

OptiPrep™ Application Sheet C38

Maintenance of cells in suspension for physical measurements

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ To access other Application Sheets referred to in the text return to the Cell Index; key Ctrl “F” and type the C-Number in the Find Box

1. Background

This is a non-centrifugal application, in which OptiPrep™ is mixed with a suspension of cells in order to prevent their sedimentation during an extended physical measurement or separation. The first published paper to report this novel application was in 2001, when Farinas, Chow and Wada [1] commented in the abstract to their paper: “*Recent developments in microfluidics have enabled the design of a lab-on-a-chip system capable of measuring cellular membrane potential. The chip accesses liquid samples sequentially by sipping from a micro-plate through a capillary, mixes the samples with cells flowing through a microchannel, contacts the cells with potential-sensitive dyes, and reads out cellular responses using fluorescence detection*”. Later Culbertson [2] noted that the use of OptiPrep™ enabled the rate of cell entry into the main chip channel of a microfluidic device to remain constant over the course of an entire experiment. The technique permits the identification of single cells with unique compositional characteristics that may presage the later development of a disease state. In its simplest form, a medium with the same density as the cell, which in no way interacts with the cell, maintains the cell in a state of continuous suspension.

Detailed descriptions of the experimentation is beyond the scope of this Application Sheet, which will confine itself in Section 2 to a brief description of solution preparation and the physical parameters of some the solutions that have been used in these studies. Section 3 summarizes the cell types that have been studied and types of physico-chemical measurements that were carried out.

2. Solutions required

- A. OptiPrep™ (shake the bottle gently before use)
- B. Buffered saline or balanced salt solution (e.g. Hank’s)

Mix OptiPrep™ with Solution B to give a solution of suitable density, according to the figures in Table 1. Table 2 summarizes a few of the conditions reported in the literature. For more information on making up solutions [see Application Sheet C01](#).

- ◆ The actual density required will depend on the cell type; it can be chosen by suspending the cells in a series of density solutions and observing the disposition of the cells after centrifugation. Because of the heterogeneity of any cell population however it is impossible to select a density that will allow all the cells to remain in suspension, some will float and some will sediment. If the cell suspension contains a variety of cell types the problem will be exaggerated. For example human peripheral blood mononuclear cells have a density range of 1.058-1.078 g/ml.

Table 1: Density of solutions produced by dilution of OptiPrep™ with an isotonic buffered saline solution

% (w/v) iodixanol	Refractive index	Density (g/ml)	% (w/v) iodixanol	Refractive index	Density (g/ml)
5.00	1.3429	1.032	11.00	1.3523	1.063
6.00	1.3444	1.037	12.00	1.3538	1.068
7.00	1.3459	1.043	13.00	1.3554	1.074
8.00	1.3475	1.048	14.00	1.3570	1.079
9.00	1.3491	1.053	15.00	1.3585	1.085
10.00	1.3507	1.058	16.00	1.3601	1.090

Table 2: Selected solution properties reported in the literature

Cell Type	Solution composition	Iodixanol (% w/v)	Density (g/ml)	Ref #
HeLa	PBS + 1% albumin	11.4	1.065	3
HeLa	Hank's Balanced Salt Solution	9.6	1.054	4
Human blood lymphocytes and THP-1 ¹ cells	Hank's Balanced Salt Solution	10.8	1.062	1
Human blood leukocytes	PBS	3-9	1.02-1.05 ²	5
Human erythrocytes	PBS	15-18	1.084-1.100	6
Human erythrocytes	PBS	17	1.095	7
Human erythrocytes	PBS/dextran ³	2.6		8
Human erythrocytes	PBS/5.5mM glucose/4% BSA	19	1.105	9
Jurkat/lymphocytes	PBS	10.8	1.062	2
Macrophages	PBS	8.4	1.052	10
Yeast	Water	19	1.105	11,12

¹ THP-1 cells are a human acute leukaemic monocytic line

² The densities were chosen to allow the cells to sediment slowly to the bottom of the chamber

³ The density is difficult to estimate because of the dextran content; the latter was added to raise the viscosity

3. Cell types and study topic

Breast cancer cells

Viability of cells in alginate particles [13]

Bronchial epithelial cells

Confocal light absorption and scattering spectroscopic (CLASS) microscopy [14]

CHO cells

Microfluidic cytometry [15]

Embryonic stem cells

Gene expression in single cells [16]

Erythrocytes

Blood viscosity changes in response to exercise [17]

Channel flow profiling [9]

Lysis kinetics [7]

Nitric oxide scavenging by haemoglobin [6,8]

Shape and mechanical properties of erythrocytes [18]

Fibroblasts

Hydrogel encapsulation [12]

Francisella tularensis

Cell sorting [10]

HeLa cells

Cell heterogeneity in metal ion responses [4]

Fluorescence-activated microfluidic devices for cell identification and sorting [3,19,20,21]

Hepatocarcinoma cells

Microfluidic cytometry [15]

Industrial effluent analysis

Membrane bioreactors [22]

Jurkat cells

Microfluidic cytometry [15]

Microfluidic devices for cell identification and sorting [2]

Leukocytes (human)

Continuous concentration of cells [23]

Microfluidic devices for cell identification and sorting; orientation-dependent elastic light scattering [5]

Lymphocytic cells (incl. lymphoblasts)

Cell membrane potential measurements using potential-sensitive dyes [1]

Cell size and density changes; response to growth factor deprivation [24]

Hydrogel encapsulation [12]

Mouse hybridoma cells

Analysis and sorting of single cells [25]

Mouse pro-B lymphoid cells

Cell size, density and deformability [26]

Rat liver lysosomes (stained with red tracker dye)

Confocal light absorption and scattering spectroscopic (CLASS) microscopy [14]

Review article

Recent advances in research involving chemical and biological reactions in microdroplets [27]

Yeast cells

Biogenic magnetization [28]

Hydrogel encapsulation [12]

Polyglycerol microgel particles for the micro-encapsulation of yeast cells [11]

4. References

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