OptiPrep™ Application Sheet S02

Preparation of gradient solutions (non-mammalian)

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.

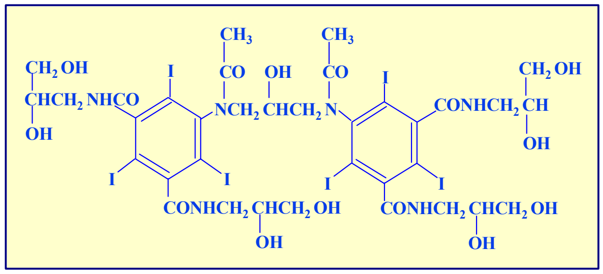

Figure 1: Molecular structure of iodixanol

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.

Homogenates of yeast and plants and the solutions used to isolate organelles from these sources frequently contain either mannitol or sorbitol at concentrations (and osmolarities) significantly higher than those used for mammalian systems. The isolation of protoplasts or spheroplasts (from plants and yeast respectively) is also carried out in such media in order to shrink the intact cell away from the cell coat. Media containing 400-600 mM mannitol or sorbitol are common but for yeast mitochondria concentrations as high as 0.8-1 M sorbitol are not unknown. Sucrose may also be used at concentrations up to 0.5 M, thus a special strategy has to be adopted to provide density solutions of the appropriate osmolality.

% (w/v) sorbitol	ρ (g/ml)	η	Π (mOsm)
4.40	1.015	1.3390	265
8.75	1.029	1.3455	525
10.5	1.035	1.3480	657
12.25	1.041	1.3505	774
17.5	1.059	1.3580	1200

Table 1: Density (ρ), refractive index (η) and osmolality (Π) of sorbitol solutions in 10 mM Tris-HCl, pH 7.4.

4. Preparation of density solutions

The general strategy is to produce a high density working solution (WS) of the correct osmolality by diluting OptiPrep™ with a sorbitol (or mannitol) containing diluent and then diluting this solution with the normal homogenization medium (HM) or organelle suspension medium. Table 1 gives the properties of sorbitol solutions in 10 mM Tris-HCl, pH 7.4. Examples of use of these diluents to produce gradient solutions of a constant osmolality are given below.

Prepare a WS of 40% (w/v) iodixanol by diluting 4 vol of OptiPrep™ with 2 vol of 12.25% (w/v) sorbitol, 30 mM Tris-HCl, pH 7.4. This has a density of 1.225 g/ml.

Dilute the WS with 8.75% (w/v) sorbitol, 10 mM Tris-HCl, pH 7.4 to provide gradient solutions of a suitable density (see Table 2). All of the solutions have an osmolality of approx 545 mOsm.

Table 2: Density (ρ) and refractive index (η) of iodixanol solutions: dilution of 40% iodixanol WS, ρ = 1.225 g/ml with the 8.75% sorbitol diluent (D).

% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + D volume ratio	% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + D volume ratio
10.00	1.078	1.3593	2.5 + 7.5	26.00	1.157	1.3821	6.5 + 3.5
12.00	1.088	1.3622	3.0 + 7.0	28.00	1.166	1.3849	7.0 + 3.0
14.00	1.098	1.3650	3.5 + 6.5	30.00	1.176	1.3878	7.5 + 2.5
16.00	1.108	1.3679	4.0 + 6.0	32.00	1.186	1.3906	8.0 + 2.0
18.00	1.117	1.3707	4.5 + 5.5	34.00	1.196	1.3935	8.5 + 1.5
20.00	1.127	1.3736	5.0 + 5.0	36.00	1.205	1.3963	9.0 + 1.0
22.00	1.137	1.3764	5.5 + 4.5	38.00	1.215	1.3992	9.5 + 0.5
24.00	1.147	1.3792	6.0 + 4.0	40.00	1.225	1.4020	

A WS prepared using the 17.5% sorbitol diluent has a density of 1.231 g/ml; when this is diluted with 12.25% sorbitol, 10 mM Tris-HCl, pH 7.4, all solutions have an osmolality of approx 756 mOsm (Table 3).

Table 3 Density (ρ) and refractive index (η) of iodixanol solutions: dilution of 40% iodixanol WS, ρ = 1.231 g/ml with the 12.25% sorbitol diluent (D)

% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + D volume ratio	% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + D volume ratio
10.00	1.088	1.3637	2.5 + 7.5	26.00	1.165	1.3848	6.5 + 3.5
12.00	1.098	1.3663	3.0 + 7.0	28.00	1.174	1.3874	7.0 + 3.0
14.00	1.108	1.3689	3.5 + 6.5	30.00	1.184	1.3900	7.5 + 2.5
16.00	1.117	1.3716	4.0 + 6.0	32.00	1.193	1.3927	8.0 + 2.0
18.00	1.127	1.3742	4.5 + 5.5	34.00	1.203	1.3953	8.5 + 1.5
20.00	1.136	1.3769	5.0 + 5.0	36.00	1.212	1.3979	9.0 + 1.0
22.00	1.146	1.3795	5.5 + 4.5	38.00	1.222	1.4006	9.5 + 0.5
24.00	1.155	1.3821	6.0 + 4.0	40.00	1.231	1.4032	

Diluents containing mannitol of the same concentration provide solutions of exactly the same density and osmolality. Sorbitol (or mannitol) solutions of 17.5%, 12.25% and 8.75% (w/v) are equivalent to 0.96 M, 0.67 M and 0.48 M respectively.

5. Concentration of buffer and other additives in the gradient

It may be important to maintain constant low concentrations (1-5 mM) of some additives such as EDTA or DTT in the gradient. In which case add them to the OptiPrep™ diluent at 3x the required gradient concentration when the WS is prepared. If the additives are also included in the WS diluent (at their required concentration) then their concentration in all density gradient solutions will be constant.

6. Other osmotic balancers

The density of any gradient solution can be calculated using Equation 1 (below), so long as the densities of the iodixanol-containing solution and of the diluent are known.

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

serumwerk bernburg

Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwwerk.com • serumwerk.de