OptiPrep™ Application Sheet V18

Purification of Class IV ((+)ssRNA) viruses: *Caliciviridae* family

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Application Sheet covers the purification of two genera of this family: *Norovirus* (Norwalk virus) and *Lagovirus* (rabbit haemorrhagic disease virus). Whether the methods described in this Application Sheet can be applied to other viruses of the same family can only be determined experimentally
- 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. General background to use of iodixanol

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. Both CsCl and 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.

2. Norwalk virus

2a. Introduction

Using recombinant bacuolvirus-infected insect cells Bertolotti-Ciarlet et al [1] have recovered full-length and mutant Norwalk virus capsid proteins from the cell supernatant. After clarification of the cell supernatant the Norwalk virus-like particles were concentrated by sedimentation and then purified in a self-generated iodixanol gradient.

2b. Solutions required

A. OptiPrep™

B. Diluent: Phosphate-buffered saline (see Section 2e: Note 1)

2c. Ultracentrifuge rotor requirements

For small volume gradients an NVT65.2 (5 ml) near-vertical rotor is recommended. However the appropriate density profile can be generated by almost any vertical or near-vertical rotor with a tube capacity of 3-12 ml and a maximum RCF of approx 350,000g_{av}. The sedimentation path length of the rotor should be no more than approx. 25 mm, thus the Beckman VTi65.1 vertical rotor, NVT65 near-vertical rotor (both 12 ml) or the TLN100 near-vertical rotor (3 ml) are acceptable alternatives (see Note 1). High performance fixed-angle rotors may only be used for the rapid formation of self-generated gradients if the tube volume is relatively small (less than 6 ml).

- For examples of self-generated density gradient profiles with these rotors see **Application Sheet V03**.

The following protocol is adapted from ref 1.

2d. Protocol

1. Harvest the culture medium from the cell monolayer.

2. Clarify the medium by centrifugation at 700 *g* for 15 min.

3. Complete the clarification by centrifugation at 20,000 *g* for 30 min.

4. Pellet the particles at 120,000 *g* for 2 h (see Section 2e: Note 2)

5. Suspend the pellet in approx 2.5 ml of Solution B and mix with OptiPrep™ to produce a final iodixanol concentration of 30% (w/v) (see Section 2e: Note 1)

6. Transfer the suspension to tubes for the NVT65.2 rotor or other suitable vertical or near-vertical rotor and centrifuge at approx. 350,000 g_{av} for 3 h at 4°C (see Section 2e: Note 3).

7. At the end of the centrifugation use either a controlled deceleration programme or turn off the brake below 2000 rpm.

8. Unload the gradient by tube puncture, upward displacement of aspiration from the meniscus in a series of equal volume fractions (15-20 fractions irrespective of the gradient volume) and analyze the fractions as required. The virus-like particles band in the top third of the gradient (see Section 2e: Note 4).

2e. Notes

1. In step 5 of the protocol the suspension containing the virus-like particles is simply mixed with an equal volume of OptiPrep™, which will mean that the buffer concentration will be reduced by 50%. If this is deemed unacceptable then first produce a Working Solution of 54% (w/v) iodixanol by mixing 5.4 vol. of OptiPrep™ with 0.6 vol. of 10xPBS. Other strategies for preparing working solutions are given in **Application Sheet V01**.
2. The ideal way of concentrating the virus is sedimentation on to a dense cushion of iodixanol, rather than pelleting, which can lead to loss of infectivity. The method is very easy to use when the subsequent purification is in a self-generated gradient. In a swinging-bucket rotor underlayer the clarified virus-containing solution with 2-3 ml of OptiPrep™ and centrifuge at 120,000 *g* for 2 h. Remove all the supernatant except for a volume equal to that of the OptiPrep™ cushion and mix well with the latter before transferring to a tube for the near-vertical or vertical rotor. For more information on concentration of virus prior to gradient purification see **Application Sheet V06**.
3. If using larger volumes it may be necessary to increase the centrifugation time.
4. For more information on harvesting gradients see **Application Sheet V04**.

3. Rabbit haemorrhagic disease virus

3a. Introduction

Previous methods for the purification of this virus from rabbit liver homogenates have been very lengthy and recoveries of high titre virus not easy to achieve. This new method, first published by Teixeira et al [2], involving a single discontinuous iodixanol gradient was developed from an earlier method for purifying rAAV by Zolotukhin et al [3]. This methodology is described in **Application Sheet V14**. This protocol is adapted from ref 2.

3b. Solutions required

A. OptiPrep™

B. 10xPhosphate-buffered saline containing 10 mM $MgCl_2$ and 25 mM KCl (10xPBS-MK)

C. Phosphate-buffered saline containing 1 mM $MgCl_2$ and 2.5 mM KCl (PBS-MK)

D. 2 M NaCl in PBS-MK

E. Working solution of 54% (w/v) iodixanol in PBS-MK: mix 9 vol. of OptiPrep with 1 vol. of Solution B.

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

$MgCl_2 \bullet 6H_2O$	0.20 g
KCl	0.19 g

To prepare Solution D:
Add 11.68 g NaCl to 10 ml of Solution B and make up to 100 ml with water.

3c. Ultracentrifuge rotor requirements

The rotor used by the Teixeira et al [2], a Beckman 75Ti fixed angle rotor is no longer commercially available; either a 70Ti or 80Ti fixed-angle rotor is the closest substitute. Using a fixed-angle rotor for a running a density gradient at high *g*-forces may not be the best choice; a vertical or near vertical-rotor is more suited to this task (see Section 3 of **Application Sheet V02** for details). In this case either the Beckman VTi65.1 or the NVT65 would the most obvious substitutes. Although no medium-volume swinging-bucket rotor can achieve the necessary *g*-force, the Beckman SW41 (maximum *g*-force 200,000 g_{av}) run for a proportionately longer time might be a good choice. Alternatively, if the total gradient volume is scaled down, rotors such as the SW55Ti can approach the required *g*-force. All these alternatives would need to be properly evaluated before using in the method described in this Application Sheet.

3d. Protocol

1. Prepare the following gradient solutions (see Section 3e, Notes 1 and 2):
   15% (w/v) iodixanol containing 1 M NaCl in PBS-MK: 1.5 vol. of Solution E + 2.7 vol. of Solution D + 1.2 vol. of Solution C.
   25% (w/v) iodixanol in PBS-MK: 2.5 vol. of Solution E + 2.9 vol. of Solution C
   40% (w/v) iodixanol in PBS-MK: 4.0 vol. of Solution E + 1.4 vol. of Solution C.5% (w/v) iodixanol in PBS-MK:

2. Produce a liver homogenate in PBS using a Dounce homogenizer and centrifuge at 900 *g* for 10 min (see Section 3e Note 3)

3. Centrifuge the post-nuclear supernatant at 4000 *g* for 20 min and then clarify by passing through a 0.2 μm filter (see Section 3e Note 4)

4. Transfer the clarified supernatant (2.5 ml) to tubes for the chosen rotor and underlayer sequentially (using a syringe and metal cannula) with 2.25 ml, 1.5 ml, 1.25 ml and 1.25 ml respectively of the 15%, 25%, 40% and 54% iodixanol solutions (see Section 3e Note 5).

5. Centrifuge at 350,000 g_{av} for 1h 20 min, using slow acceleration to and deceleration from, 2000 rpm (see Section 3e Note 6).

6. The virus bands either within the 40% iodixanol layer or at its lower interface (see Section 3e Note 7).

3e. Notes

1. For smaller or larger volume tubes scale down or up all volumes proportionally.
2. Phenol red (0.01 μg/ml) may be included in the alternate gradient layers to enhance visual identification of the layers. Diffusion of the iodixanol and, during centrifugation at 350,000 *g*, some sedimentation of the iodixanol itself, will make the interfaces less obvious.
3. Teixeira et al [2] froze the material after this step.
4. A 15% (w/v) iodixanol cushion has also been used for concentrating this virus [4].
5. For more information about the construction of discontinuous gradients see **Application Sheet V02**.
6. It may be necessary to increase the centrifugation time proportionally if the rotor cannot achieve 350,000 g_{av}.
7. Teixeira et al [2] concluded that the single short iodixanol gradient centrifugation was the method of choice compared to earlier procedures involving the use of either sucrose or CsCl gradients. It is not only a rapid procedure but also the infectivity and purity of the rabbit haemorrhagic disease virus was superior to other methods. – see also Section 1 for more information on the advantages of using iodixanol. See refs 2 and 5 for more information about the properties of the iodixanol-purified virus.

4. References

1. Bertolotti-Ciarlet, A., White, L.J., Chen, R., Prasad, B.V.V. and Estes, M.K. (2002) *Structural requirements for the assembly of Norwalk virus-like particles* J. Virol., **76**, 4044-4055
2. Teixeira, L., Marques, R.M., Aguas, A.P. and Ferreira, P.G. (2011) *A simple and rapid method for isolation of caliciviruses from liver of infected rabbits* Res. Vet. Sci., **91**, 164–166
3. Zolotukhin, S., Byrne, B.J., Mason, E., Zolotukhin, I., Potter, M., Chesnut, K., Summerford, C., Samulski, R.J. and Muzyczka, N. (1999) *Recombinant adeno-associated virus purification using novel methods improves infectious titer and yield* Gene Ther., **6,** 973-985
4. Crisci, E., Fraile, L., Moreno, N., Blanco, E., Cabezónd, R., Costa, C., Mussá, T., Baratelli, M., Martinez-Orellana, P., Ganges, L., Martínez, J., Bárcenac, J. and Montoya, M. (2012) *Chimeric calicivirus-like particles elicit specific immune responses in pigs* Vaccine **30**, 2427–2439
5. Teixeira, L., Marques, R.M., Águas, A.P. and Ferreira, P.G. (2012) *Regulatory T cells are decreased in acute RHDV lethal infection of adult rabbits* Vet. Immunol. Immunopathol., **148**, 343– 347

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