OptiPrep™ Application Sheet V38

Purification and analysis of bacteriophages

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Application Sheet summarizes the use of iodixanol gradients for the purification of:
  1. A lipid-containing marine bacteriophage PM2
  2. Bacteriophages DSSE3Φ2 and EE36Φ1 and virus-like particles from marine roseobacters
  3. Proheads from bacteriophage Φ29
  4. Bacteriophage KPP12
  5. *Podoviridae* phage C1
  6. *Pyrococcus abyssi* and *Thermococus prieurii*
  7. Bacteriophage S13'
  8. Bacteriophage CP-51
  9. Cyanophage SEIV-1
  10. Bacteriophage ΦM9
  11. *Thermotagales*-infecting virus
  12. Virus-like particles (VLPs) LPs from *E. coli (Acinetobacterphage)*
  13. Purification of the 'phage T4 base-plate complex
  14. MS-2 phage
  15. Q*β* phages

- Whether any of the methods can be applied to other bacteriophages 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 **2020Virapp** file and select the appropriate **V number.**

1. Lipid-containing bacteriophage PM2

1a. Introduction

This method for purifying PM2 was devised by Kivela et al [1,2]. Other lipid-containing bacteriophages are known to exist, such as PRD1 and ϕ6, which resemble adenoviruses (see ref 1 for details) and the iodixanol gradient described in this Application Sheet may also be applicable to these particles. The methodology may be broadly applicable to any bacteriophage, but those not containing any lipid would probably have higher densities, so the density range of the gradient for non-lipid-containing bacteriophages may require modulation for such particles.

The PM2 bacteriophage is particularly sensitive to the type of medium, which is used to purify the particles, and loss of infectivity is a major problem with the more traditional media such as CsCl and sucrose. In CsCl the PM2 bacteriophage has a banding density of approx 1.28 g/ml and in sucrose the value is approx 1.26 g/ml [1]. In 55% (w/v) sucrose (1.26 g/ml) there is a rapid loss of infectivity, the specific infectivity of the bacteriophage was reduced by as much as 98%, while the effect of CsCl was to drastically reduce the overall yield of virus recovered from the gradient rather than directly inhibit infectivity.

Because of the problems of CsCl at all concentrations and of sucrose at the high concentrations required to band the virus according to buoyant density, Kivela et al [1,2] have retained an initial 5-20% rate zonal sucrose gradient but then replaced the subsequent sucrose (or CsCl) buoyant density gradient with an iodixanol gradient. It should be noted that Dettenhoffer and Yu [3] used a rate-zonal iodixanol gradient to purify HIV1 (**for more information see Application Sheet V34)** and it may be feasible to use this also for the preliminary purification (see next page) of PM2.

The following protocol is adapted from refs 1 and 2.

1b. Solutions required

A. OptiPrep™ (60%, w/v iodixanol)

B. OptiPrep™ diluent: 100 mM NaCl, 30 mM $CaCl_2$, 120 mM Tris-HCl, pH 7.2

C. OptiPrep™ Working Solution (50% iodixanol): mix 5 vol of Solution A with 1 vol of Solution B.

D. PM2 buffer: 100 mM NaCl, 5 mM $CaCl_2$, 20 mM Tris-HCl, pH 7.2.

Prepare 100 ml of each of the following stock solutions and keep at 4°C:

1 M Tris (base)	12.1 g
1 M NaCl	5.84 g
100 mM $CaCl_2 \bullet 2H_2O$	1.47 g

To make 100 ml of Solution B:
To 30 ml water; add the following stock solutions: 10 ml of NaCl, 30 ml of $CaCl_2$ and 12 ml of Tris; adjust to pH 7.2 with HCl and make up to 100 ml.

To make 100 ml of Solution D:
To 50 ml water; add the following stock solutions: 10 ml of NaCl, 5 ml of $CaCl_2$ and 2 ml of Tris; adjust to pH 7.2 with HCl and make up to 100 ml.

1c. Ultracentrifuge rotor requirements

Swinging-bucket rotor with 4-5 ml tubes Beckman SW 50Ti, Sorvall TH660 or equivalent).

1d. Protocol

1d-1. Preliminary purification (see Note 1)

1. Precipitate the virus from the clarified fluid in 8% (w/v) polyethylene glycol (PEG 6000) at 4°C.

2. Suspend the pellet in 1 M NaCl, 10 mM $CaCl_2$ 20 mM Tris-HCl, pH 7.2 and load on to linear sucrose gradients (5-20%, w/v) in the same buffer (see Note 2).

3. Centrifuge at 75,000g_{av} for 1h 10 min at 15°C and collect the banded virus.

4. Pellet the virus at 75,000g_{av} for 3.5h min at 5°C and then resuspend the virus in Solution D (700 µl/liter of lysate).

1d-2. OptiPrep™ purification

1. Dilute Solution C with Solution D to prepare 5% and 40% (w/v) iodixanol solutions

2. Make linear 5-40% iodixanol gradients using a two-chamber gradient maker or Gradient Master™ in tubes (4-5 ml) for a swinging-bucket rotor and load the virus suspension on top (see Note 3).

3. Centrifuge at 200,000g for 16 h at 10°C.

4. PM2 bacteriophage bands at 1.16 g/ml in iodixanol. Collect the virus bands and process as required (see Note 4).

1e. Notes

1. The preliminary purification steps described briefly here are described in detail by Kivela et al [1,2]. Other strategies may be used prior to the purification in the subsequent overnight iodixanol gradient.
2. To avoid the exposure of the virus to different types of gradient media it may be possible to substitute a rate-zonal iodixanol gradient for the sucrose one. Dettenhoffer and Yu [3] used a 6-18% iodixanol gradient with centrifugation at 250,000g for 1.5 h for purifying HIV-1; **see Application Sheet V34**.
3. If neither of these devices is available create a continuous gradient by allowing a discontinuous gradient (5%, 17%, 28%, 40% iodixanol) to diffuse; for more information on making continuous gradients **see Application Sheet V02**.
4. It should be noted that any add-on purification steps such as HPLC, re-infection of microorganisms and many analytical techniques could be carried out directly on gradient fractions without the need to remove the gradient medium since iodixanol is non-ionic and very “particle-friendly”.

2. Bacteriophages DSSE3Φ2 and EE36Φ1 and virus-like particles from marine roseobacters

Zhao et al [4,5] concentrated the bacteriophages from clarified lysates of cultured roseobacter cells by polyethylene glycol precipitation; after resuspension in culture medium they were loaded on to a 10-50% (w/v) iodixanol gradient. The gradients for the purification of the DSSE3Φ2 and EE36Φ1 bacteriophages were centrifuged at 200,000 *g* for 2 h [4]; the gradients for the detection of induced virus-like particles were centrifuged for half the time [5]. Roseobacter phages were also purified in iodixanol gradients by Zhan et al [6] for DNA analysis and genome sequencing [7].

3. Proheads from bacteriophage Φ29

Iodixanol gradients were used in the analysis of the structure of bacteriophage Φ29, the details of which are outside the scope of this Application Sheet; for information see refs 8 and 9.

4. Bacteriophage KPP12

This method was developed by Fukuda et al [10]. The phage was isolated from *Pseudomonas aeruginosa* cells and after lysis of the latter, the phage was PEG precipitated. OptiPrep™ was diluted with saline to produce gradient solutions of 30%, 35% and 40% (w/v) iodixanol and the crude phage suspension layered on top of a discontinuous gradient formed from the three solutions. The phage banded in the gradient after centrifugation at 200,000 *g* for 2 h. The density range of the isoosmotic gradient was approx. 1.16–1.22 g/ml; by comparison a CsCl gradient in the range 1.3-1.7 g/ml was required for similar banding. This highlights the big advantages of using iodixanol over CsCl; solutions of the latter are not only toxic, they are also hugely hyperosmotic and dialysis is essential before further analysis. Fukuda et al [10] found that the material isolated in iodixanol gradients was extremely stable.

5. *Podoviridae* phage C1 [11]

The phage, grown in *Streptococcus* was suspended in 0.2 M NaCl, 10 mM $MgSO_4$, 20 mM Tris-HCl, pH 7.4. After concentration on to a 50% (w/v) iodixanol cushion and then purified in a 15-35% (w/v) iodixanol gradient (in the same buffer). Centrifugation was carried out at 200,000 *g* for 2 h. For convenience the gradient purification can be executed by flotation; the virus can collected along with part or all of the 50% iodixanol cushion (from the concentration step) and then diluted as required to 35% iodixanol. More information on handling viruses from concentration steps can be found in **Application Sheet V06**.

6. Archeaviruses [12]

Gorlas, A. and Geslin, C. [12] chose iodixanol as the preferred gradient for the purification and analysis of *Pyrococcus abyssi* and *Thermococcus prieurii* over the commonly-used CsCl because of the significant improvement in the recovery of infectivity. The 'phages were purified in 30-45% iodixanol gradients that were centrifuged at 180,000 *g* for 6 h.

7. Bacteriophage S13' [13]

The ability of this 'phage to infect *Staphylococcus aureus* is of great potential therapeutic value as a possible treatment for respiratory diseases caused by this bacterium. The 'phage was purified in a discontinuous iodixanol gradient (30%, 35%, 40% w/v iodixanol) centrifuged for 2h at 200,000 *g* [13] and then subjected to a second gradient of just two layers (30% and 40%) to band the virus sharply at the interface [14].

8. Bacteriophage CP-51 [15]

Klumpp et al [14] also stressed the possible use of iodixanol-purified phage-based diagnostics/ therapeutics for the *Bacillus* ACT group of pathogens

9. Cyanophage S-EIV-1 [16]

After filtration of the bacterial lysate through a 0.45 μm filter, the 'phage was concentrated by ultrafiltration loaded onto a discontinuous 20, 30, 40, 50% (w/v) iodixanol gradient and centrifuged at approx 85,000 *g* for 8 h to band the 'phage.

10. Bacteriophage ΦM9 [17]

In a similar approach to that outlined in 7 (above), the 'phage was purified first in a continuous iodixanol gradient 10-50% (w/v) iodixanol at 200,000 *g* for 2 h. Following harvesting the 'phage was suspension was adjusted to approx. 20% (w/v) iodixanol to allow application on to a discontinuous gradient of 35% and 50% iodixanol and centrifuged at 200,000 *g* for 3 h to allow concentration of the 'phage at the interface.

11. *Thermotagales*-infecting virus

Viruses that occur in the bacteria of deep-sea hydrothermal ecosystems have been investigated by Lossouarn et al [18]. The tailed hexagonal bacterium was purified in an 30-45% (w/v) iodixanol gradient (OptiPrep™ was diluted with a Tris-buffered 100 mM NaCl, 5 mM $CaCl_2$, 20 mM $MgCl_2$) at 40,000 *g* for 5 h. The position of the well-defined virus band was not stated in the paper.

12. Virus-like particles (VLPs) LPs from *E. coli* [19]

VLPs bearing the *Acinetobacter phage* AP205 capsid protein were grown in *E. coli*) and purified in an iodixanol gradient adapted from that described in **Application Sheet V10** for papillomavirus. The top-loaded discontinuous gradient of 23%, 29% and 35% (w/v) iodixanol (OptiPrep™ diluted with phosphate-buffered saline) was centrifuged at approx. 300,000 *g* for 3 h (at 16° C). *Acinetobacter phage* was also purified in studies on VLP vaccines [20].

13. Purification of the 'phage T4 base-plate complex [21]

The base-plate complex was purified in a 10-40% (w/v) iodixanol gradient (plus a 50% cushion), centrifuged at 35,000 *g* for 24 h.

14. MS-2 phage

The phage was adjusted to 20% (w/v) iodixanol; underlaid by 40% iodixanol and centrifuged at 160,000 *g* for 7 h in a swinging-bucket rotor [22]. Dai et al [23] used a top-loaded 10, 20, 30, 40, 50% (w/v) iodixanol gradient centrifuged at 100,000 *g* over night.

15. Q*β*-phages

The crude phage suspension was adjusted to 20% (w/v) iodixanol; layered over an equal volume of 40% iodixanol and centrifuged at 36,000 rpm for 18 h at 15°C to band the phage [24].

16. Bacteriophage ΦM5

The crude phage suspension was purified by layering on top of a 10, 20, 30, 40, 50% (w/v) iodixanol gradient, centrifuged at 100,000 *g* overnight [25]

15. References

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