virus – 1 (HIV-1) and lentivirus vectors

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **Axis-Shield 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 covers continuous sedimentation velocity or buoyant density pre-formed gradients and of self-generated gradients, for purification of members of the *Lentivirus* genus.
- 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

1a. Virus concentration (see Section 2)

A simple iodixanol density cushion can be used to concentrate the virus. Depending on the density of the cushion, such a technique may also partially purify the virus. A small volume of very dense cushion [1,2] was used for banding viral particles from a very large volume of virus-containing fluid. This stage can be carried out at 50,000 *g* for a short time (1.5-3 h). Should it be necessary to remove the iodixanol after recovery of the banded material, the virus can be efficiently pelleted from a small volume by gentle centrifugation for a long period [2]. Low-speed centrifugation of the original large volume on the other hand would lead to very poor recovery. These procedures lead to improved infectivity, compared to simple high-speed pelleting. Low-density cushions of 8.4% [3] and 6% [4] (w/v) iodixanol have been used to pellet HIV-1 and HIV-1 cores respectively and to separate them from more slowly-sedimenting contaminating particles and soluble proteins.

1b. Purification and analysis in a pre-formed sedimentation velocity gradient (see Section 3)

Dettenhoffer and Yu [5] developed a sedimentation velocity iodixanol gradient to purify HIV-1 virions without affecting the infectivity of the virus. In buoyant density sucrose gradients the extracellular Vif gene always co-purifies with the virus and the latter is also contaminated with cell-derived microvesicles. In rate-zonal iodixanol gradients on the other hand the HIV-1 was effectively separated both from Vif and from the microvesicles. Another important point about sucrose gradients is that although sucrose is generally less deleterious to viral infectivity than CsCl, it can nevertheless have serious effects on viral structure; in particular the loss of surface glycoproteins from retroviruses has been noted [6]. This may be related to its viscosity, which is much higher than that of iodixanol. This sedimentation velocity method is certainly the most widely used of the three gradient options.

1c. Purification and analysis in a self-generated gradient (see Section 4)

Self-generated gradients are highly reproducible and the ease of sample handling makes them very attractive. This strategy was first described using Nycodenz®; the clarified virus suspension was adjusted to 25% Nycodenz® and centrifuged in a vertical rotor at 200,000 *g* for 24 h [7,8]. The strategy has been extended to the use of iodixanol, which forms these gradients more readily [9-11].

1d. Purification and analysis in a pre-formed buoyant density gradient (see Section 5)

Warrilow et al [12] formed a 3.6 ml 20-60% (w/v) iodixanol gradient by diffusion of a multi-step discontinuous gradient; banding of HIV-1 was carried out at 150,000 *g* for 20 h.

2. Virus concentration on to a dense cushion (adapted from ref 2; see also Note 1)

♦ The notes referred to in this and the following methods can be found in Section 6

2a. Solutions required

A. OptiPrep™

B. Buffered saline solution (Hepes or phosphate-buffered)

2b. Rotor requirements

Swinging-bucket rotor (e.g. Beckman SW28) with approx 30-38 ml tubes (see Note 2)

2c. Protocol

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

2. Transfer approx 32-33 ml of the supernatant to tubes for the swinging-bucket rotor and underlayer with 5 ml of OptiPrep™. With *k*onical tubes, the volume of supernatant can be 28-29 ml with 1-2 ml of OptiPrep™ (see Note 3).

3. Centrifuge at 50,000 *g* for 1.5 h at 4° C (see Notes 4 and 5).

4. Carefully aspirate all but 3-4 ml of the supernatant; in *k*onical tubes this can be reduced to 1-2 ml.

5. Collect the banded virus in the residual supernatant, removing as little as possible of the cushion (see Note 6).

6. If the density of the virus suspension is too high to be loaded on a subsequent gradient dilute it with 1 volume of Solution B and pellet the virus at 6000 *g* for 24 h at 4°C (see Note 7).

3. HIV-1 purification in sedimentation velocity gradients (adapted from ref 5, see Note 8)

3a. 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 9-11)

3b. Rotor requirements

Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti or Sorvall TH641; see Note 122

3c. Protocol

1. Prepare a discontinuous gradient from approx. 1 ml of each density solution. This is probably best accomplished by overlayering using a peristaltic pump first to draw each 1 ml of liquid into a plastic tube and then reversing the flow to expel it gently on top of the denser layer (see Note 13).

2. Layer the concentrated virus suspension (approx 1.0 ml) on top of the gradient and centrifuge at 200,000 g_{av} for 1.5 h (see Notes 5, 14 and 15).

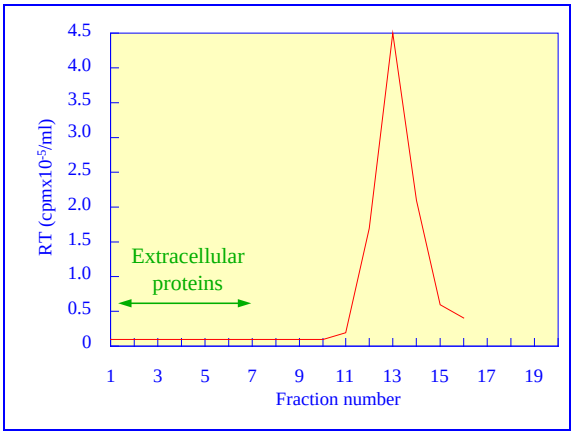

Figure 1 Banding of HIV - type 1 in iodixanol velocity gradient: RT = reverse transcriptase. Adapted from ref 1 with kind permission of the authors and the American Society for Microbiology.

3. Collect the gradient by upward displacement, low-density end first in approx 0.8-1.0 ml fractions (see Note 16). The virus bands sharply in the bottom third of the gradient (Figure 1).

4. HIV-1 purification in a self-generated gradient (adapted from ref 9, see Note 8)

4a. Rotor requirements

Vertical or near vertical rotor: e.g. Beckman VTi65.1 or NVT65 (both approx 13 ml tubes)

4b. Protocol

1. Mix equal volumes of the clarified cell supernatants and OptiPrep™ and centrifuge in the chosen vertical or near vertical rotor. Use approx 350,000 g_{av} for 3-3.5 h (see Note 17).

2. Use a slow-deceleration program or turn off the brake below 2000 rpm.

3. Collect the gradient dense end first by tube puncture or, if the tube type permits it, low density end first, by upward displacement with a dense medium or aspiration from the meniscus (see Notes 16 and 18).

5. HIV-1 purification in a pre-formed continuous gradient (adapted from ref 12, see Note 8)

5a. Solutions required

A. OptiPrep™

B. Stock 100x buffer: 2 M NaCl, 100 mM $MgCl_2$, 50 mM β-mercaptoethanol, 500 mM Tris-HCl, pH 7.4

C. Stock buffer: 20 mM NaCl, 1 mM $MgCl_2$, 0.5 mM β-mercaptoethanol, 5 mM Tris-HCl, pH 7.4

5b. Rotor requirements

Swinging-bucket rotor for approx. 4 ml tubes (e.g. Beckman SW60Ti)

5c. Protocol

1. Prepare an approx. 55% (w/v) iodixanol solution from 11 ml of OptiPrep™, 1.0 ml of water and 120 µl of Solution B. Dilute this further with Solution C to make solutions of 50%, 45%, 40%, 35%, 30%, 25% and 20% (w/v) iodixanol (see Note 19).

2. In 4 ml tubes for the swinging bucket rotor layer 0.45 ml of each of the eight iodixanol solutions, dense end first and allow the gradient to diffuse at room temperature for 4 h (see Note 19).

3. Bring the gradient to 4°C and layer approx. 0.4 ml of the virus solution on top of the gradient to fill the tube.

4. Centrifuge at 150,000 *g* for 20 h (see Note 20) using slow acceleration and deceleration (to and from approx 3000 rpm) programs, if available; if not available turn of the brake during deceleration from 3000 rpm.

5. Unload the gradient in approx 0.4 ml fractions low-density end first. HIV-1 bands in the top quarter of the gradient (see Note 16).

6. Notes

1. For more information on concentrating virus see Application Sheet V06
2. The best rotors for concentrating virus on to a cushion are swinging-bucket ones and the best tubes are the conical-bottomed "***k***onical" tubes of Beckman. The small cross-sectional area of the tube close to its bottom means that a smaller volume of cushion can be used, and recovery of the banded virus without simultaneous aspiration of the cushion itself, is facilitated.
3. Underlayering the virus-containing fluid with the cushion, using a syringe attached to a long metal cannula is certainly the preferred method. Overlayering such a small volume of cushion with a large volume of supernatant is bound to lead to mixing problems.

4. Coleman et al [2] used 2.5 h.
5. Allow the rotor to decelerate using a slow-deceleration program or turn off the brake below 2000 rpm to avoid “Coriolus” mixing of the banded virus.
6. It may be more convenient to use a syringe + long metal cannula to remove the cushion first.
7. Coleman et al [2] only used 0.22 ml of cushion and removed all of the supernatant (except for the last 0.22 ml) and then harvested all of the remaining liquid (including the cushion) and diluted the suspension 2.5x with buffer before the 24 h centrifugation. With the more convenient larger volume of cushion, the method described in Steps 4 and 5 of the protocol should allow easy harvest of the banded virus from a ***k***onical tube without removing more than 0.2 ml of cushion.
8. It is not known how widely applicable any of these methods is to the purification of other members of the *Retroviridae*, but it is highly likely that each may be used for other virus types, even though optimization of either the density range of the gradient and/or the centrifugation conditions may be needed for purity maximization.
9. Sometimes gradients of 0-18% iodixanol have been used for purifying HIV-1 (e.g. refs 13 and 14).
10. One of the practical alternatives, which might be considered, is the use of a continuous gradient rather than a multi-step discontinuous gradient. If this option is used then prepare just 6 and 18% iodixanol.
11. For more information on the preparation of density gradient solutions for viruses see Application Sheet V01.
12. 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 approx 10-15% of the gradient volume.
13. Using the more normal pipette or a syringe, considerable practice is required to be able to form discontinuous gradients of numerous small volume steps, irrespective of whether an overlayering or underlayering technique is used. Since however diffusion of iodixanol will occur during the centrifugation, it may be easier to make a continuous gradient from 6 and 18% iodixanol. For more information see Application Sheet V02.
14. If it is necessary to concentrate the virus before layering on the gradient make sure that the density of the virus suspension is low enough to permit layering on the gradient. Generally RCFs of either 100,000 *g* [14-16] or 250,000 *g* [5,17,18] have been used, occasionally 183,000 *g* [13] or 200,000 *g* [19]. Centrifugation times are usually 1.5 h [5,13,14,17-21], sometimes 3.0 h [15,16].
15. A vertical or near-vertical rotor would actually improve the resolution further because of the large surface area of the liquid in the tube during the centrifugation and the short path length would mean a much shorter centrifugation time.
16. Collection of the gradient by tube puncture may be a useful alternative. For more information on harvesting gradients see Application Sheet V04.
17. This was first carried out in a 39 ml tube vertical rotor (Beckman VTi50) at 240,000 *g* for 6 h [9], later in the 5 ml tube near-vertical NVT100 at 420,000 *g* for 3.5 h [11].
18. The method is a high resolution one – small shifts in density of V3 loop mutants compared to wild-type have been reported [9] and any released Gag is well separated in denser regions of the gradient [11].
19. Since the virus bands at a relatively low density omission of the 55% solution may be permissible. A 50% iodixanol solution may be prepared from 10 ml of OptiPrep™, 2.0 ml of water and 120 µl of Solution B. It is also likely that the number of steps might be reduced (10% iodixanol increments); formation of a continuous gradient by diffusion will take about 6 h in a 4 ml tube. Larger volume tubes will require longer diffusion times. Continuous gradients can also be made from equal volumes of the densest and lightest solutions using a two-chamber gradient maker or Gradient Master™. For more information see Application Sheet V02.
20. Shorter times at higher *g*-forces may be permissible but have not been validated.

7. References

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