OptiPrep™ Application Sheet V25

Purification of Group V ((-)ss) RNA viruses: Ebola virus capsid assembly analysis and purification of Ebola pseudovirus in self-generated gradients

- **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.

1. Background

Huang et al [1] have used the OptiPrep™ self-generated gradient method to study the assembly of Ebola virus; in particular they have used it to analyze the influence of nucleoprotein and various virion-associated proteins on the assembly of capsids in transfected 293T cells.

The procedure is particularly noteworthy for the use of larger volume tubes than is normal and this is one of the first examples of the use of the Beckman VTi50 vertical rotor (tube volume 39 ml) for creating self-generated iodixanol gradients. The tube volumes are much larger than the ones normally used for these self-generated gradients (<13 ml) and the maximum RCF of the rotor is approx 190,000g_{av} against the more routine value of 350,000g_{av}. In spite of these less favourable rotor parameters, very acceptable gradients that demonstrate only a slight flattening in the middle can be obtained after 6 h.

The method may be applicable for the intact virion. Being an enveloped virus, it would probably have a lower density than the capsid, although this has not been validated experimentally. A self-generated gradient has however been used for the purification of an Ebola pseudovirus, specifically a luciferase-expressing lentiviral vector pseudotyped with envelopes from Ebola virus [2].

The following protocol is adapted from ref 1. See Section 6 for a brief note on the self-generated gradient used for purification of the Ebola pseudovirus.

2. Solutions required

A. OptiPrep™
B. Lysis medium: 0.05% Tween 20 in phosphate-buffered saline (**see Note 1**)

3. Ultracentrifuge rotor requirements

The Beckman VTi50 vertical rotor used by Huang et al [1] accommodates 39 ml tubes. If the volume of lysate is manageable in a smaller volume rotor, then any vertical or near-vertical rotor with tube capacity of approx 12 ml and capable of approx 350,000g would be suitable, such as the Beckman VTi65.1 vertical rotor or NVT65 near-vertical rotor. These rotors have a sedimentation path length <25 mm and are more efficient than the VTi50 for gradient self-generation.

- **For more information on self-generated gradients in the VTi65.1 and NVT65 rotors consult Application Sheet V03**

4. Protocol

1. Suspend the cells in the Solution B and lyse them by freeze-thawing (three cycles).

2. Clarify the virus suspension by centrifugation at 1500 *g* for 10 min.

3. Mix the clarified lysate with an equal volume of OptiPrep™ to produce a final iodixanol concentration of 30% (w/v).

4. Transfer the suspension to tubes suitable for a vertical or near-vertical rotor. In the Beckman VTi50 centrifuge at 160,000 g_{av} (45,000 rpm) for 6 h. In the Beckman VTi65.1 vertical rotor or NVT65 near-vertical rotor use either the same RCF and centrifugation time or double the RCF and halve the centrifugation time.

5. At the end of the centrifugation use either a controlled deceleration programme or turn off the brake below 2000 rpm.

6. Unload the gradient by tube puncture, upward displacement of aspiration from the meniscus in a series of equal volume fractions (20-25 fractions irrespective of the gradient volume) and analyze the fractions as required. The capsid bands at approx 1.17 g/ml (**see Note 2**).

5. Notes

1. In step 3 of the protocol the lysate is simply mixed with an equal volume of OptiPrep™, which will mean that the buffer and Tween 20 concentrations will be reduced by 50%. If this is deemed unacceptable then first produce a Working Solution of 54% (w/v) iodixanol by mixing 5.4 vol. of OptiPrep™ with 0.6 vol. of 10xPBS containing 0.5% Tween 20.
2. For more information on harvesting gradients **see Application Sheet V04**.

6. Ebola pseudovirus

Kim et al [2] mixed the suspension with an equal volume of OptiPrep™ (i.e. the final iodixanol concentration was 30%, w/v) and centrifuged it at 421,000 *g* for 3 .5 h in a Beckman NVT100 rotor. The pseudovirus displayed a median density of approx 1.10 g/ml.

7. References

1. Huang, Y., Xu, L., Sun, Y. and Nabel, G.J. (2002) *The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein* Mol. Cell, **10**, 307-316
2. Kim, J-O., Chakrabarti, B.K., Guha-Niyogi, A., Louder, M.K., Mascola, J.R., Ganesh, L. and Nabel, G.J. (2007) *Lysis of human immunodeficiency virus type 1 by a specific secreted human phospholipase A2* J. Virol., **81**, 1441-1450