CRYOPRESERVATION

An integral part of cell culture involves the successful preservation of important cell lines. A primary factor for success is developing an appropriate protocol for a particular cell line. A common misconception among scientists is that preserving methods for a given cell type are transferable to similar cell types. Although, this may be true on occassion, it is far from the general rule. Protocols require modification for each cell type. Another key factor for successful preservation is the use of an effective cryoprotectant. The discovery of glycerol as a preservation medium led to the rapid discovery of other protectants such as methanol, ethylene glycol, and DMSO (dimethyl sulfoxide).

With any cryopreservation process, the primary objective is maximum recovery with minimal damage to the cells. The absence of an effective cryoprotectant leads to the formation of intracellular ice crystals which puncture organelles and cell membranes. Also, as water within the medium freezes, cells become dehydrated altering the salt concentration and causing morphological or biological changes after recovery. By using a proven cryoprotectant and controlling the rate of temperature change, cell loss is minimized and cell recovery is optimized.

An often overlooked aspect of serum-free cell culture is the presence of both serum and DMSO in traditional cryopreservation media. ICN's serum-free, DMSO-free preservation medium, Cellvation™, eliminates the cytotoxic risks and extends the amount of time cells can be exposed to the cryoprotectant while avoiding subcellular biochemical changes and differentiation artifacts. Additionally, the integrity of the defined serum-free environment is maintained and incidential exposure to serum is eliminated. It is equally effective for serum-supplemented cultures.

Alternatively, ICN offers Opti-Freeze™, a traditional DMSO, serum based ready-to-use preservation medium that provides optimal recovery and minimizes the damage associated with freezing and storing cells. All sterility testing is performed to USP XXIII guidelines, and each lot is negative for mycoplasma infection. Protection and recovery efficiencies are monitored using three cell types.

Cellvation™ Cryopreservation Medium

Superior cell recovery and faster cell attachment after thawing
Ready-to-use preserving medium
Contains NO serum, DMSO or other cytotoxic agents
Eliminates cytotoxic risks associated with DMSO
Reduces preparation and centrifugation steps
Recommended storage - 4-8°C

Product	Catalog No.	Quantity
Cellvation™	2030046	20 ml
	20300M2	60 ml

Opti-Freeze™ DMSO Preservation Medium

Ready-to-use preserving medium that contains serum and DMSO
Maximizes recovery while minimizing cell damage
Sterile filtered through a capsule filter of 0.2 μm
Reduces cytotoxic risks associated with DMSO
Saves time and reduces expense
Endotoxin - <1 ng/ml determined by LAL assay
Thoroughly tested for sterility and performance
Recommended storage: -20°; stable for a minimum of 3 years

Product	Catalog No.	Quantity
Opti-Freeze™	2030148	50 ml

Cryoprotectant Chemicals

196055 RT **DIMETHYL SULFOXIDE** — 25 ml, 100 ml, 500 ml, 1 liter
[67-68-5]
(DMSO)
Cell Culture Reagent
Purity: ≥99%
Ideal for use as a cryoprotectant.
NOT FOR HUMAN USE!
C_2H_6SO MW 78.13

151089 RT **ETHYLENE GLYCOL** — 250 ml, 1 liter
[107-21-1]
Purity: 99% min.
1 ml = approx. 1.11 g
$C_2H_6O_2$ MW 62.1

194672 RT **D-(+)-GLUCOSE** — 100 g, 1 kg, 5 kg
[50-99-7]
(Dextrose; Corn sugar)
Cell Culture Reagent
Anhydrous
Crystalline
$C_6H_{12}O_6$ MW 180.2

194680 RT **GLYCEROL** — 100 ml, 500 ml
[56-81-5]
Cell Culture Reagent
Highly purified
Purity: 99+%
Ideal for use as a cryoprotectant.
$C_3H_8O_3$ MW 92.09

152540 RT **D-MANNITOL, ACS** — 250 g, 500 g, 1 kg
[69-65-8]
ACS Reagent Grade
$C_6H_{14}O_6$ MW 182.2

155387 RT **METHANOL** — 500 ml, 1 liter
[67-56-1]
Spectrophotometric Grade
Purity: 99+%
1 ml = approx. 0.79 g.
CH_3OH MW 32.04

PHOSPHATE BUFFERED SALINE
(1X PBS Liquid)
Dulbecco's Formula
Storage temperature: 15-30°C
1860049 — 100 ml
1860054 — 500 ml

196056 RT **POLYVINYLPYRROLIDONE** — 100 g, 500 g
[9003-39-8]
(PVP 10)
Average MW 10,000
K Value (intrinsic viscosity): 12-18
Prepared for use as a cryoprotectant. Also, it is well suited for plant cell culture.

102787 RT **POLYVINYLPYRROLIDONE** — 100 g, 500 g, 1 kg, 5 kg
[9003-39-8]
(PVP K 90)
MW AVERAGE 360,000

151957 RT **PROPYLENE GLYCOL** — 500 ml, 1 liter
[57-55-6]
(1,2-Propanediol)
1 ml = approx. 1.04 g
$C_3H_8O_2$ MW 76.1

194747 RT **SUCROSE** — 500 g, 1 kg, 5 kg
[57-50-1]
Cell Culture Reagent
Crystalline
$C_{12}H_{22}O_{11}$ MW 342.3