OptiPrep™ Application Sheet V17

Purification of Group III (ds)RNA viruses: *Reoviridae*

- 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 Virus Index; key Ctrl "F" and type the V-Number in the Find Box.

1. Background

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 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. Compared to CsCl gradients:

- Recovery of virus from the gradient is at least ten times greater
- Particle:infectivity titer is up to 100x lower
- Infectivity measurements and many add-on techniques can be carried out without the need to dialyze the medium.

2. Methodology

There are rather few published papers reporting the use of iodixanol gradients for the purification and analysis of Class III viruses. The first by Jaafar et al [1] was concerned with study of a *Seadornavirus* (Banna virus) cores; the authors reported a multi-step purification, which incorporated a discontinuous 10, 20, 30, 40 and 55% (w/v) iodixanol (in 100 mM Tris-HCl, pH 7.5) and centrifuged at 210,000 *g* for 2 h. It was followed by a CsCl gradient. The same group (ref 2) extended the method to a new member of the group (*Dinovernavirus*) using the same iodixanol gradient but omitting the CsCl gradient. Banding at the 40-55% (w/v) iodixanol interface was observed in both cases. Later Attoui et al [3] used an OptiPrep™ cushion to concentrate Liao ning virus (another *Seadornavirus*).

Rotavirus was investigated using a continuous iodixanol gradient [4] centrifuged at 80,000 *g* for 16 h. Gradient solutions were prepared by dilution of OptiPrep™ with buffered 0.25 M sucrose. The majority of the dsRNA banded in the ρ = 1.11-1.15 g/ml region (approx. 16.5-25% w/v iodixanol). The authors considered that this was consistent with the nascent sub-viral particles residing in neoplasms and that they were associated with lipid droplets. Gradients were similar to those used by other workers to show that the hepatitis C virus isolated from liver samples for example were also associated with lipid [5]. For more information on the use for such gradients see **Application Sheet V19**

A *Birnavirus* (Espirito Santo virus) has been purified in a 12-35% (w/v) iodixanol gradient centrifuged for 16 h at 76,000 *g* and then re-centrifuged through a second gradient of 20-35% (w/v) iodixanol for 3 h at 90,000 *g* [6].

An interesting aspect of the replication of rotaviruses is that this process occurs in cytoplasmic inclusion bodies termed viroplasms. It is known that these viroplasms interact (both physically and

functionally) with lipid droplets (LDs). Iodixanol gradients have been used to purify these particles, which have a density of 1.11-1.15 g/ml [7-8].

3. References

1. Jaafar, F.M., Attoui, H., Mertens, P.P.C., de Micco, P. and de Lamballerie, X. (2005) *Structural organization of an encephalitic human isolate of Banna virus (genus Seadornavirus, family Reoviridae)* J. Gen. Virol., **86**, 1147-1157
2. Attoui, H., Jaafar, F.M., Belhouchet, M., Biagini, P., Cantaloube, J-F., de Micco, P. and de Lamballerie, X. (2005) *Expansion of family reoviridae to include nine-segmented dsRNA viruses: isolation and characterization of a new virus designated aedes pseudoscutellaris reovirus assigned to a proposed genes (Dinovernavirus)* Virology, **343**, 212-223
3. Attoui, H., Jaafar, F.M., Belhouchet, M., Tao, S., Chen, B., Liang, G., Tesh, R.B., de Micco, P. and de Lamballerie, X. (2006) *Liao ning, a new Chinese seadornavirus that replicates in transformed and embryonic mammalian cells* J. Gen. Virol., **87**, 199-208
4. Cheung, W., Gill, M., Esposito, A., Kaminski, C.F., Courousse, N., Chwetzoff, S., Trugnan, G. Keshavan, N., Lever, A. and Desselberger, U. (2010) *Rotaviruses associate with cellular lipid droplet components to replicate in viroplasms, and compounds disrupting or blocking lipid droplets inhibit viroplasm formation and viral replication* J. Virol., **84**, 6782-6798
5. Nielsen, S.U., Bassendine, M.F., Martin, C., Lowther, D., Purcell, P.J., King, B.J., Neely, D., Toms, G.L. (2008) *Characterization of hepatitis C RNA-containing particles from human liver by density and size* J. Gen. Virol., **89**, 2507-2517
6. Vancini, R., Paredes, A., Ribeiro, M., Blackburn, K., Ferreira, D., Kononchik, Jr. J.P., Hernandez, R. and Brown, D. (2012) *Espirito Santo virus: a new Birnavirus that replicates in insect cells* J. Virol., 86, 2390-2399
7. Lever, A. and Desselberger, U. (2016) *Rotavirus replication and the role of cellular lipid droplets: New therapeutic targets?* J. Formosan Med. Assoc., **115**, 389-394
8. Cheung, W., Gaunt, E., Lever, A. and Desselberger, U. (2016) *Rotavirus replication: the role of lipid droplets in viral gastroenteritis*, Elsevier Inc pp 175-187

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