# OptiPrep™ Application Sheet V28

## Purification of Group V ((-)ss) RNA viruses: *Paramyxoviridae* – *Paramyxovirinae* and *Pneumovirinae*

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Application Sheet covers the purification of human respiratory syncytial virus (Section 2), measles virus (Section 3), Newcastle disease virus (Section 4), swine paramyxovirus (Section 5) and henipavirus (Section 6).
- **To access other Application Sheets** referred to in the text: return to the **2020Virapp** file and select the appropriate **V number.**

### 1. Background

The *Paramyxoviridae* family is divided into two subfamilies: *Paramyxovirinae* and *Pneumovirinae*. The *Paramyxovirinae* comprises a large number of genuses including *Avulavirus* (Newcastle disease virus) and *Morbillivirus*, which includes measles virus but also other important forms such as Rinderpest virus and canine distemper virus. A new genus *Respirovirus* has recently been added, in which has been placed newly isolated swine paramyxoviruses. The *Pneumovirinae* comprises both human and bovine respiratory syncytial virus and the avian pneumovirus. This Application Sheet covers the isolation of such viruses. Although different methodologies have been developed for specific viruses, it is very likely that, since all paramyxoviruses have a similar structure then it is likely that the individual methods are more widely applicable; it must be stressed however that this has not been tested.

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; in particular the loss of surface glycoproteins from retroviruses has been noted [1]. This may be related to its viscosity, which, in solutions of the same density, is much higher than that of iodixanol.

Like CsCl, 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 and assembly analysis much more convenient than the use of either CsCl or sucrose.

### 2. Analysis of respiratory syncytial virus (adapted from ref 2)

#### 2a. Solutions required (see box ⇒ and Section 2d, Note 1)

**A.** OptiPrep™
**B.** 150 mM NaCl, 50 mM Tris-HCl, pH 7.5
**C.** 50% (w/v) PEG6000 in Solution B
**D.** 1 M $MgSO_4$, 0.25 M sucrose, 100 mM Tris-HCl, pH 7.5
**E.** 100 mM $MgSO_4$, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5
**F.** 100 mM $MgSO_4$, 150 mM NaCl, 50 mM Tris-HCl, pH 7.5

Make a 1 M Tris-HCl, pH 7.5 stock buffer (12.1 g Tris/100 ml)

**Buffers B and F**
Dissolve 0.876 g NaCl in 50 ml of water; add 5 ml of Tris buffer; check the pH and adjust if necessary and make up to 100 ml. For Buffer F only, supplement with 2.46 g of $MgSO_4 \bullet 7H_2O$

**Buffer D** (100ml)
Dissolve 24 .6 g of $MgSO_4 \bullet 7H_2O$ and 8.5 g of sucrose in 60 ml water; add 10 ml of 1 M Tris-HCl; pH 7.5 check pH and make up to 100 ml

**Buffer E** (100 ml)
Dissolve 2.46 g of $MgSO_4 \bullet 7H_2O$ and 8.5 g of sucrose in 60 ml water; add 1 ml of 1 M Tris-HCl; pH 7.5 check pH and make up to 100 ml

**3b. Rotor requirements**
Swinging-bucket rotor with approx 13 ml tubes (e.g. Beckman SW41Ti)

**2c. Protocol** (carry out all operations at 4°C)

1. Prepare a 52% (w/v) iodixanol solution by mixing 5.2 vol. of OptiPrep™ with 0.6 vol. of Solution D and 0.2 vol. of water. Dilute some of the 52% iodixanol solution with Solution E to produce solutions of 36% and 20% (w/v) iodixanol (**see Section 2d, Note 2**).

2. Clarify the virus-containing culture fluid by centrifugation at 3000 *g* for 20 min.

3. Concentrate the virus by adding Solution C to the 3000 *g* supernatant (1 vol. + 4 vol. respectively) and stir gently at 4°C for 90 min.

4. Centrifuge the virus at 3250 *g* for 20 min.

5. Remove the supernatant and re-centrifuge the pellet to remove the remaining fluid; suspend the pellet in 1.0 ml of Solution F.

6. In tubes for the swinging-bucket rotor prepare a discontinuous gradient from approx 4.0 ml each of the three iodixanol solutions (52%, 36% and 20%). For more information on preparing discontinuous gradients **see Application Sheet V02.**

7. Layer the virus on top of the gradient and centrifuge at approx. 150,000 *g* for 90 min. Use a slow deceleration program or allow the rotor to decelerate from 3000 rpm without the brake.

8. During the centrifugation produce continuous gradients from equal volumes (approx 5.5 ml) of 20% and 52% iodixanol using either a two-chamber gradient maker or a Gradient Master™. For more information on preparing continuous gradients **see Application Sheet V02**.

9. Recover the band of virus from 20-30% interface of the discontinuous gradient and dilute with 2 vol. of Solution F (**see Section 2d, Note 3**).

10. Layer the virus suspension on top of the prepared continuous gradient and centrifuge at 150,000 *g* for 18 h. Use a slow deceleration program or allow the rotor to decelerate from 3000 rpm without the brake.

11. Collect the gradient by aspiration from the meniscus, upward displacement with a dense medium or tube puncture (for more information on harvesting gradients **see Application Sheet V04**) and analyze the fractions (**see Section 2d, Note 4**).

**2d. Notes**

1. The sucrose solutions were treated with 0.1% diethylpyrocarbonate (DEPC) at 37°C for 24 h to inactivate any RNases and then heated to 60°C for 3 days to remove the DEPC. Check pH and volume of solutions after this treatment and readjust if required [3].
2. Sufficient volumes of the three iodixanol solutions must be made for at least four gradients; two discontinuous gradients (Step 6) and two continuous gradients (Step 8). If neither of the devices for preparing continuous gradients are available (Step 8), then make up discontinuous gradients as described in Step 6 and allow them to diffuse overnight at 4°C.
3. This virus harvest is to be reloaded on to the second gradient in Step 10 so its density must be sufficiently low to allow this. When recovering the band sample as little of the 36% iodixanol layer as possible.
4. The method has also been used by Murawski et al [4]. Whether any of the methods used for other paramyxoviruses, described briefly in the following sections, are also applicable to respiratory syncytial virus can only be determined by experimental validation.

**3. Measles virus**

**3a. Methods**

Hallek et al [5] purified measles virus, grown in Vero cells; after lysis of the cells the virus-containing fluid was clarified at 1,500 *g* for 10 min and the supernatant applied to a continuous 6-36% (w/v) iodixanol gradient, centrifuged at approx 200,000 *g* for 2h.

Brindley and Plemper [6] also cultivated the virus in Vero cells and after lysing the cells in 100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.8, the lysate was clarified at 5,000 *g*, for 20 min at 4°C to pellet nuclei and cell debris. Two solutions of 10% and 30% (w/v) iodixanol were prepared in the lysis buffer (**see Application Sheet V01 for details on gradient solution preparation**) and used for preparing a discontinuous gradient. After loading the lysate on top, the tubes were centrifuged at 100,000 *g* for 90 min. The concentrated virus was harvested from the interface in the minimum volume of liquid and, followed by loading on a continuous 10%-30% (w/v) iodixanol gradient and re-centrifuged at 100,000 *g* for 14 h. All operations were carried out at 4°C. The gradient was collected in equivolume fractions and analyzed (**see Section 3b Notes 1 and 2**).

Liljeroos et al [7] also used a discontinuous gradient to concentrate the virus. Iodixanol solutions were prepared using a Tris buffered 180 mM NaCl (without EDTA) and the virus sedimented on to a 2 ml 54% (w/v) iodixanol cushion through an 8 ml layer of 20% (w/v) iodixanol by centrifugation at 134,000 *g* for 4 h. The harvested band was diluted with the buffered saline and concentrated in an Amicon spin column before being loaded on a second discontinuous gradient of 15%, 25%, 35% and 54% (w/v) iodixanol and centrifuged at the same speed for approx. 15 h (**see Section 3b Notes 1 and 2**).

- **For information on preparation of gradients see Application Sheet V02**

**3b. Notes**

1. When a virus is banded at an interface between two layers of iodixanol and it is subsequently overlaid on to a second similar gradient great care has to be taken in harvesting the banded material so that removal of too much of the denser layer is avoided. This can be facilitated by removal of the bulk of the higher density layer first, before removing the bulk of the low density layer. **For more information on harvesting of gradients see Application Sheet V04**.
2. Although the harvest can be diluted and reduced in volume by ultrafiltration to permit reloading in a low-density medium on top of a second gradient, a useful alternative would be to perform the second gradient by flotation. The sample is adjusted to a higher density and loaded at the bottom of a second discontinuous or continuous gradient.

**4. Newcastle disease virus (NDV)**

Biswas et al [8] investigated the interaction between NDV and human serum using a continuous 10-26% (w/v) iodixanol gradient, centrifuged at approx. 80,000 $g_{av}$ for 2 h. The separation in the gradient probably encompassed some degree resolution according to sedimentation rate. The fusion and matrix proteins banded towards the middle of the gradient and human serum caused a small high density shift of the proteins and the appearance of a significant amount of aggregated virus towards the bottom of the gradient. Complement C3 fractions from the serum had a broader distribution, also in the middle of the gradient, overlapping the fusion and matrix proteins, but did not appear in the very dense region. In the absence of virus these complement proteins banded only in a low density region.

**5. Swine paramyxoviruses**

Two separate swine paramyxoviruses have been purified in 14-26 (w/v) iodixanol gradients prepared by dilution of OptiPrep™ with PBS [9]. The cell lysates were layered one top of the gradient and centrifuged for at 250,000 *g* for 1.5 h in a swinging-bucket rotor. The banding density of the viruses was not stated.

**6. Henipavirus**

Henipavirus [10] has been purified by the same velocity iodixanol gradient as that described for HIV (**see Application Sheet V34**)

- **For information on gradient analysis see Application Sheet V05**

## 6. References


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