DENSITY GRADIENT MEDIA

OptiPrep™ Preparation of gradient solutions

Measurement and calculation of densities

The density of OptiPrep™ solutions can be determined directly and accurately by weighing a known volume using a density bottle. Weighing a series of aliquots dispensed from an automatic pipette (calibrated with water) on an electronic balance is a permissible but less accurate alternative. However the most convenient way of determining the density is to measure the refractive index. Another method for determining the density of a solution is to measure its absorbance. If a solution of iodixanol of known density is mixed with a diluent (whose density is also known), the density of the mixture can be calculated from the following equation:

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

D = Density of mixture; V = volume of iodixanol solution; d = density of iodixanol solution; V_1 = volume of diluent; d_1 = density of diluent.

OptiPrep™ working and gradient solutions for mammalian cells

An illustrative example for preparing working solutions for cells, based on a cell suspension medium (CSM) containing 0.85% (w/v) NaCl, 10 mM Tricine-NaOH, pH 7.4, is given in the Table 1 below. Table 2 describes the density of gradient solutions prepared by dilution of the working solution with CSM.

Hepes-buffered media provide solutions of identical densities and refractive indices. The 40% iodixanol Working Solution contains 10 mM Tricine-NaOH, pH 7.4, hence dilutions of this solution with the CSM will contain the same buffer concentration. If it was also necessary to maintain a low concentration of another reagent (e.g. 1 mM $CaCl_2$) in the gradient, the OptiPrep™ diluent used to prepare the Working Solution would also contain 3 mM $CaCl_2$. Low concentrations of such additives will not significantly change the osmolality of the gradient solutions.

If it is not necessary to maintain a constant ionic and buffer composition in the gradient, isoosmotic solutions can be prepared simply by diluting OptiPrep™ with an isoosmotic salt medium. This diluent can be the standard buffered 0.85 (w/v) NaCl or it can be a routine culture medium such as DMEM or RPMI. All these media (in the absence of added serum) have densities of 1.005-1.006 g/ml (Table 3). A culture medium containing 10% serum has a slightly higher density at approx 1.009 g/ml.

Because most mammalian cells have densities below 1.12 g/ml, a convenient Working Solution (ρ = 1.163 g/ml) containing 30% iodixanol can also be made from equal volumes of OptiPrep™ and a buffered saline or culture medium.

Table 1
OptiPrep™ Working Solutions for mammalian cells

Cell suspension medium (CSM): 0.85% (w/v) NaCl, 10 mM Tricine-NaOH, pH 7.4

To prepare a 40% (w/v) iodixanol Working Solution (ρ = 1.215 g/ml; η = 1.3976):
Diluent required: 0.85% (w/v) NaCl, 30 mM Tricine-NaOH, pH 7.4.
Mix 2 vol of OptiPrep™ with 1 vol of diluent.

To prepare a 30% (w/v) iodixanol Working Solution (ρ = 1.163 g/ml; η = 1.3820):
Diluent required: 0.85% (w/v) NaCl, 20 mM Tricine-NaOH, pH 7.4.
Mix 1 vol of OptiPrep™ with 1 vol of diluent.

Dilute Working Solutions with CSM to make gradient solutions (Table 2)

Table 2
Properties of iodixanol-NaCl/Tricine solutions

% iodixanol	η	ρ (g/ml)	WS (ml)	CSM (ml)
6.00	1.3444	1.037	0.6	3.4
8.00	1.3475	1.048	0.8	3.2
10.00	1.3507	1.058	1.0	3.0
12.00	1.3538	1.069	1.2	2.8
14.00	1.3569	1.079	1.4	2.6
16.00	1.3601	1.090	1.6	2.4
18.00	1.3632	1.100	1.8	2.2
20.00	1.3663	1.111	2.0	2.0
22.00	1.3694	1.121	2.2	1.8
24.00	1.3726	1.132	2.4	1.6
26.00	1.3757	1.142	2.6	1.4
28.00	1.3788	1.153	2.8	1.2
30.00	1.3820	1.163	3.0	1.0
32.00	1.3851	1.174	3.2	0.8
34.00	1.3882	1.184	3.4	0.6
36.00	1.3914	1.195	3.6	0.4
38.00	1.3945	1.205	3.8	0.2
40.00	1.3976	1.215	4.0	0

WS = 40% (w/v) iodixanol working solution; CSM = cell suspension medium η = ref. index; ρ = density. The osmolality of the gradient solutions is in the range 285-300 mOsm.

Table 3
Properties of iodixanol-DMEM solutions

% iodixanol	η	ρ (g/ml)	WS (ml)	DMEM(ml)
6.00	1.3448	1.038	0.6	3.4
8.00	1.3479	1.049	0.8	3.2
10.00	1.3510	1.059	1.0	3.0
12.00	1.3541	1.070	1.2	2.8
14.00	1.3572	1.080	1.4	2.6
16.00	1.3603	1.090	1.6	2.4
18.00	1.3633	1.101	1.8	2.2
20.00	1.3664	1.111	2.0	2.0
22.00	1.3695	1.122	2.2	1.8
24.00	1.3726	1.132	2.4	1.6
26.00	1.3757	1.143	2.6	1.4
28.00	1.3788	1.153	2.8	1.2
30.00	1.3819	1.163	3.0	1.0
32.00	1.3850	1.174	3.2	0.8
34.00	1.3881	1.184	3.4	0.6
36.00	1.3912	1.195	3.6	0.4
38.00	1.3943	1.205	3.8	0.2
40.00	1.3974	1.216	4.0	0

WS = 40% iodixanol working solution (4 vol of OptiPrep™ + 2 vol DMEM), η = refractive index; ρ = density. The osmolality of the gradient solutions is in the range 290-305 mOsm.

In some instances media customized to the maintenance of viability of a particular cell type are recommended (e.g. Gey's Solution for hepatocytes or University of Wisconsin Solution for Islets of Langerhans). Such media are described fully in the appropriate OptiPrep™ Application Sheets.

OptiPrep™ working and gradient solutions for mammalian organelles

A widely-used general purpose homogenization medium (HM) is 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) can also be used as the basis for preparing the gradient solutions. For the preparation of some organelles there are minor variations which will not affect the overall density of the solutions. For fractionating the endoplasmic reticulum, the EDTA is often omitted and for peroxisomes, 0.1% (v/v) ethanol is included in the HM. For the isolation of nuclei however a medium containing KCl and $MgCl_2$ is recommended for the retention of structural and functional integrity; a common one is 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.8. While for mitochondria it may be desirable to use mannitol as an osmotic balancer rather than sucrose.

Table 4 describes the methods for preparing Working Solutions based on these HMs and Tables 5-7 describe the properties of the gradient solutions produced from them. As with the preparation of density gradient solutions for cells, sometimes OptiPrep™ is simply diluted with an appropriate homogenisation medium.

As OptiPrep™ (for dilution purposes) is approximately isoosmotic, all diluents should contain normal concentrations of osmotic balancer (e.g. 0.25 M sucrose). In contrast 2.5 M sucrose (i.e. a viscous solution close to saturation) is required to produce a working solution from Percoll®. Other organic buffers of the same concentration can be used with no change to the density or osmotic properties of the solutions.

Table 4
OptiPrep™ Working Solutions for mammalian organelles

Homogenization media (HM):
General purpose (HM1): 0.25 M sucrose, 1 mM EDTA 10 mM Tris-HCl, pH 7.4 (ρ = 1.030 g/ml)
Nuclei (HM2): 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.8 (ρ = 1.033 g/ml)
Mitochondria (HM3): 4.4% (w/v) mannitol, 1 mM EDTA 10 mM Tris-HCl, pH 7.4 (ρ = 1.015 g/ml)

To prepare a 50% (w/v) iodixanol Working Solution (WS1-3):
Mix 5 vol. of OptiPrep™ with 1 vol. of the following diluents.
For WS1: 0.25 M sucrose, 6 mM EDTA, 60 mM Tris-HCl, pH 7.4.
For WS2: 150 mM KCl, 30 mM $MgCl_2$, 120 mM Tris-HCl, pH 7.8
For WS3: 4.4% (w/v) mannitol, 6 mM EDTA 60 mM Tris-HCl, pH 7.4

WS1: ρ = 1.272 g/ml; η = 1.4147
WS2: ρ = 1.269 g/ml; η = 1.4148
WS3: ρ = 1.269 g/ml; η = 1.4139

Dilute WS with appropriate HM to make gradient solutions (Tables 5-7)

Table 5
Properties of iodixanol-sucrose solutions

% iodixanol	η	ρ (g/ml)	WS1 (ml)	HM1(ml)
6.00	1.3534	1.059	1.2	8.8
8.00	1.3562	1.069	1.6	8.4
10.00	1.3589	1.079	2.0	8.0
12.00	1.3617	1.088	2.4	7.6
14.00	1.3645	1.098	2.8	7.2
16.00	1.3673	1.107	3.2	6.8
18.00	1.3701	1.117	3.6	6.4
20.00	1.3729	1.127	4.0	6.0
22.00	1.3757	1.136	4.4	5.6
24.00	1.3785	1.146	4.8	5.2
26.00	1.3813	1.156	5.2	4.8
28.00	1.3840	1.165	5.6	4.4
30.00	1.3868	1.175	6.0	4.0
32.00	1.3896	1.185	6.4	3.6
34.00	1.3924	1.194	6.8	3.2
36.00	1.3952	1.204	7.2	2.8
38.00	1.3980	1.214	7.6	2.4
40.00	1.4008	1.223	8.0	2.0
42.00	1.4036	1.233	8.4	1.6
44.00	1.4064	1.243	8.8	1.2
46.00	1.4091	1.252	9.2	0.8
48.00	1.4119	1.262	9.6	0.4
50.00	1.4147	1.272		

WS = working solution, HM = homogenization medium; η = ref. inde
ρ = density The osmolality of the gradient solutions is in the range 295-310 mOsm.

Table 6
Properties of iodixanol-sucrose-KCl-$MgCl_2$ solutions

% iodixanol	η	ρ (g/ml)	WS2 (ml)	HM2(ml)
10.0	1.3604	1.080	2.0	8.0
20.0	1.3740	1.127	4.0	6.0
30.0	1.3876	1.175	6.0	4.0
40.0	1.4012	1.222	8.0	2.0
50.0	1.4148	1.269		

WS = working solution, HM = homogenization medium; η = ref. inde
ρ = density The osmolality of the gradient solutions is in the range 320-360 mOsm.

Table 7
Properties of iodixanol-mannitol solutions

% iodixanol	η	ρ (g/ml)	WS3 (ml)	HM3(ml)
10.0	1.3548	1.065	2.0	8.0
20.0	1.3796	1.116	4.0	6.0
30.0	1.3844	1.167	6.0	4.0
40.0	1.3991	1.218	8.0	2.0
50.0	1.4139	1.269		

WS = working solution, HM = homogenization medium; η = ref.inde
ρ = density The osmolality of the gradient solutions is in the range 292-310 mOsm.

OptiPrep™ working and gradient solutions for non-mammalian organelles

Homogenates of yeast and plant tissue and the solutions used to isolate organelles from these sources frequently contain either mannitol or sorbitol at concentrations (and osmolalities) significantly higher than those used for mammalian systems. The isolation of protoplasts or spheroplasts 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. The relationship between osmolality and concentration of sorbitol solutions in water is given in Table 8. Sucrose may also be used at up to 0.5 M.

Table 8
Density (ρ), refractive index (η) and osmolality (σ) of sorbitol solutions in 10 mM Tris-HCl, pH 7.4

% sorbitol	η	ρ (g/ml)	σ (mOsm)
4.40	1.3390	1.015	265
8.75	1.3455	1.029	525
10.50	1.3480	1.035	657
12.25	1.3505	1.041	774
17.50	1.3580	1.059	1200

The general strategy is to produce a Working Solution of the correct osmolality by diluting OptiPrep™ with a sorbitol (or mannitol) containing diluent and then diluting this solution with a solution of the same osmolality. Table 9 describes the preparation and properties of sorbitol solutions in 10 mM Tris-HCl, pH 7.4 of osmolalities of approx 550 and 750 mOsm. The densities of gradient solutions with these two osmolalities are given in Tables 10 and 11 respectively.

Web:

www.axis-shield-density-gradient-media.com

AXIS-SHIELD

PO Box 6863 Rodelokka
N-0504 Oslo
Norway
Phone: +47 24 05 60 00
Fax: +47 24 05 60 10
Email: bjh@no.axis-shield.com or
john@jgrescon.fsbusiness.co.uk

Table 9
OptiPrep™ Working Solutions (sorbitol) for non-mammalian organelles

A 550 mOsm Working Solution
Suspension medium (SMA): 8.75% (w/v) sorbitol, 1 mM EDTA,10 mM Tris-HCl, pH 7.4

To prepare a 40% (w/v) iodixanol Working Solution (WSA)
Diluent required: 12.25% sorbitol, 3 mM EDTA, 30 mM Tris-HCl, pH 7.4.
Mix 2 vol of OptiPrep™ with 1 vol of diluent

Properties of 40% iodixanol WSA
ρ = 1.225 g/ml; η = 1.4020; σ = 545 mOsm.

Dilute WSA with SMA to make solutions of lower densities (Table 10)

B 750 mOsm Working Solution
Suspension medium (SMB): 12.25% (w/v) sorbitol, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4

To prepare a 40% (w/v) iodixanol Working Solution (WSB)
Diluent required: 17.5% sorbitol, 3 mM EDTA, 30 mM Tris-HCl, pH 7.4.
Mix 2 vol of OptiPrep™ with 1 vol of diluent

Properties of 40% iodixanol WSB
ρ = 1.231 g/ml; η = 1.4032; σ = 756 mOsm.

Dilute WSB with SMB to make solutions of lower densities (Table 11)

Table 10
Properties of iodixanol-sorbitol gradient solutions (550 mOsm)

% iodixanol	η	ρ (g/ml)	WSA (ml)	SMA (ml)
6.00	1.3536	1.059	0.6	3.4
8.00	1.3565	1.068	0.8	3.2
10.00	1.3596	1.078	1.0	3.0
12.00	1.3622	1.088	1.2	2.8
14.00	1.3650	1.098	1.4	2.6
16.00	1.3679	1.108	1.6	2.4
18.00	1.3707	1.117	1.8	2.2
20.00	1.3736	1.127	2.0	2.0
22.00	1.3764	1.127	2.2	1.8
24.00	1.3792	1.147	2.4	1.6
26.00	1.3821	1.157	2.6	1.4
28.00	1.3849	1.166	2.8	1.2
30.00	1.3878	1.176	3.0	1.0
32.00	1.3906	1.186	3.2	0.8
34.00	1.3935	1.196	3.4	0.6
36.00	1.3963	1.205	3.6	0.4
38.00	1.3992	1.215	3.8	0.2
40.00	1.4020	1.225	4.0	0

WS = working solution, SM = suspension medium; η = ref. index ρ = density The osmolality of the gradient solutions is in the range 545-560 mOsm.

Table 11
Properties of iodixanol-sorbitol gradient solutions (750 mOsm)

% iodixanol	η	ρ (g/ml)	WSB (ml)	SMB (ml)
6.00	1.3584	1.070	0.6	3.4
8.00	1.3610	1.079	0.8	3.2
10.00	1.3637	1.089	1.0	3.0
12.00	1.3663	1.098	1.2	2.8
14.00	1.3689	1.108	1.4	2.6
16.00	1.3716	1.117	1.6	2.4
18.00	1.3742	1.127	1.8	2.2
20.00	1.3769	1.136	2.0	2.0
22.00	1.3795	1.146	2.2	1.8
24.00	1.3821	1.155	2.4	1.6
26.00	1.3848	1.165	2.6	1.4
28.00	1.3874	1.174	2.8	1.2
30.00	1.3900	1.184	3.0	1.0
32.00	1.3927	1.193	3.2	0.8
34.00	1.3953	1.203	3.4	0.6
36.00	1.3979	1.212	3.6	0.4
38.00	1.4006	1.222	3.8	0.2
40.00	1.4032	1.231	4.0	0

WS = working solution, SM = suspension medium; η = ref. index; ρ = density. The osmolality of the gradient solutions is in the range 755-850 mOsm.

For more information regarding OptiPrep™ and its applications see the OptiPrep™ Application CD or online at:: www.axis-shield-density-gradient-media.com

References are available on request

Percoll is a trademark of GE Healthcare companies

AXIS-SHIELD
Innovation for Life