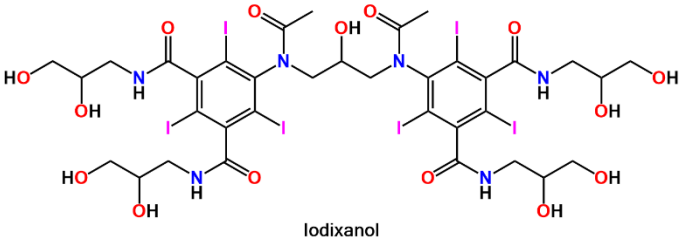
Optiprep™

PRODUCT DESCRIPTION

OptiPrep™ is a ready-made, sterile and endotoxin tested solution of Iodixanol, designed for the in vitro isolation of biological particles.
The systematic name of Iodixanol is 5,5'-[(2-hydroxy-1-3 propanediyl)-bis(acetylamino)] bis [N,N'- bis(2,3dihydroxypropyl-2,4,6-triiodo-1,3-benzenecarboxamide].



Iodixanol
M_w = 1550 g/mol

Composition:

Iodixanol: 60% (w/v) in water

Physical-chemical characteristics:

Density: 1.320 ± 0.001 g/ml (20°C)
Osmolality: 170 ± 15 mOsm

OPTIPREP™ WORKING AND GRADIENT SOLUTIONS FOR CELLS

An illustrative example of 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 Table 1. 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. Low concentrations of additives (e.g. 1-5 mM $MgCl_2$ or 1-2 mM EDTA) can be included without significant effect on the density or osmolality of the solutions.

Table 1: OptiPrep™ Working Solutions for mammalian cells

Cell suspension medium (CSM): 0.85% (w/v) NaCl, 10 mM Tricine-NaOH, pH 7.4 (ρ = 1.006 g/ml)
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 Solution with CSM to make gradient solutions (Table 2)

Table 2: Properties of Iodixanol-NaCl/Tricine solutions[1]

% Iodixanol (w/v)	η	ρ (g/ml)	40% Iodixanol 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

[1] WS = working solution CSM = cell suspension medium
η = refractive index ρ = density
The osmolality of the gradient solutions is in the range 285-300 mOsm.

Alternatively, isoosmotic solutions can be prepared simply by diluting OptiPrep™ with a buffered 0.85 (w/v) NaCl or a routine culture medium such as Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute Medium (RPMI). All these media (in the absence of added serum) have densities of 1.006-1.007 g/ml (Table 3). A culture medium containing 10% serum has a slightly higher density at approximately 1.009 g/ml.

Table 3: Properties of Iodixanol-DMEM solutions[1]

% Iodixanol (w/v)	η	ρ (g/ml)	40% Iodixanol 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

[1] WS = 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.

OPTIPREP™ WORKING AND GRADIENT SOLUTIONS FOR MAMMALIAN ORGANELLES

Table 4 describes the preparation of a general purpose Working Solution for mammalian organelles and also modifications for mitochondria and nuclei. Other minor variations such as omission of the EDTA or inclusion of 0.1% (v/v) ethanol or 1-5 mM DTT, or use of alternative buffers will not affect the density of osmolality of the solutions.

Table 4: OptiPrep™ Working Solutions for mammalian organelles

Homogenization media (HM1-3):
Nuclei (HM1): 0.25 M Sucrose, 25 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-HCl pH 7.8 (ρ = 1.033 g/ml)
General purpose (HM2): 0.25 M Sucrose, 1 mM EDTA 10 mM Tris-HCl pH 7.4, (ρ = 1.030 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 one of the following diluents:
Nuclei (WS1): 150 mM KCl, 30 mM $MgCl_2$, 120 mM Tris-HCl pH 7.8 (ρ = 1.012 g/ml)
General purpose (WS2): 0.25 M Sucrose, 6 mM EDTA, 60 mM Tris-HCl pH 7.4. (ρ = 1.032 g/ml)
Mitochondria (WS3): 4.4% (w/v) Mannitol, 6 mM EDTA 60 mM Tris-HCl pH 7.4 (ρ = 1.017 g/ml)

WS1: ρ = 1.269 g/ml; η = 1.4148
WS2: ρ = 1.272 g/ml; η = 1.4147
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-KCl-$MgCl_2$ solutions[1]

% Iodixanol (w/v)	η	ρ (g/ml)	50% Iodixanol WS1 (ml)	HM1 (ml)
10.00	1.3604	1.080	2.0	8.0
20.00	1.3740	1.127	4.0	6.0
30.00	1.3876	1.175	6.0	4.0
40.00	1.4012	1.222	8.0	2.0
50.00	1.4148	1.269	10.0	0.0

[1] WS = working solution HM = homogenization medium
η = refractive index ρ = density
The osmolality of the gradient solutions is in the range 320-360 mOsm.

Table 6: Properties of Iodixanol-Sucrose solutions[1]

% Iodixanol (w/v)	η	ρ (g/ml)	50% Iodixanol WS2 (ml)	HM2 (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	10.0	0.0

[1] WS = working solution HM = homogenization medium
η = refractive index ρ = density
The osmolality of the gradient solutions is in the range 295-310 mOsm.

Table 7: Properties of Iodixanol-Mannitol solutions[1]

% Iodixanol (w/v)	η	ρ (g/ml)	50% Iodixanol WS3 (ml)	HM3 (ml)
10.00	1.3548	1.065	2.0	8.0
20.00	1.3696	1.116	4.0	6.0
30.00	1.3844	1.167	6.0	4.0
40.00	1.3991	1.218	8.0	2.0
50.00	1.4139	1.269	10.0	0.0

[1] WS = working solution HM = homogenization medium
η = refractive index ρ = density
The osmolality of the gradient solutions is in the range 292-310 mOsm.

OPTIPREP™ WORKING AND GRADIENT SOLUTIONS FOR NON-MAMMALIAN ORGANELLES

Media containing 400-600 mM Mannitol or Sorbitol are common but for yeast organelles. The relationship between osmolality and concentration of Sorbitol solutions in water is given in Table 8.
The general strategy for production of gradient solutions of the correct osmolality is described in Tables 9-11.

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

% sorbitol (w/v)	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

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 (approx 550 mOsm)[1]

% Iodixanol (w/v)	η	ρ (g/ml)	40% Iodixanol 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	1.137	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

[1] WS = working solution SM = suspension medium
η = refractive index ρ = density
The osmolality of the gradient solutions is in the range 545-560 mOsm.

Table 11: Properties of Iodixanol-Sorbitol gradient solutions (approx 750 mOsm)[1]

% Iodixanol (w/v)	η	ρ (g/ml)	40% Iodixanol 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

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

STABILITY AND STORAGE

OptiPrep™ is stable for 3 years provided the solution is kept sterile and protected from light. Prolonged exposure to direct sunlight leads to release of iodine from the molecule. This effect is negligible when working with these solutions on a day to day basis. OptiPrep™ should be stored between 4°C - 24°C.
Before use invert the bottles several times to mix the contents.

ORDERING INFORMATION

OptiPrep™ prod. No. 1893 1 x 250 ml

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