OptiPrep™ Application Sheet V13

Purification of Group I (ds)DNA viruses (non-mammalian sources): *Iridoviridae*

- 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.
- Note that the purification of viruses that grow in other non-mammalian cells (e.g. algae, protozoa, marine arthropods, plant cells, etc) is summarized in **Application Sheet V37**).

1. Background

This Application Sheet describes the purification of a Group I (ds)DNA iridovirus from the Singapore grouper.

In all comparative studies between CsCl and iodixanol, it has been shown that 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 enveloped viruses has been noted [2]. This may be related to its viscosity, which is much higher than that of iodixanol. Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast, many add-on techniques can be performed and cells infected with virus, without dialysis of iodixanol.

2. Iridovirus purification

The following protocol is adapted from ref 1. The virus was grown in grouper embryonic cells and the cells were lysed in a buffered NaCl solution, but the gradient solutions were prepared by dilution of OptiPrep™ with isoosmotic buffered sucrose.

Keep the following stock solutions at 4°C:

1 M Tris (free base),	12.1 g per 100 ml
100 mM $EDTANa_2 \bullet 2H_2O$	3.72 g per 100 ml
1 M NaCl	5.84 g per 100 ml

Solution B: To 50 ml water, add 5.0 ml each of Tris and EDTA, and 10 ml of NaCl stock solutions; adjust to pH 7.4 with 1 M HCl; make up to 100 ml

Solution C: Dissolve 7.4 g sucrose in 50 ml of water; add 4 ml each of the Tris and EDTA stock solutions; adjust to pH 7.4 with 1 M HCl; make up to 100 ml

Solution D: make up as Solution C using 2 ml each of the Tris and EDTA solutions

2a. Solutions required

A. OptiPrep™
B. Cell lysis buffer: 0.1 M NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4
C. OptiPrep™ dilution buffer: 7.4% (w/v) sucrose, 4 mM EDTA, 40 mM Tris-HCl, pH 7.4 (see Section 2d Note 1)
D. Dilution buffer for gradient stock solution: 7.4% (w/v) sucrose, 2 mM EDTA, 20 mM Tris-HCl, pH 7.4

2b. Ultracentrifuge rotor requirements

Swinging-bucket rotor (e.g. Beckman SW28 or SW41Ti) and a vertical or near-vertical rotor (e.g. Beckman Vti65.1 or NVT65) – see Section 2d Note 2.

2c. Protocol

1. Homogenize the cells using Dounce homogenizer or similar device to release the virus and clarify and centrifuge at 1500 *g* for 20 min to remove cellular debris. The supernatant may be clarified by passage through a 0.22 μm filter.

2. Dilute the OptiPrep™ with an equal volume of Solution C to produce an isoosmotic 30% (w/v) iodixanol solution containing 2 mM EDTA and 20 mM Tris-HCl, pH 7.4; then diluted this stock solution with Solution D to produce 5%, 10% and 20% (w/v) iodixanol solutions.

3. In the chosen swinging-bucket rotor layer equal volumes of the, 5%, 10%, 20% and 30% (w/v) iodixanol and layer the crude virus suspension on top. See **Application Sheet V02** for more information about making these gradients.

4. Centrifuge at 55,000 *g* for 1 h. The virus should band at the 20%/30% iodixanol interface.

5. Remove the solutions from above the virus band, then aspirate the virus using a syringe and metal cannula, taking as little of the 30% (w/v) solution as possible (see **Application Sheet V04** for information about harvesting material from gradients)

6. Dilute the virus suspension with 25% (w/v) iodixanol (gradient stock diluted with Solution D) and transfer to tubes for the chosen vertical or near-vertical rotor and centrifuge at approx 350,000 *g* for 2-3 h (see Section 2d Note 2).

7. Collect the visible band of virus.

2d. Notes

1. The production of a stock solution of 30% (w/v) iodixanol that contains 2mM EDTA and 20 mM Tris-HCl, pH 7.4 in order to make the gradient solutions ensures not only that all the gradient solutions are approx. isoosmotic, but also they all contain 2 mM EDTA and 20 mM Tris. If this is regarded as unnecessary then simply dilute the OptiPrep™ with Solution D. For more information on the preparation of density gradient solutions see **Application Sheet V01**.
2. Wu et al [2] used a Beckman SW41 rotor for creating a self-generated gradient and centrifuged the tubes at 87,000 *g* overnight. Most commonly (see **Application Sheet V03**) a vertical or near-vertical rotor is used at a much higher *g*-force for much shorter time (as recommended). The type of gradient that forms at 87,000 *g* overnight in a swinging-bucket rotor has not been investigated. The operator must choose between the two alternatives.

3. References

1. Wu, J., Chan, R., Wenk, M.R. and Hew, C-L. (2010) *Lipidomic study of intracellular Singapore grouper iridovirus* Virology **399**, 248–256
2. 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 435-445Dettenhoffer, M. and Yu, X-F. (1999) *J. Virol.*, **73**, 1460-1467

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