OptiPrep™ Application Sheet V15

Purification of Group II (ss)DNA viruses: *Protoparvovirus* - murine minute virus

- 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 **2020Virapp** file and select the appropriate **V number.**
- The **OptiPrep™ Reference List (RV02-1), Part B** contains a list of papers describing the use of iodixanol gradients for all **Group II viruses (except rAAV)**; to access return to the initial list of Folders and select "**Reference Lists**".

1. Background

Density gradient centrifugation has always played an important part in the concentration and purification of virus particles but the gradient media that have been used most prominently, sucrose and CsCl, pose a number of problems. Both media are highly hyperosmotic at the densities used to band viruses (sucrose solutions are also very viscous) and generally have to be removed either by pelleting the virus or by dialysis, prior to further processing or analysis. CsCl also leads to poor recoveries and low infectivity of virus isolates.

Because of the very low water activity of CsCl solutions, viruses tend to have significantly higher density in this medium compared to media such as sucrose or any of the iodinated density gradient media, although the magnitude of this difference varies from virus to virus. Many viruses in CsCl have a density of approx 1.34 g/ml, in iodixanol the density range is generally 1.16-1.22 g/ml, although some viruses may be as low as 1.14 g/ml or as high as 1.26 g/ml. MMV is one of the denser ones banding at approx 1.26 g/ml.

In studies where the iodixanol and CsCl gradients have been compared:

- Recovery of virus from the gradient is at least ten times greater
- Particle:infectivity titer is up to 100x lower
- Infectivity measurements and many add-on techniques can be carried out without the need to dialyze the medium.

2. Protocols

In the method devised by Cotmore and Tattershall [1], the virus was released from infected cells by three cycles of freeze-thawing in 50 mM Tris-HCl, 0.5 mM EDTA, pH 8.7. Virus was purified in a discontinuous gradient of iodixanol.

- The gradient is able to discriminate mature virions from viral DNA, which is much lighter – the low density of DNA banding is a characteristic only of iodixanol gradients

2a. Solutions required (see Section 2d Note 1)

A. OptiPrep™
B. 10xPhosphate-buffered saline containing 10 mM $MgCl_2$ and 25 mM KCl, pH 7.2
C. 5 mM EDTA, 500 mM Tris-HCl, pH 7.8
D. 0.5 mM EDTA, 50 mM Tris-HCl, pH 7.8

Keep the following stock solution at 4°C:
1 M Tris (free base): 12.1 g per 100 ml water
100 mM EDTA (Na_2•$2H_2O$): 3.72 g per 100 ml water

To prepare Solution B: add the following to 100 ml of 10xPBS, pH 7.2:

$MgCl_2$•$6H_2O$	0.20 g
KCl	0.19 g

To prepare Solution C:
To 50 ml of Tris stock add 5 ml of EDTA stock; adjust to pH 7.8 with 2 M HCl and make up to 100 ml with water

2b. Ultracentrifuge rotor requirements

Swinging-bucket rotor with approx. 13 ml tubes, such as Beckman SW41Ti

2c. Protocol

1. Prepare the following iodixanol gradient solutions in EDTA-Tris: 15% (w/v) iodixanol, mix 15 vol. OptiPrep™ with 6 vol. of Solution C and 39 vol. of water; 35% (w/v) iodixanol, mix 35 vol. OptiPrep™ with 6 vol. of Solution C and 19 vol. of water (**see Section 2d Note 2**).

2. Prepare the following iodixanol gradient solutions in PBS-Mg-K: 45% (w/v) iodixanol, mix 45 vol. OptiPrep™ with 5 vol. of Solution B and 10 vol. of water; 55% (w/v) iodixanol, mix 55 vol. OptiPrep™ with 5 vol. of Solution B (**see Section 2d Note 2**).

3. Release the virus from the cells in Solution D by three cycles of freeze-thawing.

4. Clarify the suspension by centrifugation at 15,000 *g* for 30 min at 4°C.

5. Prepare discontinuous gradients from 1 ml of 55%, 2 ml of 45%, 2 ml of 35% and 1.5 ml of 15% iodixanol (**see Section 2d Notes 3 and 4**). For more information on the construction of discontinuous gradients **see Application Sheet V02**.

6. Layer approx. 6 ml of the clarified virus suspension on top of the gradient, to fill the tube according to the manufacturer's specifications.

7. Centrifuge at approx. 150,000 g_{av} for 18 h at 18°C. Allow the rotor to decelerate to zero using a controlled deceleration program, or turn off the brake below 2000 rpm.

8. Harvest the gradient in 0.5-1 ml fractions dense-end first. For more information about harvesting gradients **see Application Sheet V04**.

2d. Notes

1. In the original methodology [1], all of the gradient solutions were prepared in PBS. Later [2] improved resolution was obtained by making up the denser two solutions in PBS, 1 mM $MgCl_2$, 2 mM KCl, pH 7.2 and the lighter two solutions in 50 mM Tris-HCl (pH 8.7), 0.5 mM EDTA. This strategy was also reported in subsequent publications [3-11]. Farr et al [12] used two types of gradient, one at pH 7.5 in which gradient solutions contained PBS, 1 mM $MgCl_2$, 2 mM KCl, pH 7.2 pH 7.5 or 50 mM MES (pH 5.5) 120 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl. The authors compared the tryptic digestion of virions at pH 5.5 and 7.5. Wild-type virions after trypsin treatment banded close the 45%/55% iodixanol boundary at both pHs. However a threonine-substituted variant shifted the banding in the pH 7.5 gradient to a lower density. Plevka et al [13] also studied similar gradients (**see Note 3**).
2. Smaller volume modified gradients of 0.5 ml 55% iodixanol and 1 ml each of 45%, 35%, 25% iodixanol and 0.5 ml of 15% iodixanol in 5 ml tubes (e.g. Beckman SW50.1 or SW55Ti) are centrifuged at 140,000 g_{av} for 20 h.
3. Cotmore and Tattershall [14] used similar iodixanol gradients of 55% (0.75 ml), 45% (1.5 ml), 35% (1 ml) and 15% (0.75 ml) in PBS containing 5 mM KCl and 1 mM $MgCl_2$ (in 5 ml tubes) in an *in vitro* analysis of genome uncoating. The gradients resolve fully infectious virions from less dense empty particles. Studies showed that mutant forms displayed a distinctive shift towards less dense profiles.

3. References

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