OptiPrep™ Application Sheet M01

Preparation of gradient solutions (for macromolecules and macromolecular complexes)

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.

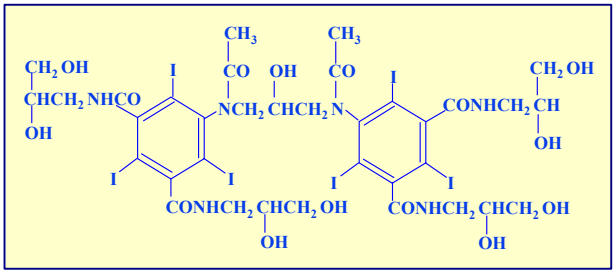

Figure 1: Molecular structure of iodixanol

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. Thus, measured values of osmolality may be 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

Macromolecules and macromolecular complexes cover such a diverse range of particles that it is not possible to give all the possible recipes for the preparation of gradient solutions from OptiPrep™. Instead, the preparation of gradient solutions from two types of general-purpose working solution, based on the use of either NaCl or sucrose as osmotic balancer, are described in this section. Specific examples of gradient solutes for the purification of nucleic acids, proteins, nucleoprotein particles and lipoproteins are briefly discussed in Section 5.

If it is important to maintain the concentration of a particular buffer or additive constant throughout the gradient, then the general strategy is to start by making a dense working solution. For example make a 50% (w/v) iodixanol working solution (WS) by diluting 5 vol. of OptiPrep™ with a 1 vol. of a diluent containing 6x the required concentrations of buffer and additives. The working solution will then contain the correct concentration of additives; this can then be further diluted with the normal medium to provide solutions of lower density. Note that the concentration of any osmotic balancer (e.g. NaCl or sucrose) is not similarly increased six-fold; if it were then the solution would be grossly hyperosmotic. The WS can also be added directly to a sample to adjust its density. 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). In each case the WS contains the same concentration of buffer or buffer + EDTA respectively.

Macromolecules and macromolecular complexes traditionally have been purified in gradients containing high concentrations of sucrose, glycerol, alkali metal salts (e.g. KBr and NaCl) or heavy metal salts (e.g. CsCl). The particles have therefore been isolated in grossly hyperosmotic conditions. OptiPrep™ offers the opportunity to isolate them under more or less isoosmotic conditions. Note therefore that in iodixanol gradients, the macromolecules may have lower densities.

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

5. Macromolecule-specific buffers

Very often the buffers used in the gradients are specific to the type of macromolecule of macromolecular complex under investigation. Some of the published examples are briefly described in this section.

5a. Nucleic acids

Gradients containing 1 mM EDTA, 10 mM NaCl, 10 mM Tris-HCl, pH 7.5 are not uncommon. See ref 1 for more information of the effect of gradient composition on the banding density of nucleic acids in iodinated density gradient media.

5b. Ribonucleoproteins

Gradients for the study of ribonucleoprotein complexes from both *Xenopus* and mammalian sources often contain quite high levels of KCl: for example 0.3 M KCl, 2 mM $MgCl_2$ in a 10 or 20 mM HEPES of Tris buffer [2-4], although lower concentrations of 115 mM KCl have also been used [5]. There is nevertheless a wide variety of (often complex) media that are used in gradients for the isolation of ribonucleoproteins and there are several excellent reviews on the fractionation of these complexes in a variety of media [6-8] that give details of the required gradient composition.

5c. DNA-protein complexes

The banding of DNA-protein complexes usually occurs in gradients containing salt since the complexes are unstable in its absence (e.g. 0.14 M NaCl, 1 mM DTT, 0.1 mM EDTA, 10 mM Tris-HCl, pH 7.5). Gradients for studying pre-integration complexes; gradients often contain buffered 5 mM $MgCl_2$, 6 mM EDTA, 150 mM KCl with [9] or without DTT [10].

5d. Proteins

Soluble proteins have been banded in gradients produced by dilution of OptiPrep™ with a simple HEPES-buffered saline, but often other reagents that may stabilize the protein are included. Basi and

Rebois [11] for example included 20 mM HEPES-NaOH, pH 8.0, 1 mM EDTA, 1 mM DTT, 2 mM $MgSO_4$ and 0.1% Lubrol PX in iodixanol gradients for studying the sedimentation of proteins. At the concentrations used, these reagents will have little effect on the density or osmolarity of the gradient. Other gradient studies on proteins have used 35 mM PIPES, 0.5 mM $MgSO_4$, 0.1 mM EGTA, 0.5 mM EDTA [12] and 100 mM NaCl, 1 mM EDTA buffered with 50 mM Tris [13].

5e. Lipoproteins

Simple dilutions of OptiPrep™ with HEPES-buffered saline suffice for the fractionation of lipoproteins and antioxidants may be included at the discretion of the operator.

6. 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

7. References

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