OptiPrep™ Application Sheet C01

Preparation of gradient solutions (cells)

1. OptiPrep™

OptiPrep™ is a sterile 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.

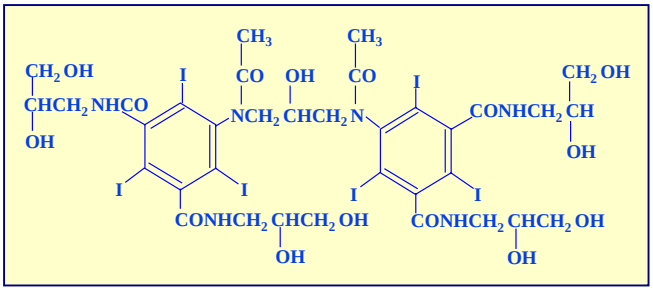

Figure 1: Molecular structure of iodixanol

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 gradient solutions

The following methodology is based on use of 0.85% (w/v) NaCl, 10 mM Tricine-NaOH, pH 7.4 as a cell suspension medium. To keep the buffer concentration constant throughout any gradient a 40% (w/v) iodixanol working solution (WS) is first prepared by mixing 4 vol. of OptiPrep™ with 2 vol. of Solution A that contains 3x the required buffer concentration (see Box 1). Gradient solutions of the appropriate density are then produced from the WS by further dilution with Solution B. Densities of solutions produced in this manner are given in Table 1.

BOX 1

Keep HEPES (free acid) or Tricine as a 100 mM stock solution at 4°C; Tricine (1.79g) or HEPES (2.38g) per 100 ml water.

Solution A: Dissolve 0.85g NaCl in 50 ml water; add 30 ml of Tricine or HEPES stock solution; adjust to pH 7.4 with 1 M NaOH and make up to 100 ml.

Solution B: Dissolve 0.85g NaCl in 50 ml water; add 10 ml of Tricine or HEPES stock solution; adjust to pH 7.4 with 1 M NaOH and make up to 100 ml.

Table 1: Density and refractive index of iodixanol solutions*

Density (ρ)	% Iodixanol	WS + Soln. B	RI (η)	Density (ρ)	% Iodixanol	WS + Soln. B	RI (η)
1.037	6.0	0.6 + 3.4	1.3444	1.132	24.0	2.4 + 1.6	1.3726
1.048	8.0	0.8 + 3.2	1.3475	1.142	26.0	2.6 + 1.4	1.3757
1.058	10.0	1.0 + 3.0	1.3507	1.153	28.0	2.8 + 1.2	1.3788
1.069	12.0	1.2 + 2.8	1.3538	1.163	30.0	3.0 + 1.0	1.3820
1.079	14.0	1.4 + 2.6	1.3569	1.174	32.0	3.2 + 0.8	1.3851
1.090	16.0	1.6 + 2.4	1.3601	1.184	34.0	3.4 + 0.6	1.3882
1.100	18.0	1.8 + 2.2	1.3632	1.195	36.0	3.6 + 0.4	1.3914
1.111	20.0	2.0 + 2.0	1.3663	1.205	38.0	3.8 + 0.2	1.3945
1.121	22.0	2.2 + 1.8	1.3694	1.215	40.0		1.3976

* Density values are in $g.ml^{-1}$, iodixanol concentrations are % (w/v), WS + Soln. B figures are the volume ratios of a 40% (w/v) iodixanol and working solution and Solution B (see text above), RI = refractive index

Any other organic buffer (e.g. Tricine) may be substituted for HEPES. Low concentrations (1-2 mM) of other additives such as Mg^{2+} or Ca^{2+} in Solution B can also be maintained constant in the gradient by inclusion in Solution A at 3-6 mM. The density and refractive index of such solutions will

only be marginally altered from the figures in Table 1. The osmolality of all these gradient solutions will be in the range 285-305 mOsm. If maintenance of a constant buffer and/or divalent cation concentration is deemed unnecessary the OptiPrep™ may simply be diluted directly with Solution B (with or without low concentrations of divalent cations). There will only be marginal differences in the density and osmolality of the gradient solutions (compared to those in Table 1).

5. Use of balanced salt and culture media

Working solutions and gradient solutions may also be prepared by diluting OptiPrep™ directly with any cell suspension media such as Hanks Balanced Salt Solution (HBSS) or serum-free culture medium (e.g. RPMI or DMEM) which have the same density as Solution B (approx. 1.006 g/ml). The density of the gradient solutions may be marginally higher (up by approx. 0.001 g/ml) than those given in Table 1. Refractive indices may also be slightly higher (by 0.0001-0.0004). The osmolality of all solutions will again be in the range 285-305 mOsm.

6. Use of serum-containing media

A standard cell culture medium containing 10% (v/v) serum may also be used to dilute OptiPrep™ directly; because the density of the diluent (approx 1.009 g/ml) is higher than that of any balanced salt medium, the density of the gradient solutions will be correspondingly higher. Table 2 compares the densities of selected gradient solutions produced by RPMI with and without the serum.

	Density (g/ml)	
% iodixanol	No serum	10% serum
10	1.058	1.061
15	1.084	1.087
20	1.110	1.113
25	1.137	1.139
30	1.163	1.165
35	1.189	1.191
40	1.215	1.216

Table 2. Comparison of density of solutions produced by the dilution of OptiPrep™ with RPMI, with and without 10% (v/v) serum

7. Adjusting the density of a cell suspension

The 40% (w/v) iodixanol working solution is also suitable for adding to a cell suspension in order to adjust its density. Although in many cases it is acceptable to add OptiPrep™ directly to a cell suspension. The advantage of adding OptiPrep™ is that smaller volumes are required to achieve the appropriate density, a disadvantage (particularly if relatively large volumes need to be added), may be that it is unbuffered. In such cases a buffered working solution may be preferred.

8. Customized cell suspension media

Occasionally it will be necessary to use a medium, whose composition is quite different to either a simple buffered saline or a balanced salt solution, for example the double-strength University of Wisconsin (UWS) solution used for isolation of pancreatic Islets of Langerhans. As long as the density of the diluent is known then the equation below is used to calculate the density of any solution produced from the diluent and a working or stock solution of iodixanol.

Equation:

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

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

- Sometimes maintaining a suitable ionic strength in the gradient media is more important than keeping them precisely isoosmotic. In the purification of monocytes for example the diluent should contain 1% NaCl rather than 0.85%.