

# OptiPrep™ Application Sheet V09

## Purification of Group I (ds)DNA viruses: *Herpesviridae* and *Asfaveridae* in a pre-formed gradient

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ **The OptiPrep™ Mini-Review (MV03)** “Purification and analysis of *Herpesviridae* viruses and herpes virus vectors” summarizes the published methods and provides a full bibliography of all published papers reporting the use of iodixanol gradients for their purification; to access return to the initial list of Folders and select “**Mini-Reviews**”.
- ◆ Use of a self-generated gradient strategy for Herpes virus is described in **Application Sheet V08**.
- ◆ 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. Background

There are presently eight members of the *Herpesviridae* family of viruses (HHV1-8), including human herpes virus 1 and 2 (HHV-1 and HHV-2), the Epstein-Barr virus (HHV-4), human cytomegalovirus and Kaposi’s sarcoma associated herpes virus (KSHV). They enveloped viruses and share a common structure. These and others have been purified in pre-formed continuous or discontinuous Nycodenz® or iodixanol gradients. The use of sucrose gradients is also common, but the use of this gradient solute for any enveloped virus has serious disadvantages:

- ◆ Palker [1] was one of the first to point out that the movement of virus particles through a sucrose gradient caused loss of surface glycoproteins from enveloped viruses
- ◆ Zhu and Yuan [2] noted that most of the glycoprotein gB was stripped from the surface of KSHV in sucrose gradients (even if the severity of any surface hydrodynamic shear was reduced by using low *g*-forces), while this was not observed in Nycodenz® gradients
- ◆ Use of iodixanol gradients overcame the problem of the cellular vesicle contamination of HHV-6A that was observed in sucrose gradients [3]

### 2. Clarification of virus suspension

Virus-containing suspensions are usually clarified (separated from cellular debris) by low-speed centrifugation, often followed by filtration through a 0.45 µm filter. Centrifugation speeds are usually carried out at approx. 4000 *g* for 30 min (e.g. refs 2 and 4), sometimes 8000 *g* for 15 min [5], which may be in addition to the 4000 *g* step [2].

### 3. Concentration of virus

Concentration of virus particles by rapid sedimentation from a saline solution can cause serious loss of infectivity and there are a number of strategies that minimize this problem. Xiao et al [6] pelleted Epstein-Barr virus at 22,000 *g* overnight rather than a higher *g*-force for a shorter time. There are many instances of the use of a low-density cushion through which the virus sediments: 5 ml of 5% sucrose at approx. 100,000 *g* for 1 h [2], 20% sorbitol, at 64,000 *g* for 1 h [7]; 10% Nycodenz® at 35,000 *g* [4] or 20% iodixanol at 141,000 *g* for 1 h [8].

To avoid entirely the pelleting of the virus, banding on to a dense cushion is often a strategy used for a number of viruses. Garrigues et al [9] first concentrated KSHV on a 50% (w/v) iodixanol cushion. This has also been used for Rhadinovirus [10]. **More information on methods for virus concentration is provided in Application Sheet V06.**

### 4. Gradient solutions

It is highly likely that any method describing the use of a Nycodenz® gradient can be transposed directly to an iodixanol gradient of the same % (w/v) concentration range. Certainly, it is much more

simple to prepare sterile gradient solutions from OptiPrep™ than from powdered Nycodenz®. The preparation of solutions from both sources is described below.

#### 4a. Buffer preparation

The concentrated virus is commonly suspended in PBS [2] or a Tris-HCl buffered 100 mM NaCl containing EDTA at pH 7.2-7.4; the Tris concentration varies from 10 mM [6] to 50 mM [7] and that of the EDTA from 1 mM [6] to 10 mM [7]. The chosen gradient medium (Nycodenz® or iodixanol) is usually made up in the same medium or sometimes in 1 mM potassium phosphate, pH 7.4 [11,12].

#### 4b. Nycodenz®

To make up a stock solution of 50% (w/v) Nycodenz® place 50 ml of buffer in a 150 ml beaker on a heated magnetic stirrer set at approx. 50°C and add 50 g of Nycodenz® powder in small amounts until dissolved. Allow the solution to cool to room temperature and make up to 100 ml with buffer. Filter sterilize if required.

Nycodenz® solutions in water are hyperosmotic above approx 30% (w/v) and 50% (w/v) Nycodenz® has an osmolality of approx. 485 mOsm, thus if the stock solution is made up in a buffer containing 100 mM NaCl, the resultant solution will be > 700 mOsm. To avoid such high osmolalities the NaCl should be omitted and the stock solution of 50% (w/v) Nycodenz® made up in, for example, 10 mM EDTA, 50 mM Tris-HCl, pH 7.4. Subsequent dilutions of the stock are made with the complete buffer. A 35% (w/v) Nycodenz® solution in 0.5 mM phosphate was diluted with 0.25 M sucrose, 0.5 mM phosphate [13]; all these solutions will be approx. isoosmotic.

#### 4c. Iodixanol

As dilution of OptiPrep™ (60% w/v iodixanol) with any volume of an isoosmotic salt solution will produce a solution that is also isoosmotic, it can be diluted directly with the chosen buffer, but if it is important to keep the concentrations of EDTA and Tris constant then a 50% (w/v) iodixanol stock should be prepared from 5 vol. of OptiPrep™ and 1 vol. of a 6x EDTA/Tris buffer. Iodixanol solutions are certainly easier to prepare than Nycodenz® solutions and they can probably be used as a substitute in Nycodenz® methods, but no direct comparative study has been made.

#### 5. Ultracentrifuge rotor requirements

The routine requirement is for a swinging-bucket rotor capable of 100,00-140,000  $g_{av}$ , the Beckman SW28, SW28.1, SW41Ti or equivalent rotors are commonly used.

#### 6. Gradient preparation (all Nycodenz® and iodixanol concentrations are %, w/v)

**Discontinuous gradients** of the following formats have been used: 20% and 35% Nycodenz® [2] and 20% and 40% Nycodenz® [6]. More discriminating multi-step iodixanol gradients were introduced by Garrigues et al [9] for KSHV and subsequently used by Hahn et al [14]; these comprised 20,25, 30 and 40% (w/v) iodixanol. For *Herpes simplex* a broader range of 20,30,40 and 50% (w/v) iodixanol was used [8].

**Continuous gradients** are more widely used; a commonly used one is 1 ml or 2 ml each of 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40% and 42% Nycodenz® [10,11,15,16] which is allowed to diffuse overnight at 4°C. It is probably easier to construct such a gradient from equal volumes of 24% and 42% Nycodenz® using a two-chamber gradient maker or Gradient Master™. Other continuous gradients are: 20-35% [2], 10-50% [7,17], 10-40% [18], 15-35% [13].

Iodixanol gradients of 5-25% (w/v) have been reported [3].

- ◆ **For more information about the preparation of both discontinuous and continuous gradients see Application Sheet V02.**

## 7. Gradient centrifugation

Commonly 0.5-2.0 ml of virus suspension is layered on top of the gradient and centrifuged at approx 70-120,000 g for 2-4, usually at 4°C. Use a slow acceleration and deceleration program if they are available – if not, turn off the brake during deceleration from 3000 rpm.

## 8. Gradient analysis

With discontinuous gradients the virus may be aspirated from the interface between the two gradient solutions. Unload continuous gradients in small equal volume fractions either low- or high-density end first (commonly 20-25 fractions are collected). The precise banding position of the gradient may depend on the virus type or any pre-gradient treatments of the virus. The density of HHV is normally in the range 1.13-1.15 g/ml (equivalent to approx. 24-29% Nycodenz®, but being an enveloped virus its precise density may also depend on the osmolality of the gradient (see Sections 4b and 4c). In the case of HHV-5, the gradient was able to resolve virus particles from dense bodies [7] and recombinant HHV-4 was found to contain a denser fraction (1.18-1.20 g/ml) consistent with de-enveloped particles or partially damaged virus that was not observed with the wild-type virus [10]. For more information about unloading methods see [Application Sheet V04](#).

- ◆ Iodixanol gradients have also been used for the purification of African swine fever virus; no detailed methodology was provided [19,20] but it is highly likely that one of the pre-formed gradients described in this Application Sheet would be effective.

## 9. References

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