

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)

- OptiPrep™
- 10xPhosphate-buffered saline containing 10 mM MgCl₂ and 25 mM KCl, pH 7.2
- 5 mM EDTA, 500 mM Tris-HCl, pH 7.8
- 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 (Na₂•2H₂O): 3.72 g per 100 ml water

To prepare Solution B: add the following to 100 ml of 10xPBS, pH 7.2:
 MgCl₂•6H₂O 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 MgCl₂, 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 MgCl₂, 2 mM KCl, pH 7.2 pH 7.5 or 50 mM MES (pH 5.5) 120 mM NaCl, 1 mM MgCl₂, 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 MgCl₂ (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

1. Cotmore, S.F. and Tattersall, P. (2005) *Encapsidation of minute virus of mice DNA: Aspects of the translocation mechanism revealed by the structure of partially packaged genomes* *Virology*, **336**, 100-112
2. D'Abramo Jr., A.M., Ali, A.A., Wang, F., Cotmore, S.F. and Tattersall, P. (2005) *Host range mutants of minute virus of mice with a single VP2 amino acid change require additional silent mutations that regulate NS2 accumulation* *Virology*, **340**, 143-154
3. Paglino, J., Burnett, E. and Tattershall, P. (2007) *Exploring the contribution of distal P4 promoter elements to the oncospecificity of minute virus of mice* *Virology*, **361**, 174-184

4. Cotmore, S.F., Hafenstein, S. and Tattersall, P. (2010) *Depletion of virion-associated divalent cations induces parvovirus minute virus of mice to eject its genome in a 3'-to-5' direction from an otherwise intact viral particle* J. Virol., **84**, 1945-1956
5. Ruiz, Z., Mihaylov, I.S., Cotmore, S.F. and Tattersall, P. (2011) *Recruitment of DNA replication and damage response proteins to viral replication centers during infection with NS2 mutants of Minute Virus of mice (MVM)* Virology **410**, 375–384
6. Li, L., Cotmore, S.F. and Tattersall, P. (2012) *Maintenance of the flip sequence orientation of the ears in the parvoviral left-end hairpin is a nonessential consequence of the critical asymmetry in the hairpin stem* J. Virol., **86**, 12187-12197
7. Li, L., Cotmore, S.F. and Tattersall, P. (2013) *Parvoviral left-end hairpin ears are essential during infection for establishing a functional intranuclear transcription template and for efficient progeny genome encapsidation* J. Virol., **87**, 10501–10514
8. Halder, S., Cotmore, S., Heimburg-Molinari, J., Smith, D.F., Cummings, R.D., Chen, X., Trollope, A.J., North, S.J., Haslam, S.M., Dell, A., Tattersall, P., McKenna, R. and Agbandje-McKenna, M. (2014) *Profiling of glycan receptors for minute virus of mice in permissive cell lines towards understanding the mechanism of cell recognition* PLoS One, **9**: e86909
9. Rostovsky, I. and Davis, C. (2015) *Induction of an embryonic mouse innate immune response following inoculation in utero with minute virus of mice* J. Virol., **89**, 2182-2191
10. Mihaylov, I.S., Cotmore, S.F. and Tattersall, P. (2014) *Complementation for an essential ancillary non-structural protein function across parvovirus genera* Virology, **468-470**, 226–237
11. Rostovsky, I. and Davis, C. (2015) *Induction of an embryonic mouse innate immune response following inoculation in utero with minute virus of mice* J. Virol., **89**, 2182-2191
12. Farr, G.A., Cotmore, S.F. and Tattersall, P. (2006) *VP2 cleavage and the leucine ring at the base of the fivefold cylinder control pH-dependent externalization of both the VP1 N terminus and the genome of minute virus of mice* J. Virol., **80**, 161-171
13. Plevka, P., Hafenstein, S., Li, L., D'Abramo, A., Cotmore, S.F., Rossmann, M.G. and Tattersall, P. (2011) *Structure of a packaging-defective mutant of minute virus of mice indicates that the genome is packaged via a pore at a 5-Fold axis* J. Virol., **85**, 4822–4827
14. Cotmore, S.F. and Tattersall, P. (2012) *Mutations at the base of the icosahedral five-fold cylinders of minute virus of mice induce 3'-to-5' genome uncoating and critically impair entry functions* J. Virol., **86**, 69-80
15. Lang, S.I., Boelz, S., Stroh-Dege, A.Y., Rommelaere, J., Dinsart, C. and Cornelis, J.J. (2005) *The infectivity and lytic activity of minute virus of mice wild-type and derived vector particles are strikingly different* J. Virol., **79**, 289-298
16. Grekova, S., Zawatzky, R., Hörlein, R., Cziepluch, C., Mincberg, M., Davis, C., Rommelaere, J. and Daeffler, L. (2010) *Activation of an antiviral response in normal but not transformed mouse cells: a new determinant of minute virus of mice oncotropism* J. Virol., **84**, 516-531
17. Grekova, S.P., Raykov, Z., Zawatzky, R., Rommelaere, J. and Koch, U. (2012) *Activation of a glioma-specific immune response by oncolytic parvovirus Minute Virus of Mice infection* Cancer Gene Ther., **19**, 468-475

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