OptiPrep™ Application Sheet V11

Purification of Group I (ds)DNA viruses: *Polyomaviridae*

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- 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 that of 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 much more convenient than the use of either CsCl or sucrose.

The protocol described in this OptiPrep™ Application Sheet (Section 2) was first developed in 2004 for papillomavirus vector purification [2-4]; in 2009 however, Pastrana et al [5] applied the methodology to the purification of the polyomavirus associated with Merkel cell carcinoma. Although there is an earlier report by Nakanishi et al [6] in which the 39-33-27% (w/v) iodixanol gradient was used for other types of polyomavirus (SV-40, JC virus, BK virus and B-lymphotropic papovavirus). The iodixanol solutions are prepared in PBS supplemented with additional NaCl, KCl and divalent cations. Section 2 describes the principal steps involved in the gradient procedure.

- Section 3 summarizes other gradient strategies and includes a few details from many of the more recent published papers.
- Section 4 contains some brief comments regarding the purification of SV40
- Section 5 is the reference list

2. Three-step discontinuous gradient method

This method has been adapted from refs 4, 5 and 7; other gradients are described in Section 3.

2a. Solutions required (see Note 1 in Section 2e)

A. OptiPrep™

B. 10xPBS

C. OptiPrep™ diluent: 3.125 M NaCl, 4.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 10.5 mM KCl

D. 39% (w/v) Iodixanol Working Solution (WS): mix 3.9 vol. Solution A, 0.6 vol. of Solution B, 1.2 vol. of Solution C and 0.3 vol. of water (see Note 2)

E. WS Diluent: mix 0.6 vol. of Solution B, 1.2 vol. of Solution C and 4.2 vol. of water

Keep the following stock solutions at 4°C:
5 M NaCl: 292 g per 1000 ml of water
2 M $MgCl_2 \bullet 6H_2O$: 40.6 g per 100 ml of water

Solution C: Dissolve 0.13 g $CaCl_2 \bullet 2H_2O$ and 0.16 g KCl in 50 ml water, add 125 ml and 0.25 ml respectively of the NaCl and $MgCl_2$ stock solutions and make up to 200 ml.

2b. Pre-gradient protocols

This Application Sheet is concerned primarily with the density gradient purification of polyomavirus. The pre-gradient protocols may vary from laboratory to laboratory and are outside the scope of this text. It is worth noting however that Pastrana et al [5] considered that most polyoma viral

capsids are too fragile to allow processing immediately after release from the host cells by a detergent lysis buffer. These workers therefore devised a maturation regimen in which the cells, suspended in PBS (supplemented with 9.5 mM $MgCl_2$), containing 0.4% Triton X100, a 0.1% RNase A/T1 cocktail (and an antibiotic), are incubated at 37°C overnight. For more details of the maturation protocol see ref 5. Nakanishi et al [6] used a medium containing 10 mM Tris–HCl pH 7.5, 2 mM $MgCl_2$, 0.25% Brij 58 and 5 U/ml of Benzonase incubated for only 30 min at 37 °C.

These pre-gradient protocols [2-4] were originally developed for human papillomavirus and also used Brij 58 as the detergent. However Buck and Thomson [7] now consider that Triton X-100 is the detergent of choice. Although the cell lysis capacity of Triton X-100 and Brij 58 are similar, solutions of Triton X-100 are considerably more stable than those of Brij 58, which have to be re-made every few weeks. Moreover Buck and Thomson [7] noted that Triton X-100 is superior to Brij 58 for polyoma vectors.

Sunyaev et al [8] released JC virus from the cells using a mechanical homogenization device.

2c. Rotor requirements

Swinging-bucket rotor with approx 5 ml tubes (e.g. Beckman SW50.1 or SW55Ti or Sorvall AH650) for 5 ml gradients (**see Note 3**)

2d. Protocol

1. Dilute Solution D with Solution E to produce a 27% (w/v) iodixanol solution and in tubes for the 5 ml swinging-bucket rotor prepare 4.2 ml continuous gradients from equal volumes of Solution D and the 27% iodixanol solution using a two-chamber gradient maker or a Gradient Master™ (**see Note 4**). For more information about the preparation of continuous gradients **see Application Sheet V02**.

2. **OR** make up two iodixanol solutions of 27% and 33% (w/v) and prepare, by underlayering, a discontinuous gradient from 1.4 ml each of these two solutions and Solution D. Allow the gradients to diffuse at room temperature for 3-4 h (see Note 4). For more information on the preparation of continuous from discontinuous gradients **see Application Sheet V02**.

3. Bring the cell lysate to 4°C; adjust the salt concentration to 0.85 M by addition of 0.17 vol. of 5 M NaCl and incubate for 10-20 min (**see Note 5**).

4. Clarify the suspension by centrifugation at 5000 *g* for 10 min in a microfuge (**see Note 6**).

5. Aspirate and keep the virus-containing supernatant.

6. Resuspend the pellet in approx 0.25 ml of Solution E and repeat step 4.

7. Aspirate the supernatant; combine with the first supernatant and repeat step 4 (**see Note 7**).

8. Layer the clarified virus suspension on top of the continuous iodixanol gradients and centrifuge at 234,000 *g* for 3.5 h at 16°C. Use a slow acceleration and deceleration program up to and down from 2000 rpm. If such a facility is not available, turn off the brake during deceleration below 2000 rpm (**see Note 8**).

9. Collect the gradient by tube puncture or, if the band is sufficiently well defined, retrieve the banded virus (about half to two thirds of the way down the tube) using a syringe (**see Note 9**).

2e. Notes

1. The mode of preparing the solutions ensures that the concentrations of buffer and ions are constant throughout the gradient. Any suitable buffer can be used for suspending the virus and for making

the gradient solutions and its composition may vary from laboratory to laboratory. 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 for example be a cell culture medium (e.g. DMEM or RPMI) rather than a balanced salt solution. **Application Sheet V01 gives more details on the making up of gradient solutions**.

2. In the original papillomavirus method [2-4], a 46% (w/v) iodixanol solution was used as the stock solution from which three gradient solutions of 39%, 33% and 27% (w/v) iodixanol were prepared. In this adaptation the 39% (w/v) stock solution is used as densest gradient solution and the source of the lower density solutions.
3. Larger volume tubes are permissible (e.g. in the Beckman SW41) but the time will need increasing to compensate for the longer sedimentation path and lower RCF (see Note 7) 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.
4. For larger rotor tubes scale up all volumes proportionately.
5. The volumes used for the cell lysis should be small enough to allow for loading on to the density gradients. Buck et al [2,4] used 0.65 ml per 10^8 cells in their original papillomavirus methodology. Although the efficacy of the density gradient is considered to be due, at least partly, to sedimentation velocity, the volume of sample can be as much as 2/3 of the gradients volume. This may reflect a very rapid sedimentation of the viral particles to the gradient interface.
6. Higher and lower centrifugation speeds have been used for the clarification step, e.g. 8,000 *g* [8] and 1,500 *g* [9] for 15 min. In the latter example filtration through a
7. If the volume of virus suspension is too large, sediment it on to a small cushion (approx. 0.5 ml) of Solution D; a conical-bottomed Beckman *k*onical™ tube is ideal for this. When aspirating the banded virus make sure that the final iodixanol concentration is no more than approx. 15% (w/v), to facilitate layering on to the gradient. For more information on concentrating virus suspensions see Application Sheet V24. Hamilton et al [9] centrifuged the clarified supernatant at 105,000 *g* for 90 min to concentrate JC and BK virus as a pellet before resuspending it in buffer. Sunyaev et al [8] pelleted JC virus through a 40% sucrose cushion at 100,000 *g* for 5 h, followed by treatment with 0.25% deoxycholate.
8. Larger volume rotors can be used at lower RCFs for longer times, e.g. the SW41 at 200,000 *g* for 4.75 h [4].
9. **For more information on harvesting gradients see Application Sheet V04**.

3. Comments and recently published papers

Sunyaev et al [8] also used a discontinuous gradient, covering a similar density range (25-40% iodixanol), in which the viral particles were loaded in one of the median layers, centrifuged at 180,000 *g* for 17 h in a fixed-angle rotor (Beckman 50.2Ti rotor). Median sample loading has been used for high resolution of certain cell organelles, but it has been used relatively rarely for viruses. Median loading allows lighter and denser particles to move in opposite directions during the centrifugation, thus minimizing aggregation of different particles. A simple two layer gradient (26% and 32% w/v iodixanol) centrifuged at 165,000 *g* for 4 h was used to band Merkel carcinoma polyomavirus at the interface between the two layers (the virus was released from cells by freeze-thawing) [9]. Hamilton et al [10], like Sunyaev et al [8], used a much longer centrifugation time (160,000 *g* for 16 h) with a 15-36% (w/v) continuous iodixanol.

Using the purification methodology described in this Application Sheet Schowalter et al [11] described the identification of two previously unknown skin-tropic polyoma viruses and Feng et al [12] reported the banding of fully-encapsidated Merkel cell carcinoma virions at a density of 1.24 g/ml, while those of JC virus banded at approx. 1.20 g/ml. The values were significantly lower than those observed in CsCl and sucrose gradients because of the much lower osmolality of iodixanol gradients. The authors emphasized the superiority of iodixanol gradients over those of CsCl or sucrose for studies on these viruses.

- Other published papers reporting the use of similar OptiPrep™-based methods for polyomavirus, pseudovirions and virus-like particles are given in refs 13-31.

- Notably the method isolated encapsidated virions from the plasma of transplant patients [32].
- Polyoma-like particles have been isolated from *Nicotiana benthamiana* leaves in a 20%, 30%, 40%, 50% (w/v) iodixanol gradient at 140,000*g*, for 3 h [33]
- In a recent large study of polyoma contamination of ground beef samples Peretti et al [34] concentrated detergent-soluble capsids on to a 39% (w/v) iodixanol cushion (1.5 ml) by centrifugation at 110,000 *g* for 2 h, then carefully removed all the supernatant except for the bottom 1.5 ml. The viral material now in 3 ml of 19.5% (w/v) iodixanol was then loaded on to the regular 27%-33%-39% (w/v) iodixanol density gradient and centrifuged for 5 h at 234,000*g*. The authors concluded that this strategy was applicable to "any DNA virus with a detergent-soluble capsid that is impermeant to nucleases and capable of migrating down an OptiPrep ultracentrifuge gradient".
- Hurdis et al [35] investigated the structure of the genome and minor capsid proteins using cryo-electron microscopy
- Liu et al [36] studied the mechanisms of Merkel Cell polyoma infection.
- Nguyen et al [37] investigated the association of polyomavirus with pruritic and dyskeratotic dermatoses.

4. Summary of methods for purification of Simian Virus 40 (SV40)

Nakanishi et al [6] used an identical gradient to that described in Section 2, using a slightly lower *g*- force of 190,000 *g*. In the most frequently used method however [38] the cells were first lysed in a buffered saline containing 0.5% Brij58, which was then clarified at 16,000 *g* for 10 min. The supernatant was loaded on to a 20%, 40% discontinuous iodixanol gradient and centrifuged at approx. 240,000 *g* for 2 g. The virus banded at the interface. The same gradient has been reported in several published papers [39-43], or 20% and 50% [44, 45] or occasionally 20% and 55% (w/v) iodixanol [46, 47]. The centrifugation conditions have generally been 220-235,000 g for 2 h, although in refs 46 and 47 the *g*-force was lower – 160,000 *g*).

- Other OptiPrep™ Application Sheets that may be useful are:
- Concentration of virus particles: see **Application Sheet V06**
- Harvesting gradients: see **Application Sheet V04**
- Analysis of gradients: see **Application Sheet V05**

5. References

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