

OptiPrep™ Application Sheet V22

Purification of Group IV ((+)ss) RNA viruses: *Togaviridae*: *Alphavirus* and *Rubivirus*

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ Whether any of the methods described in this Application Sheet can be applied to any *Togaviridae* alphavirus with similar morphology, macromolecular composition and size, other than those described, 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. Introduction

Regarding *Togaviridae* alphaviruses, the majority of publications reporting the use of iodixanol gradients are concerned with Semliki Forest virus, for which detailed protocols are given in Section 2. Other *Togaviridae* alphaviruses have also been prepared and analyzed in iodixanol gradients and subsequent sections briefly present methodologies for Sindbis virus (Section 3), Venezuelan equine encephalitis virus (Section 4), Chikungunya virus (Section 5) and Rubella (Section 6)

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 retroviruses has been noted [1]. 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. Semliki forest virus

The protocol below is adapted from refs 2 and 3 (see also Section 2e).

2a. Solutions required

- A. OptiPrep™
- B. OptiPrep™ diluent: 3.0 mM EDTA, 0.3 M Tris-HCl, pH 7.4
- C. Suspension medium: 0.1 M NaCl, 0.5 mM EDTA, 50 mM Tris-HCl, pH 7.4
- D. Iodixanol working solution: 50% iodixanol, 0.5 mM EDTA, 50 mM Tris-HCl, pH 7.4; mix 5 vol. of OptiPrep™ with 1 vol. of Solution B (see Note 1)

Keep the following stock solutions at 4°C:

1 M Tris (free base),	12.1 g per 100 ml
100 mM EDTANa ₂ •2H ₂ O	3.72 g per 100 ml
1 M NaCl	5.84 g per 100 ml

Solution B: To 50 ml water add 30 ml and 3.0 ml respectively of Tris and EDTA stock solutions; adjust to pH 7.4 with HCl; make up to 100 ml.

Solution C: To 50 ml water, add 10 ml, 5.0 ml and 0.5 ml respectively each of NaCl, Tris and EDTA stock solutions; adjust to pH 7.4 with 1 M HCl; make up to 100 ml.

2b. Ultracentrifuge rotor requirements

Swinging-bucket rotor with 13-14 ml tubes (e.g. Beckman SW41Ti or Sorvall TH641).

2c. Protocol

1. Produce solutions of 5% and 30% (w/v) iodixanol by diluting Solution D with Solution C (see Notes 2 and 3).
2. Harvest the virus-containing supernatant from the cells.
3. Clarify the suspension by centrifugation at 1500 g for 20 min.

4. Concentrate the virus suspension by pelleting it through a 5% iodixanol barrier at 160,000 g_{av} for 1 h (see Note 4).
5. Allow the pellet to disperse itself overnight at 4°C in 1-2 ml of Solution C (see Notes 4 and 5).
6. Using a two-chamber gradient maker or a Gradient Master™ prepare a continuous gradient from approx 6 ml each of the 5% and 30% iodixanol solutions (see Notes 6-8).
7. Layer the crude virus suspension (1.0-1.5 ml) on top of the gradient and centrifuge at 160,000 g_{av} for 1.5 h at 4°C.
8. Collect the gradient by upward displacement, low-density end first in approx 0.8-1.0 ml fractions (see Note 9). The virus bands sharply towards the bottom of the gradient.

2d. Notes

1. The production of a working solution as described ensures that the buffer and EDTA concentration is constant throughout the gradient. If Solution B also contains 0.6 M NaCl, the NaCl concentration will also be constant but the dense part of the gradient will be hyperosmotic. For more information on the preparation of density gradient solutions see Application Sheet V01.
2. If a gradient making device is unavailable, then make up solutions of 5%, 11%, 17%, 24% and 30% (w/v) iodixanol (see Note 6).
3. For the isolation of Gag particles Hammarstedt et al [1] used a 5-20% (w/v) iodixanol gradient.
4. The ideal way of concentrating the virus is sedimentation on to a dense cushion of iodixanol, rather than pelleting. This however may be less convenient when, as in this case, the concentration of iodixanol in the viral suspension needs to be <4% (w/v) to permit loading on to the gradient. When recovering the band of virus as little as possible of the cushion must be aspirated. It may however be permissible to raise the density of the top of the gradient to 7.5% (w/v) iodixanol without compromising the purification. In which case the stringent requirement for a virus suspension of <4% iodixanol need not apply. For more information on concentration of virus prior to gradient purification see Application Sheet V06.
5. The overnight dispersal of the pellet avoids the often harsh shear conditions required to resuspend virus pellets and consequently may give improved retention of infectivity.
6. Alternatively make a discontinuous gradient from equal volumes of the 5%, 11%, 17%, 24% and 30% iodixanol solutions (see Note 2) and allow the formation of a continuous gradient by diffusion (approx. 5 h at room temperature, or overnight at 4°C). For more information on making gradients see Application Sheet V02.
7. If forming a gradient by diffusion confirm that it is continuous by checking the density of a blank gradient. For more information about density measurement see Application Sheet V05.
8. If larger volumes of crude virus are to be purified then proportionately larger volume gradients must be used.
9. Collection of the gradient by tube puncture may be a useful alternative. For more information on harvesting gradients see Application Sheet V04.

2e. SFV sedimentation velocity separation

The relatively short centrifugation time employed in the method described above may be because it is based, at least in part, on sedimentation velocity. More recently SFV has been purified by a method that more clearly relies on sedimentation velocity for its efficacy [4]. The virus was first concentrated by pelleting through a sucrose cushion (see Application Sheet V06 for other methods of concentration). A 7.5%-27.5% (w/v) iodixanol gradient was generated by diffusion overnight at 4°C from a multiple step discontinuous gradient (0.65 ml each of 7.5% and 10% iodixanol and 0.5 ml each of 11.25%, 12.5%, 13.75%, 15%.....27.5% iodixanol). No more than 0.25 ml of concentrated virus was placed on top and centrifuged in 14 ml tubes at 85,000 g for 30 min. It is highly likely that an approx. 13 ml continuous gradient generated from equal volumes of 7.5% and 27.5% (w/v) iodixanol

would substitute for the diffused multi-step gradient (see [Application Sheet V02](#) for pre-formed continuous gradient methodology).

3. Sindbis virus

Sindbis virus nucleocapsids have been analyzed in a continuous iodixanol gradient. After cell lysis in a hypoosmotic buffer containing 4% Triton X100, a post-nuclear supernatant was loaded onto a continuous isoosmotic 0-30% (w/v) iodixanol gradient containing 0.1% Triton X100, 100 mM NaCl, 1 mM EDTA and 50 mM Tris-HCl, pH 7.4 and centrifuged at approx. 120,000 g for 2.5 h (approx 14 ml tubes). The nucleocapsids peaked in fractions approximately two-thirds of the distance from the top of the gradient [5-8]. Iodixanol gradients are also able to resolve two types of virus, the denser of which is more infectious [9].

Snyder et al [10] also reported the use of a 0 to 30% (w/v) iodixanol gradient in a standard buffered saline containing 1 mM EDTA; the gradient was top-loaded with a crude virus suspension and centrifuged at approx 125,000 g for 2 h. Both Sindbis and Chikungunya virus (see Section 5) were purified using this gradient.

4. Venezuelan equine encephalitis virus

Prior to the isolation of pre-viral nucleocapsids the virus was concentrated by pelleting through a 25% (w/v) iodixanol cushion at 115,000 g for 3 h (see [Application Sheet V06](#) for methods of concentration). The pre-viral nucleocapsids were then purified in a 10-60% (w/v) iodixanol gradient under the same centrifugation conditions; all procedures were carried out at 4°C [11].

Because of the safety problems regarding the development of attenuated HIV as a vaccine, Jurgens et al [12] investigated the use of the genome from the Venezuelan equine encephalitis virus, modified to express SHIV89.6P genes encoding the Gag and Env proteins of HIV. Essentially the same methodology of an iodixanol cushion and subsequent gradient was used as described in ref 11; the only significant difference being the slightly narrower density range of the gradient (10-50% w/v iodixanol).

Ref 13 also reports the use of iodixanol gradients for the final stage purification of this virus.

5. Chikungunya virus

The regulation of Chikungunya virus-like particle production by the E2 proteins has been studied by Akahata and Nabel [14] using a self-generated iodixanol gradient that was first introduced to study HIV tropism. A crude suspension of particles was adjusted to 30% (w/v) iodixanol and centrifuged at 160,000 g_{av} (45,000 rpm) for 6 h in a Beckman VTi50 vertical rotor, to generate a gradient that spanned the range 1.01-1.22 g/ml. These studies on the Chikungunya virus revealed that the capsid protein banded at significantly higher density than the virus-like particles. This method was also used by Urakami et al [15]. **For more information on self-generated iodixanol gradients see [OptiPrep™ Application Sheet V03](#).**

6. Rubella virus

PEG-pelleted Rubella virus has been purified on 0-54% (w/v) iodixanol gradients (containing 20 mM Tris-HCl, pH 8.0, 120 mM NaCl, 1 mM EDTA) centrifuged at 175,000 g for 2 h [16]; the authors demonstrated the significant difference in the organization of Rubella structural proteins compared to those of other viruses in the *Togaviridae* alphavirus group.

7. References

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