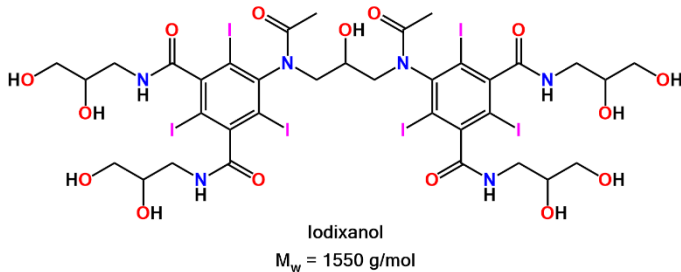


PRODUCT DESCRIPTION

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].



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 MgCl₂ 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 solutions¹

% 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 solutions¹

% 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 or 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-MgCl₂ solutions¹

% 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.

