Preparation of density gradient solutions

1. OptiPrep™

OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml. Iodixanol is a non-ionic molecule with a molecular mass of 1550 (see Figure 1).

2. Handling OptiPrep™

Exposure (several months) of iodixanol solutions to direct sunlight will cause a slow release of iodine (solution turns yellow); OptiPrep™ should therefore be stored away from strong sunlight. On standing, iodixanol may "settle out" of concentrated solutions, which should be well mixed before use.

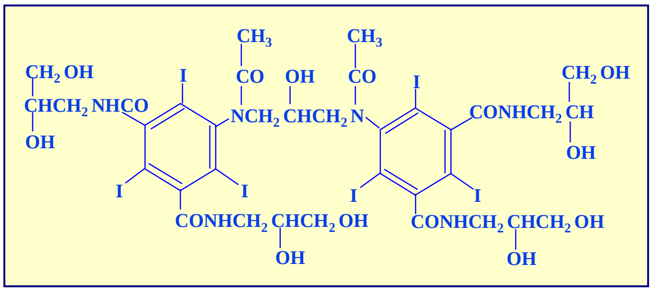

Figure 1: Molecular structure of iodixanol

3. Osmolality

The observed osmolality of OptiPrep™ depends on the mode of measurement (vapour pressure or freezing point); moreover the situation is complicated by the tendency of the iodixanol molecules to associate non-covalently in a concentrated aqueous solution. Measured values for its osmolality are thus lower than might be expected. Importantly however, when OptiPrep™ is diluted with a buffered isoosmotic solution, the iodixanol oligomers dissociate and all dilutions are isoosmotic. Under normal operating conditions therefore OptiPrep™ behaves as if it had an osmolality of approx 290 mOsm.

4. Preparation of density solutions

Traditionally viruses have been purified in gradients containing high concentrations of sucrose, glycerol or CsCl. The particles have therefore been isolated in grossly hyperosmotic conditions. OptiPrep™ offers the opportunity to isolate them under isoosmotic conditions. In many instances the density of a virus in iodixanol will be considerably lower than that in CsCl and slightly lower than that in sucrose or glycerol.

Commonly the solutions used to suspend viruses are the same as, or similar to, those used for mammalian cells, such as phosphate-buffered saline or a Tris-buffered saline, although sometimes a 0.25 M sucrose based buffer is used. The solutions may also contain low concentrations of useful additives such as EDTA (1 mM), KCl (2.5 mM) or $MgCl_2$ (1 mM). If it is important to maintain the concentration of the buffer and additives constant throughout the gradient, then the general strategy is to start by making a dense working solution (WS). For example make a 50% (w/v) iodixanol working solution by diluting 5 vol. of OptiPrep™ with a 1 vol. of a diluent containing 6x the required concentrations of buffer and additives. Note that the concentration of the osmotic balancer (NaCl or sucrose) is not similarly increased six-fold; if it were then the solution would be hyperosmotic. The WS will then contain the correct concentration of buffer and additives and be approximately isoosmotic; this can then be further diluted with the normal medium to provide solutions of lower density. The WS can also be added directly to a sample to adjust its density. Iodixanol solutions produced in this manner will be in the range 285-305 mOsm. The use of alternative organic buffers at similar concentrations will have no significant effect on the density and osmolality of the solutions.

Tables 1 and 2 give the density of solutions produced by dilution of a 50% (w/v) iodixanol WS with either 0.85% NaCl, 10 mM Tris-HCl, pH 7.4 (Table 1) or 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4 (Table 2).

Table 1: Density and refractive index of iodixanol solutions (0.85% NaCl diluent)*

Density (ρ)	% Iodixanol	WS + Diluent	RI (η)	Density (ρ)	% Iodixanol	WS + Soln. B	RI (η)
1.058	10.0	1.0 + 4.0	1.3507	1.174	32.0	3.2 + 1.8	1.3851
1.069	12.0	1.2 + 3.8	1.3538	1.184	34.0	3.4 + 1.6	1.3882
1.079	14.0	1.4 + 3.6	1.3569	1.195	36.0	3.6 + 1.4	1.3914
1.090	16.0	1.6 + 3.4	1.3601	1.205	38.0	3.8 + 1.2	1.3945
1.100	18.0	1.8 + 3.2	1.3632	1.215	40.0	4.0 + 1.0	1.3976
1.111	20.0	2.0 + 3.0	1.3663	1.226	42.0	4.2 + 0.8	1.4008
1.121	22.0	2.2 + 2.8	1.3694	1.236	44.0	4.4 + 0.6	1.4039
1.132	24.0	2.4 + 2.6	1.3726	1.246	46.0	4.6 + 0.4	1.4070
1.142	26.0	2.6 + 2.4	1.3757	1.257	48.0	4.8 + 0.2	1.4100
1.153	28.0	2.8 + 2.2	1.3788	1.267	50.0		1.4132
1.163	30.0	3.0 + 2.0	1.3820				

* Density values are in g.ml^{-1}, iodixanol concentrations are % (w/v), WS + diluent figures are the volume ratios of a 50% (w/v) iodixanol WS and diluent (see text above), RI = refractive index

Table2: Density and refractive index of iodixanol solutions (0.25 M sucrose diluent)*

Density (ρ)	% Iodixanol	WS + diluent	RI (η)	Density (ρ)	% iodixanol	WS + diluent	RI (η)
1.078	10.00	1.0 + 4.0	1.3589	1.185	32.00	3.2 + 1.8	1.3896
1.088	12.00	1.2 + 3.8	1.3617	1.194	34.00	3.4 + 1.6	1.3924
1.098	14.00	1.4 + 3.6	1.3645	1.204	36.00	3.6 + 1.4	1.3952
1.107	16.00	1.6 + 3.4	1.3673	1.214	38.00	3.8 + 1.2	1.3980
1.117	18.00	1.8 + 3.2	1.3701	1.223	40.00	4.0 + 1.0	1.4008
1.127	20.00	2.0 + 3.0	1.3729	1.233	42.00	4.2 + 0.8	1.4036
1.136	22.00	2.2 + 2.8	1.3757	1.243	44.00	4.4 + 0.6	1.4064
1.146	24.00	2.4 + 2.6	1.3785	1.252	46.00	4.6 + 0.4	1.4091
1.156	26.00	2.6 + 2.4	1.3813	1.262	48.00	4.8 + 0.2	1.4119
1.165	28.00	2.8 + 2.2	1.3840	1.272	50.00		1.4147
1.175	30.00	3.0 + 2.0	1.3868				

* Density values are in g.ml^{-1}, iodixanol concentrations are % (w/v), WS + diluent figures are the volume ratios of a 50% (w/v) iodixanol WS and diluent (see text above), RI = refractive index

To maintain a constant buffer concentration in the solutions (Table 1), the 40% iodixanol WS was produced by diluting 4 vol. of OptiPrep™ with 2 vol. of 0.85% NaCl, 30 mM Tris-HCl, pH 7.4. Similarly the 50% iodixanol WS in Table 2 was produced by diluting 5 vol. of OptiPrep™ with 1 vol. of 0.25 M sucrose, 6 mM EDTA, 60 mM Tris-HCl, pH 7.4

5 Calculation of density

As long as the density of the diluent is known then Equation 1 can be used to calculate the density of any solution produced from the diluent and a working or stock solution of iodixanol.

Equation 1:

$$D = \frac{Vd + V_1 d_1}{V + V_1}$$

D = density of mixture; V = volume of iodixanol stock solution; d = density iodixanol stock solution; V_1 = volume of diluent; d_1 = density of diluent