

# OptiPrep™ Application Sheet V01

## 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.

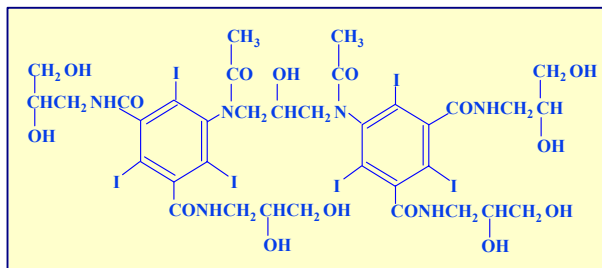


Figure 1: Molecular structure of iodixanol

### 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 phosphate-, Tris- or HEPES-buffered buffered saline (or 0.25 M sucrose). The solutions may contain low concentrations of additives such as EDTA (1 mM), KCl (2.5 mM) or MgCl<sub>2</sub> (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). To maintain a constant buffer concentration in the solutions (Table 1), the 50% iodixanol WS was produced by diluting 5 vol. of OptiPrep™ with 1 vol. of 0.85% NaCl, 60 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.

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

Density ( $\rho$ )	% Iodixanol	WS + Diluent	RI ( $\eta$ )	Density ( $\rho$ )	% Iodixanol	WS + Soln. B	RI ( $\eta$ )
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  $\text{g.ml}^{-1}$ , 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 ( $\rho$ )	% Iodixanol	WS + diluent	RI ( $\eta$ )	Density ( $\rho$ )	% iodixanol	WS + diluent	RI ( $\eta$ )
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  $\text{g.ml}^{-1}$ , 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

## 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:**

$$D = \frac{Vd + V_1d_1}{V + V_1}$$

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