OptiPrep™ Application Sheet V29

Purification and analysis of Group VI (ss)RNA-RT viruses: *Retroviridae*: *Alpharetrovirus*: Rous sarcoma virus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review (MV04)** "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 buoyant density pre-formed gradient for purification of Rous sarcoma virus, which belongs to the *Alpharetrovirus* genus of retroviruses.
- 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 more convenient than the use of either CsCl or sucrose.

In the protocol described in this OptiPrep™ Application Sheet, RSV is banded according to its buoyant density and it is adapted from ref 2. The pre-gradient strategy is adapted from ref 3.

2. Solutions required (see Note 1)

A. OptiPrep™

B. OptiPrep™ diluent:, 300 mM NaCl, 6 mM EDTA, 60 mM HEPES-NaOH (pH 7.5)

C. Working Solution (50%, w/v iodixanol): Mix 5 vol. of Solution A with 1 vol. of Solution B (see Note 1)

D. Working solution diluent: 50 mM NaCl, 1 mM EDTA, 10 mM HEPES-NaOH (pH 7.5)

Prepare 100 ml of each of the following stock solutions and keep at 4°C:

500 mM HEPES (free acid)	11.9 g
1 M NaCl	5.84 g
100 mM EDTA (Na_2•$2H_2O$)	3.72 g

Solution B: To 30 ml of water; add 12 ml, 30 ml and 6 ml respectively of the HEPES, NaCl and EDTA stock solutions; adjust to pH 7.5 with NaOH and make up to 100 ml.

Solution D: To 50 ml of water; add 2 ml, 5 ml and 1 ml respectively of the HEPES, NaCl and EDTA stock solutions; adjust to pH 7.5 with NaOH and make up to 100 ml

3. Ultracentrifuge 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 13-14 ml tubes (e.g. Beckman SW41Ti or Sorvall TH641) (**see Note 2**).

4. Protocol

1. Once the virus has been released from the cells clarify the suspension by low speed centrifugation (approx. 5000 *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 Notes 3 and 4**).

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

6. During the centrifugation make solutions of 10% and 40% (w/v) iodixanol (mix Solution C and Solution D 1:4 and 4:1 respectively). Then use equal volumes (5-6 ml) of the two iodixanol solutions in a two-chamber gradient maker or a Gradient Master™ to make a continuous gradient in a tube for the 14 ml swinging-bucket rotor (**see Note 5**).

7. Resupend the virus pellet in 1-2 ml of 5% (w/v) iodixanol (1 vol. of Solution C + 9 vol. of Solution D) and layer on top of the 10-40% iodixanol gradient.

8. Centrifuge at 160,000 g_{av} for 4 h at 4°C.

9. Unload the gradient either by upward displacement, aspiration from the meniscus or by tube puncture in 0.5-1.0 ml fractions. If the RSV forms a visible band (about 2/3rds down the gradient), it can alternatively be recovered using a syringe and metal cannula (**see Notes 6 and 7**).

5. Notes

1. The mode of preparing the solutions ensures that the concentrations of NaCl, 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. It might be a routine phosphate buffered saline [4] or a cell culture medium (e.g. DMEM or RPMI) supplemented with any additives as required. For more details on the making up of gradient solutions **see Application Sheet V01**.
2. Larger volume gradients are permissible (e.g. in the Beckman SW28) 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 VTi50 or VTi65.1), the shorter sedimentation path length will permit shorter centrifugation times.
3. Vogt and Simon [3] sedimented the RSV through a 15% 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. In this case, the iodixanol concentration must be <10% (w/v). The use of Beckman "*k*onical" tubes overcomes this problem to some extent. Moreover, as long as the purification is based on buoyant density and sedimentation velocity then the sample volume is not really important. For buoyant density banding the sample may alternatively be layered beneath the gradient, in which case the contamination from the cushion is irrelevant. **For more information on concentration of virus prior to gradient purification see Application Sheet V06.**

5. A continuous gradient can alternatively be constructed by allowing a discontinuous gradient (10%, 20%, 30% and 40% iodixanol layers) to diffuse. **For more information on making gradients see Application Sheet V02.**
6. **For more information on harvesting gradients see Application Sheet V04**.
7. The median density of wild-type RSV is approx 1.14 g/ml [2]. The banding density of a capsid (CA)-deleted Gag mutant was 1.16 g/ml, while that of a matrix (MA)-deleted mutant was considerably lower – 1.09 g/ml [2], indicating that the MA domain is required for proper HSV assembly while the major homology region (included in CA) is not. Iodixanol gradients were also used to purify Rous sarcoma virus in a study of monoclonal antibody binding to the receptor-binding site [4].

6. References

1. Palker, T.J. (1990) *Mapping of epitopes on human T-cell leukemia virus type 1 envelope glycoprotein* In: Human Retrovirology: HTLV (ed. Blattner, W.A.) Raven Press, NY, pp 435-445
2. Lee, E-G., Yeo, A., Kraemer, B., Wickens. M. and Linial, M. (1999) *The Gag domains required for avian retroviral RNA encapsidation determined by using two independent assays* J. Virol.**, 73**, 6282-6292
3. Vogt, V.M. and Simon, M.N. (1999) *Mass determination of Rous sarcoma virus virions by scanning transmission electron microscopy* J. Virol., **73**, 7050-7055
4. Ochsenbauer-Jambor, C., Delos, S. E., Accavitti, M. A., White, J. M. and Hunter, E. (2002) *Novel monoclonal antibody directed at the receptor binding site on the avian sarcoma and leukosis virus env complex* J. Virol.**, 76**, 7518-7527

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