OptiPrep™ Application Sheet V16

Purification of Group II (ss)DNA viruses: *Parvovirinae* - parvovirus

- 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.**
- This Application Sheet describes a gradient that was first developed by Zolotukhin et al [1] for the purification of rAAV and it has been widely applied also to parvovirus. Section 4 describes some of the variations to the methodology.
- In 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

Viral vectors that are of potential use in gene therapy would clearly benefit from isolation methods which are both effective and cause little or no damage to the viral particles. Density gradient centrifugation has always played an important part in the concentration and purification of virus particles but the main gradient media that have been used (sucrose and CsCl), pose a number of problems. Both are highly hyperosmotic at the densities used to band viruses (sucrose solutions are also very viscous). Both 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 parvovirus and rAAV 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 1.24 g/ml.

- OptiPrep™ is widely regarded as the gradient medium of choice for purification of viruses and viural vectors. Compared to CsCl gradients recovery of virus from the gradient is at least ten times greater and the particle:infectivity titer is usually at least 100x lower. Infectivity measurements and many add-on techniques can be carried out without the need to dialyze the medium
- Lock et al [1] compared the use of iodixanol and CsCl methods for the purification of rAAV and noted the considerable improved transduction (both *in vitro* and importantly *in vivo*) of the iodixanol-purified material. Moreover only the iodixanol gradient separated infectious particles from empty capsids, which the authors deemed "a desirable property for reducing toxicity and unwanted immune responses during preclinical studies".
- Brandenburger et al [2] who used the method described below for parvovirus also commented that the recovery of the virus is more efficient from iodixanol gradients that from CsCl gradients; that there is no loss of infectivity and that iodixanol prevents aggregation of virus particles. The authors also noted that no dialysis of the isolated virus was required prior to downstream processing.
- In 2001 Bloom et al [3] reported the use of the Zolotukhin et al [4] rAAV purification method, for the purification of Aleutian mink disease parvovirus and since that time many other papers have referenced essentially the same method for other parvoviruses, often with some modifications [5-16].
- The centrifugation conditions of the gradient were modified by Farr and Tattersall [17] to the use of a swinging-bucket rotor (Beckman SW41Ti) at approx 150,000 g_{av} for 18 h (see also ref. 18). Paglino et al [19] described these gradient conditions as effective for the separation of full and empty capsids. Other modifications of the centrifugation conditions include the use of 110,000 *g* for 6.5 h at 25°C [20].

2. Discontinuous gradient method

The method is adapted from that devised by Zolotukhin et al [4] for r-AAV and described by Bloom et al for parvovirus [4]

2a. Solutions required (see box)

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.

2b. Ultracentrifuge rotor requirements

Fixed-angle rotor with approx 39 ml sealed tubes capable of approx 350,000 *g* (e.g. Beckman 70Ti or Sorvall T865; **(see Section 3, Note 1)**.

2c. Protocol

1. Prepare the following gradient solutions **(see Section 3 Notes 2 and 3):**
   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.

2. Clarify the cell lysate clarified by centrifugation at 4000 *g* for 20 min.

3. Underlayer 10-15 ml of clarified lysate with 9 ml of 15% iodixanol; 6 ml of 25% iodixanol, 5 ml of 40% iodixanol and 5 ml of the 54% iodixanol working solution (**see Section 3, Note 1**). Use a long metal cannula (0.8 mm i.d.) attached to a syringe or via tubing to a peristaltic pump to load the tubes (**see Section 3, Notes 4 and 5**).

4. Centrifuge at 350,000 g_{av} for 1 h at 18°C. Use a slow acceleration and deceleration programme (up to and below 2000 rpm) if this facility is available on the centrifuge, or turn off the brake below 2000 rpm during deceleration (**see Section 3, Note 5**).

5. Either collect the whole gradient (Figure 1) in 1-2 ml fractions dense end first or use a syringe inserted at the 40%/54% interface to aspirate 4 ml of the 40% layer (**see Section 3, Notes 6-8**).

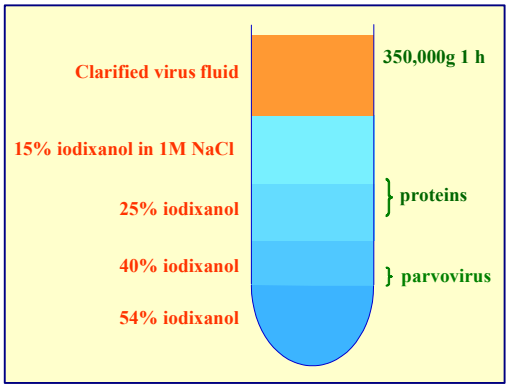

Figure 1 Parvovirus banding in discontinuous iodixanol gradient

3. Notes

1. Smaller capacity rotors may be substituted. For smaller volume tubes scale down all volumes proportionately.
2. Aggregation of parvovirus with proteins in the cell lysate can pose a serious problem to its isolation as the aggregates are heterogeneous and consequently exhibit a broad range of densities. Inclusion of 1 M NaCl in the 15% iodixanol prevents this aggregation and allows the virus to be isolated as a single band above the 40%/54% iodixanol interface (**see Figure 1**).
3. Phenol red (0.01 µg/ml) may be included in the alternate gradient layers to aid the layering process.

4. If large volumes of solutions are used in creation of this gradient, the use of a peristaltic pump to introduce the iodixanol solutions makes this task easier. For more information on preparing discontinuous gradients see **Application Sheet V02**.
5. The gradients described in refs 17 and 18 were reduced to a total volume of 6 ml with a 5 ml sample layer. The lower *g*-force of 150,000 *g* (for a longer time) may reduce any interfacial aggregation of particles and proteins.
6. All of the contaminating proteins in the lysate band at the 25%/40% iodixanol interface.
7. For more information about harvesting gradients see **Application Sheet V04**.
8. Ion exchange, affinity chromatography, incubations with cells or SDS-PAGE can be carried out directly on the iodixanol-containing fractions. Only electron microscopy may require removal of the iodixanol. For more information about gradient analysis see **Application Sheet V05**

4. Self-generated iodixanol gradients

Hendrie et al [21] used a self-generated iodixanol gradient to purify parvovirus. Cells were lysed in a routine Tris-buffered saline by freeze/thawing and after removal of cell debris at 4100 *g* for 30 min the supernatant was adjusted to 1 M NaCl. It was then mixed with OptiPrep™ to a final iodixanol concentration of 36% (w/v) and centrifuged at 290,000 *g* for 3 h. All operations were carried out at 4°C. The virus banded sharply at approx 1.29 g/ml.

- **For more information about self-generated gradients see Application Sheet V03**

5. References

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