OptiPrep™ Application Sheet V07

Purification of Group I (ds)DNA viruses: adenovirus and removal of helper virus

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- Whether this method can be applied to other *Adenoviridae* members with similar morphology, macromolecular composition and size 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.
- See Section 6 for details of a self-generated gradient for separation of helper-dependent adenoviral vector from the helper virus.
- A short bibliographical review of the literature is given in Section 7.

1. Background

Adenovirus is important on two accounts, firstly most children by the age of 15 have been infected with at least one type of adenovirus and it is responsible for a wide variety of upper respiratory tract infections. Secondly it is a popular choice as a viral vector for potential use in gene therapy. The latter in particular 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 gradient media that have been used most prominently, sucrose and CsCl, pose a number of problems. Both media are highly hyperosmotic at the densities used to band viruses (sucrose solutions are also very viscous) and generally 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 adenovirus 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 the purification of other vectors such as rAAV and there is huge bibliography of papers reporting the use of OptiPrep™. Now the method has been extended to adenovirus [1], again with major increases (five- to tenfold) in infectivity titer over CsCl [2]. Infectivity measurements and many add-on techniques can be carried out without the need to dialyze the medium. The following methodology is adapted from ref 2.

- **See Note 1 for details of a modified gradient used by Arpiainen et al [3]**

2. 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.

3. Ultracentrifuge rotor requirements

Swinging-bucket rotor with approx 14 ml tubes, e.g. Beckman SW41Ti or SW40Ti

4. Protocol

1. Prepare the following gradient solutions:
   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. Disrupt the virus-containing cells by 4 cycles of freeze-thawing: snap-freeze the cells in liquid N_2 or dry ice-methanol; thaw at room temperature, then vortex the suspension twice for 30 sec, with a 15 sec rest on ice between each vortexing.

3. Clarify the cell lysate clarified by centrifugation at 2000 *g* for 10 min.

4. Underlayer 4 ml of clarified lysate with 1 ml of 15% iodixanol; 3 ml of 25% iodixanol, 3 ml of 40% iodixanol and 1 ml of 54% iodixanol working solution (see Figure 1 and Notes 2 and 3).

5. Fill the tubes with a small volume of low-density mineral oil on the top and centrifuge at 100,000 g_{av} for 6-16 h at 4°C (see Notes 4-7).

6. Either collect the whole gradient (see Figure 1) in 1 ml fractions dense end first or use a syringe (attached to a 22 gauge needle) inserted just beneath the adenovirus band to remove the band directly in 0.5-1.0 ml (see Notes 8 and 9).

5. Notes

1. Arpiainen et al [3] used a modified gradient of 15%, 30% and 40% (w/v) iodixanol with centrifugation at 100,000 *g* at 4°C for 14-16 h.
2. For larger or smaller volume tubes scale up or down the volume of the layers and sample proportionally. Peng et al [4] used 6.5 ml of the clarified lysate, 2 ml each of the 15%, 25% and 40% iodixanol solutions and 0.5 ml of 54% iodixanol.
3. Peng et al [4] created the gradient by under-layering the sample, starting with the most dense solution. This is the reverse of the more usual underlayering strategy (i.e. starting with the least dense solution). For information on preparing discontinuous gradients **see Application Sheet V02**.

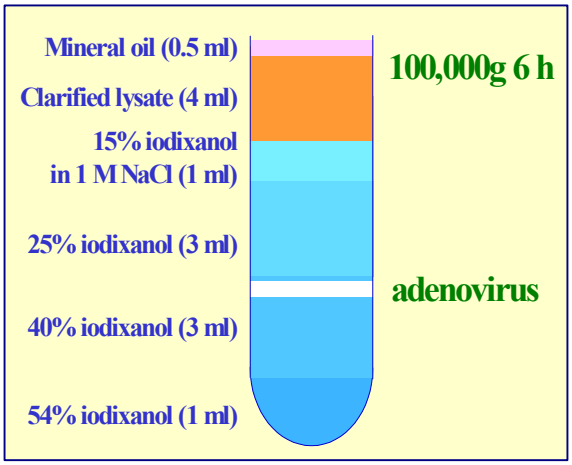

Figure 1: Diagrammatical representation of gradient set up and adenovirus banding position

4. Six hours is the minimum requirement at 100,000g. Although many swinging-bucket rotors are capable of higher g-forces, this relatively low g-force may minimize aggregation of the virus particles at the first interface.
5. Soluble proteins band broadly in the 25% iodixanol layer and their encroachment of the adenovirus band might be reduced by centrifuging for 6 h rather than 16 h.
6. The adenovirus bands at the top of the 40% iodixanol layer, sometimes as a doublet. All of the contaminating proteins in the lysate band broadly in the 25% iodixanol and may reach the 25%/40% iodixanol interface.
7. Peng et al [4] used approx. 200,000 *g* for only 1 h. Under these conditions the adenovirus bands at the 25%/40% interface [4].
8. For more information on the collection of gradients and the recovery of banded material see **Application Sheet V04**.

9. In many cases it is unnecessary to remove the iodixanol before processing the sample further. If it is however a requirement of any downstream procedures, size exclusion chromatography is often the method of choice [4]. For more information see **Application Sheet V05**.

6. Separation of helper-dependent adenoviral vector from the helper virus

An excellent example of use the very shallow iodixanol gradients that can be formed by self generation in a vertical rotor was devised by Dormond et al [5] for the separation of helper-dependent adenoviral vectors (HDV) from the helper virus (HV). Very often the difference in density between these two types of particle is very small and only a self-generated gradient is able to offer an easy solution to the problem of their discrimination. In this case the difference in banding position differed by little more than 0.01 g/ml, yet the peak separation of the sharply banding particles was three fractions. The authors reported that by using two rounds of gradient centrifugation the helper virus contamination was reduced from 2.57% to 0.03%. The absence of any interface where particles could aggregate is clearly a big benefit in the use of self-generated gradients. In the method, the virus suspension was adjusted to 38.7% (w/v) iodixanol and centrifuged (in approx 13 ml tubes) at 180,000 *g* for 3 h at 4°C in a vertical rotor. The HV banded at 40.3-41.6% iodixanol, while the HDV banded at 37.6-39.4% [6]. Iodixanol was removed by size exclusion chromatography [6]. **For more information on self-generated gradients see Application Sheet V03.**

7. Bibliographical review

1. Manninen et al [1] used this adenovirus purification method in their studies on the role of caveolin-1 in apical membrane transport by generating caveolin-1-deficient Madin-Darby canine kidney (MDCK) cells using virus-mediated RNA interference.
2. Elevation of the peroxisome proliferators-activated receptor γ co-activator PGC-1α by adenovirus mediated gene transfer, which increased transcription of the xenobiotic-metabolizing enzyme CYP2A5 transcription [3].
3. Using an iodixanol discontinuous gradient Peng et al [4] reported a fourfold improvement in yield of an adenovector with RGD-modified fibre proteins, compared to the CsCl method.
4. Targeting of caveolin to lipid bodies in adipocytes that express high levels of caveolins [7].
5. The role of LN5 in the spreading, proliferation, wound-edge migration, and apical–basal polarization of MDCK cells [8].
6. To test whether synapsin plays a role in Glut4 traffic, by expression of a site 1 phosphorylation mutant (S10A synapsin) in 3T3-L1 adipocytes [9].
7. Deletion of transcription domain CR3 of adenoviral E1A13S protein and anti-tumour activity in drug-resistant cells [10].
8. Study of the use of cell-derived isolates for the production of seasonal influenza vaccine [11].
9. Iodixanol gradient purification used in adenovirus targeting to prostate-specific membrane antigen.[12].
10. El-Andaloussi et al [13] have described the oncolytic potential of iodixanol-purified adenovirus-parvovirus chimeras.
11. Several authors have commented on the advantages of using a biologically-friendly density gradient medium (OptiPrep™), rather than a toxic heavy metal salt such as CsCl: for direct *in vitro* and *in vivo* experimentation and potential clinical applications; the improved function:particle ratio; the rapid execution of the methodology and the avoidance of desalting techniques [14-16].
12. Papers have described new transfection methods [17], the advantage of adenovirus-parvovirus chimeras in oncolysis [18] and rAAV co-infection [19].
13. More recently iodixanol gradient-purified recombinant adenovirus vectors have been used in gene transfer studies in cardiomyocytes and rat heart. Koivisto et al [20] studied the activation transcription factor 3 (ATF3), a stress-activated immediate early gene that may have both a detrimental and a cardioprotective role in the heart, using cardiomyocytes. Moilanenet al [21] investigated the function of the WD-repeat domain 12 (WDR12) in early-onset myocardial infarction (MI), using adult rat heart. Papers covering treatment of lung carcinoma [22], osteoclast function [23] and oxidant induction in prostate carcinoma treatment [24].

8. References

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