OptiPrep™ Application Sheet V33

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae*: *Gammaretrovirus*: Moloney murine leukemia virus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **Application Sheet V06** provides a summary of the OptiPrep™ virus purification methodology.
- The **OptiPrep™ Reference List (RV06)** provides a full bibliography of all published papers reporting the use of iodixanol gradients for the purification of **Group VI viruses**; to access return to the initial list of Folders and select "**Reference Lists**".
- This Application Sheet describes the use of continuous sedimentation velocity or buoyant density pre-formed gradients for purification of members of the *Gammaretrovirus* 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 2020Virapp file and select the appropriate** V number.

1. Background

Dettenhoffer and Yu [1] developed a sedimentation velocity iodixanol gradient to purify HIV-1 virions without affecting the infectivity of the virus. Furthermore the iodixanol gradient was shown to provide better resolution from vesicle and macromolecular contaminants than a buoyant density sucrose gradient. 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 certain important aspects of viral function; in particular the loss of surface glycoproteins from retroviruses has been noted [2]. This may be related to its viscosity, which is much higher than iodixanol. This iodixanol gradient strategy has now been extended to the Moloney murine leukemia virus (MLV) by Onafuwa-Nuga et al [3] and Leblanc et al [4].

Prior to loading on to the gradient Onafuwa-Nuga et al [3] pelleted the virions before resuspension in DMEM containing 10% calf serum. High-speed pelleting and resuspension of the pellet by shearing forces, although effective and simple, can however lead to a significant loss of infectivity. Better recovery of viral infectivity can be obtained by sedimentation on to a dense iodixanol cushion. A small volume of dense cushion [5,6] can be used for banding retroviruses (50,000 *g* for 1.5-3 h) from a very large volume of virus-containing fluid. However, the top of the sedimentation-velocity gradient used for purifying the virus has a low density (6% iodixanol), so when recovering the virus band from the dense cushion it is necessary to ensure that the iodixanol concentration in the virus suspension is <5%. Coleman et al [6] overcame this problem by diluting the harvest with approx 4 vol. of buffer (total volume approx 4 ml); this permits the virus to be efficiently pelleted by very gentle centrifugation for 24 h [6]. Low-speed centrifugation of the original large volume of culture fluid, on the other hand, would lead to a very poor recovery.

The virus has also been purified on the basis of its buoyant density using OptiPrep™ [7].

The strategies of high-density cushion concentration (Section 2) and purification in a sedimentation velocity gradient (Section 3) and buoyant density gradient (Section 4) will be described in this Application Sheet.

2. Virus concentration (adapted from refs 5 and 6)

♦ The notes referred to in the following methods can be found in Section 5

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 1**)

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 Notes 2 and 3**).

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

4. Carefully aspirate all but 2-3 ml of the supernatant; in *k*onical tubes this can be reduced to 1-2 ml (**see Note 6**).

5. Collect the banded virus in the residual supernatant, removing as little as possible of the cushion.

6. If the density of the virus suspension is too high to be loaded on the 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. Purification in sedimentation velocity gradients (adapted from ref 3)

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 8 and 9**)

3b. Rotor requirements

Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti or Sorvall TH641; **see Note 10**)

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 11**).

2. Layer the virus suspension (< 2.0 ml) on top of the gradient and centrifuge at 100,000 g_{av} for 1 h at 4°C (**see Notes 12 and 13**). Use slow acceleration and deceleration programs if they are available or turn off the brake during deceleration from 3000 rpm.

3. Collect the gradient by upward displacement, low-density end first in approx 0.8-1.0 ml fractions (**see Note 14**). The virus bands just below the middle of the gradient.

4. Purification in buoyant density gradients (adapted from ref 7)

4a. Solutions required

A. OptiPrep™

B. Phosphate-buffered saline

C. Gradient solutions: dilute OptiPrep™ with Solution B to give solutions of 10% and 30% (w/v) iodixanol (20 ml of each).

4b. Rotor requirements

Swinging-bucket rotor with 38-39 ml tubes, e.g. Beckman SW28 (**see Note 15**)

4c. Protocol

1. Using a two-chamber gradient maker or a Gradient Master™ prepare a gradient in tubes for the swinging-bucket rotor using 17 ml each of the 10% and 30% iodixanol solutions (**see Note 15**). For more information on making continuous gradients **see Application Sheet V02**.

2. Layer 3-4 ml of the concentrated virus on top of the gradient and centrifuge at 100,000 *g* for 4 h; use a slow-deceleration program or turn off the brake during deceleration from 3000 rpm (**see Note 15**).

3. Collect the gradient by tube puncture or aspiration from the meniscus in approx 5.0 ml fractions (**see Note 15**). The virus bands in the middle third of the gradient.

5. Notes

1. The best rotors for concentrating virus are certainly 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.
2. The alternative to banding is simply to pellet the virus at 50,000 *g* for 1.5 h.
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 [6] 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 [6] 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 step 4 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. One of the practical alternatives, which might be considered, is the use of a continuous gradient rather than a multi-step discontinuous gradient (see Note 5). If this option is used then prepare just 6 and 18% iodixanol.
9. For more information on the preparation of density gradient solutions for viruses **see Application Sheet V01**.
10. 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 15% of the gradient volume.
11. 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. The recommended use of a pump may facilitate the process. Since however diffusion of iodixanol will occur during the centrifugation, it may be even easier to make a continuous gradient from equal volumes of the densest and lightest solutions. **For more information see Application Sheet V02**.
12. 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. **For more information see Application Sheet V06**.

13. More recently Eckwahl et al [7] used a gradient of 8-22% (w/v) iodixanol centrifuged at 88,000 *g* for 1 h: the virus banded very sharply at approx. 17-18% iodixanol.
14. Collection of the gradient by tube puncture may be a useful alternative. **For more information on harvesting gradients see Application Sheet V04**.
15. Smaller volume rotors (e.g. Beckman SW41Ti, tube volume approx. 13 ml) can almost certainly be used; scale down the volumes of gradient solutions and sample proportionately.
16. Ref 8 reports the collection of just three fractions of 12.5 ml, 10 ml and 14.5 ml (high-density end first) – the virus banding in the middle fraction.

6. References

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