OptiPrep™ Application Sheet V10

Purification of Group I (ds)DNA viruses: human papillomavirus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **The OptiPrep™ Mini-Review (MV05) "Purification and analysis of papillomaviruses"** provides a full bibliography of all published papers reporting the use of iodixanol gradients for the purification of papillomavirus; to access return to the initial list of Folders and select "**Mini-Reviews**".
- Human papillomavirus is a small non-enveloped DNA virus and the method described in this OptiPrep™ Application Sheet may be applicable to other similar papillomaviruses.
- 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 for papillomavirus vector purification, which is adapted from refs 2-4, has also been used for the purification of pseudovirus carrying a secreted alkaline phosphatase (SEAP) reporter gene [5]. The iodixanol solutions are prepared in PBS supplemented with additional NaCl, KCl and divalent cations. Note that ref. 4 contains an excellent detailed account of all the methodology associated with culture and transfection of 293TT cells, harvesting and maturation of virus, virus purification and assay.

2. Solutions required (see Note 1, in Section 6)

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.

3. Pre-gradient protocols

This Application Sheet is concerned primarily with the density gradient purification of papillomavirus, but it is important to point out that there are a number of pre-gradient protocols that contribute significantly to the success of the purification procedure. These may vary from laboratory to laboratory (**see Note 3**) and their detailed consideration is outside the scope of this text. It is worth noting however that Buck et al [4] considered that most of the viral capsids were too fragile to allow processing immediately after release from the host cells by a detergent lysis buffer and consequently

devised a maturation regimen in which the cells, suspended in PBS (supplemented with 9.5 mM $MgCl_2$), containing 0.35% Brij 58, 0.1% Benzonase and 0.1% Plasmid Safe, are incubated at 37°C for at least 16 h. After this, the lysate is clarified by salt extraction prior to density gradient centrifugation. For more details of the maturation protocol see refs 3 and 4. However Buck and Thomson [6] now consider that Triton X-100 is the detergent of choice. This detergent is used at a concentration of 0.4%. 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. See ref 6 for more details.

4. 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 4**)

5. 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 5**). 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 Notes 5 and 6**). 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 7**).

4. Clarify the suspension by centrifugation at 5000 *g* for 10 min in a microfuge (**see Note 8**).

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 9**).

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 10**).

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 Notes 11 and 12**).

6. 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 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. Cell lysis may also be achieved by freeze-thawing: Pejawar-Gaddy et al [7] for example used this technique with the cells suspended in 150 mM NaCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.0; clarified the suspension at 8000 *g* for 20 min and delipidated the supernatant before pelleting the virus particles through a 40% sucrose cushion.
4. 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.
5. For larger rotor tubes scale up all volumes proportionately.
6. Sometimes the sample is layered on a discontinuous gradient, without prior diffusion [7,8]. There are also small variations in the concentrations of iodixanol used for the discontinuous iodixanol gradient; for example 26%, 32% and 38% iodixanol [7]. Pejawar-Gaddy et al [7] reported that the capsomeres banded at the 26%/32% interface and the capsids at 32%/38% interface.
7. 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. 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.
8. Higher centrifugation speeds have been used for the clarification step, e.g. 16,300 *g* [9].
9. 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 V06**.
10. There is some variation in the *g*-force used for the gradient separation, occasionally higher values are used, e.g. 300,000 *g* [7]. 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].
11. For more information on harvesting gradients **see Application Sheet V04**.
12. In spite of the toxicity of CsCl gradients (see Section 1) there are many examples in the published literature of purification schedules that use sequential iodixanol and CsCl gradients.

7. References

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