OptiPrep™ Application Sheet S01

Preparation of gradient solutions (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.

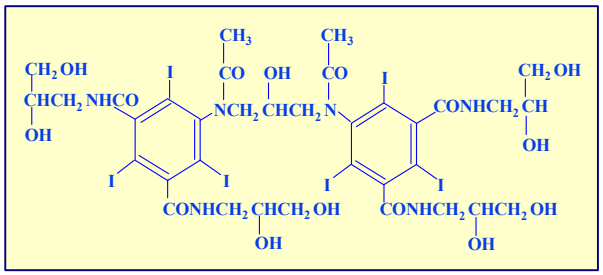

Figure 1: Molecular structure of iodixanol

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.

4. Preparation of density solutions for all organelles, except nuclei

The recommended procedure for the production of density gradient solutions or for adjustment of the density of organelle suspensions is to use a working solution (WS) whose composition is compatible with the particles to be separated. The following methodology is based on the use of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4 as homogenization medium (HM). To keep the concentrations of EDTA and buffer constant in the gradient constant first prepare a 50% (w/v) iodixanol working solution by mixing 5 vol. of OptiPrep™ with 1 vol. of 0.25 M sucrose, 6mM EDTA, 60 mM Tris-HCl, pH 7.4. The gradient solutions are then produced from the Working Solution (WS) by dilution with the HM according to Table 1.

% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + HM volume ratio	% (w/v) iodixanol	Density (ρ) g/ml	Refr. Index (η)	WS + HM volume ratio
10.00	1.078	1.3589	2.0 + 8.0	32.00	1.185	1.3896	6.4 + 3.6
12.00	1.088	1.3617	2.4 + 7.6	34.00	1.194	1.3924	6.8 + 3.2
14.00	1.098	1.3645	2.8 + 7.2	36.00	1.204	1.3952	7.2 + 2.8
16.00	1.107	1.3673	3.2 + 6.8	38.00	1.214	1.3980	7.6 + 2.4
18.00	1.117	1.3701	3.6 + 6.4	40.00	1.223	1.4008	8.0 + 2.0
20.00	1.127	1.3729	4.0 + 6.0	42.00	1.233	1.4036	8.4 + 1.6
22.00	1.136	1.3757	4.4 + 5.6	44.00	1.243	1.4064	8.8 + 1.2
24.00	1.146	1.3785	4.8 + 5.2	46.00	1.252	1.4091	9.2 + 0.8
26.00	1.156	1.3813	5.2 + 4.8	48.00	1.262	1.4119	9.6 + 0.4
28.00	1.165	1.3840	5.6 + 4.4	50.00	1.272	1.4147	
30.00	1.175	1.3868	6.0 + 4.0				

Table 1: Density (ρ) and refractive index (η) of iodixanol solutions: dilution of 50% iodixanol WS with HM (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4)

The osmolality of all the dilutions is in the range 295-310 mOsm. The use of alternative organic buffers at similar concentrations will have no significant effect on the density and osmolality of the WS. The concentration of buffer and EDTA in all of the gradient solutions will be the same as in the HM. If a low concentration (1-5 mM) of any other additive (e.g. DTT or a detergent) needs to be kept constant in the gradient, this can also be added to the OptiPrep™ diluent at the appropriate concentration.

- It may be permissible to produce density solutions simply by diluting OptiPrep™ with homogenization medium. The osmolality will be satisfactory but the concentration of buffer and additives in the gradient will decrease as the iodixanol concentration increases.

- The 50% (w/v) iodixanol WS is also suitable for adding to the homogenate or differential centrifugation fraction (suspended in buffered 0.25 M sucrose) in order to adjust its density; although it may be acceptable to add OptiPrep™ directly.

% w/v iodixanol	ρ (g/ml)	RI (η)	WS (ml)	HM (ml)
10	1.065	1.3548	2.00	8.00
20	1.116	1.3796	4.00	6.00
30	1.167	1.3844	6.00	4.00
40	1.218	1.3991	8.00	2.00
50	1.269	1.4139		

Table 2 Density (ρ) and refractive index (η) of mannitol-containing iodixanol solutions: dilution of 50% (w/v) iodixanol Working Solution (WS) with HM

5. Other non-ionic osmotic balancers

Occasionally mannitol (or sorbitol) may be preferred over sucrose as an osmotic balancer for mammalian systems. Mannitol in particular is widely used in media for the isolation of mitochondria and sometimes it is used for suspending cells when a non-ionic medium is required. Isoosmotic density solutions based on an HM containing 4.4% (w/v) mannitol (or sorbitol), 10 mM Tris-HCl, pH 7.4 (ρ = 1.015 g/ml) are produced in the same manner as those based on 0.25 M sucrose. A 50% (w/v) iodixanol WS is produced by diluting 5 vol of OptiPrep™ with 1 vol of 4.4% (w/v) mannitol, 60 mM Tris-HCl, pH 7.4. This is then diluted further with HM. The properties of a few selected dilutions are given in Table 2. The osmolality of solutions is 290-310 mOsm.

6. Preparation of density solutions for nuclei

The majority of homogenization solutions for the isolation of nuclei contain KCl and $MgCl_2$ as opposed to EDTA. An homogenization medium (HM) of 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.8 is often recommended. Mix 5 vol. of OptiPrep™ with 1 vol. of 150 mM KCl, 30 mM $MgCl_2$, 120 mM Tris-HCl, pH 7.8, to produce a WS with a density of 1.269 g/ml and osmolality of 320 mOsm. Dilute the WS with HM (ρ=1.033 g/ml) to provide solutions of the appropriate density (see Table 3).

% (w/v) iodixanol	ρ (g/ml)	RI (η)	WS (ml)	HM (ml)
10.0	1.080	1.3604	2.0	8.0
20.0	1.127	1.3740	4.0	6.0
30.0	1.175	1.3876	6.0	4.0
40.0	1.222	1.4012	8.0	2.0
50.0	1.269	1.4148		

Table 3 Density (ρ) and refractive index (η) of iodixanol-sucrose-KCl-$MgCl_2$ solutions: dilution of 50% (w/v) iodixanol WS with HM

7. Homogenization media containing ionic osmotic balancers

Although the use of non-ionic osmotic balancers such as sucrose (or mannitol) is more or less a tradition in organelle isolation, there has been trend over the last ten years to move to the use of ionic osmotic balancers (KCl or NaCl) either on their own or in combination with sucrose, particularly for cultured cells. Solutions with a higher ionic strength may be particularly useful for cells in which the proteins of the cytoskeleton tend to form a gel during homogenization. Some examples are (the buffer is given as the final component in each example): 0.25 M sucrose, 130 mM KCl, 5 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.4; 130 mM KCl, 25 mM NaCl, 1 mM EGTA, 25 mM Tris-HCl, pH 7.4; 120 mM NaCl, 20 mM KCl, 1 mM EGTA, 1 mM EDTA, 10 mM Tris-HCl, pH 7.5 and 0.25 M sucrose, 78 mM KCl, 4 mM $MgCl_2$, 8.32 mM $CaCl_2$, 10 mM EGTA, 50 mM Hepes-KOH, pH 7.0

8. Density calculations

The density of any gradient solution can be calculated using Equation 1, 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

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