Cryopreservation

New Products

Cell Freezing Media and Reagents

ECACC Cell Lines

Protocol for the Cryopreservation of Cell Lines

Cryoware
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Introduction 3

New Products 4
CryoStor™ 4
HypoThermosol® 5
Sericin 6

Cell Freezing Media and Reagents 8

ECACC Cell Lines 11

Protocol for the Cryopreservation of Cell Lines 14

Cryoware 16
Cryovial Color Coders 16
Cryo Containers 16
Cryo Storage Racks 17
Cryo Vials 18
Benchtop Coolers 19

Cryo Accessories 19
Aldrich® wide-mouth dewar flasks 19
Barnstead/Thermolyne® Dewars, Biocane™ Series 20
Barnstead/Thermolyne® Dewars, Locator® Series 21
Nalgene® Dewars 21
Taylor-Wharton Dewars 21
Taylor-Wharton Dewar Accessories 22
Wide-mouth Dewars 22
Wide-mouth Dewars with Silvered Glass Liner 22

Technical content: Don Finley, M. Sc.
Freezing living cells with the hope of full recovery began with the freezing of sperm in glycerol in 1949. Later DMSO was shown to be a better preservative as it can better permeate cells like red blood cells. Cryopreservation for all practical purposes is limited to individual cells or small clumps of cells. So preserving mammalian cells, plant cells, blood, sperm, and embryos is routine practice for clinicians and researchers. DMSO has proven to be the most robust cryopreservative agent and the most widely used. DMSO has some drawbacks in that it is cytotoxic and given that it permeates cells, it is difficult to completely eliminate. There are a number of non-permeating cryoprotectants that have also proven effective such as hydroxyethyl starch, PVP and CMC. Typically non-permeating cryoprotectants are not as efficient at preserving cell viability and are often used in concert with DMSO to enhance the efficiency of cryoprotection.

There are two common methods of cryopreservation, controlled rate freezing and vitrification. Controlled rate freezing typically involves placing the cells in a cryoprotectant media and cooling at a rate of −1 to −3 °C per minute, to about −80 °C and finally storing at −196 °C in liquid nitrogen. In this method, ice crystals form outside of cells first, and the higher concentrations of solutes that are excluded from ice crystals draw water from the cells and prevent or minimize freezing inside the cells. Controlled rate freezing is the most commonly practiced method, as it has proven to be robust and widely applicable. However, the osmotic changes and ice crystals formed using this technique can lead to reduced recovery and cryopreservation-induced delayed-onset cell death. Vitrification involves rapid freezing in higher concentration of cryoprotectants. The higher concentrations of cryoprotectants and rapid freezing prevent the formation of crystalline ice and promote the formation of an amorphous ice or glass. Unfortunately, vitrification is not without some problems as near toxic concentrations of DMSO are often required to promote the formation of a glass. Furthermore at this time the conditions required to achieve vitrification vary from application to application.

Sigma offers a wide selection of equipment and reagents for your cryopreservation needs that include DMSO, prepared freezing media and Mr. Frosty Freezers. In addition, we recently introduced CryoStor™ a completely optimizable serum free cryopreservation system, HypoThermosol®, a highly protective media for storage of cells and tissues at 2–8 °C and will soon offer sericin, a protein from silkworm that has proved to be a valuable FBS replacement in cryopreservation.

References
New Products

CryoStor™

The CryoStor line of serum free cryoprotective products allow higher recovery rates than using media with 10% FBS and DMSO (Figure 1). CryoStor allows researchers to optimize, and if necessary, minimize the amount of DMSO needed (Figure 2). The CryoStor line is serum-free so concerns of viruses, BSE or other FBS/protein-based contaminations are eliminated. In addition, CryoStor is robust and has been tested with the following cell types.

- Liver Cells (Hepatocytes & C3A) Cardiomyocytes
- Stem Cells Hybridomas
- PBMC Pancreatic Islets
- Cord Blood Skeletal Muscle Cells (SKMC)
- T-Cells Smooth Muscle Cells (CASMС)
- Lymphocytes PBSC
- Coronary Artery Endothelial Cells (CAEC) Chondrocytes
- Cancer Cells (PC3, LNCaP) CHO
- Skeletal Myoblasts C2C12
- Neuronal Cells Ocular (BCE, HCE)
- Keratinocytes (A431) Engineered Tissue
- Renal Cells (RPTEC, MDCK) Organ / Tissue
- Fibroblasts (NHDF, 3T3) Vascular Tissue
- Transfected Cells Hair Follicle
- HEK Dendritic Cells

CryoStor is ideal for cryopreservation applications that must be serum free and where high recovery is critical.

CryoStor™ cell cryopreservation media

CryoStor, a series of cell-specific, optimized preservation media, is uniquely formulated to address the molecular and biological aspects of cells during the cryopreservation process thereby directly reducing the level of Cryopreservation-Induced Delayed-Onset Cell Death and improving post-thaw cell viability and function.

- Store at: 2–8 °C

- CS2
  CryoStor CS2 is formulated to contain 2% dimethyl sulfoxide (DMSO). Suggested when reducing DMSO is of primary concern.
  C3124-100ML 100 mL

- CS5
  CryoStor CS5 is formulated to contain 5% dimethyl sulfoxide (DMSO). Recommended for cryopreservation of most cell types.
  C2999-100ML 100 mL

- CS10
  CryoStor™ CS10 is a uniquely formulated cryopreservation medium containing 10% dimethyl sulfoxide (DMSO). Recommended for the preservation of hepatocytes, tissue samples and other extremely sensitive cell types.
  C2874-100ML 100 mL

Figure 1. Viability of NHDF cells following exposure to cell culture media + 5% DMSO or CryoStor + 5% DMSO (CS5). Solution efficacy was investigated following a 10 minute hold at 2–8 °C, following ice-nucleation, and after LN2 storage. For each of the test conditions, samples were removed, thawed, diluted with media and plated. Relative cell viability was determined 1 day post-thaw and overall viability was compared to 37 °C non-cryopreserved control cultures.

Figure 2. Post-thaw viability of cells following cryopreservation with varying concentrations of DMSO in either cell culture media or CryoStor. Relative cell viability was determined 1 day post-thaw as described in the methods and overall viability was compared to 37 °C non-cryopreserved control cultures. DMSO concentration and vehicle solution impact cell viability post-thaw.
**HypoThermosol®**

**HypoThermosol®-FRS Preservation Solution**

HypoThermosol is an optimized hypothermic (2–8 °C) preservation media that enables improved and extended preservation of cells, tissues and organs. HypoThermosol-FRS is uniquely formulated to address the molecular-biological response of cells during the hypothermic preservation process. HypoThermosol-FRS includes key ions at concentrations that balance the intracellular state at hypothermic temperatures. Additional components include pH buffers, energy substrates, free radical scavengers, and osmotic/oncotic stabilizers.

*store at 2–8 °C*

**H4416-100ML** 100 mL

**Figures 1 and 2** clearly show that HypoThermosol outperforms commonly used hypothermic preservation media on Human Mesenchymal Stem Cells (hMSC) and on Human Dermal Fibroblasts. In addition to these cell lines, hypothermosol has been tested on the following cell lines, Tissues and organs:

- Liver Cells (Hepatocytes & C3A) Cardiomyocytes
- Stem Cells Hybridomas
- PBMC Pancreatic Islets
- Cord Blood Skeletal Muscle Cells (SKMC)
- T-Cells Smooth Muscle Cells (CASMC)
- Lymphocytes PBSC
- Coronary Artery Endothelial Cells (CAEC) Chondrocytes
- Cancer Cells (PC3, LNCaP) CHO
- Skeletal Myoblasts C2C12
- Neuronal Cells Ocular (BCE, HCE)
- Keratinocytes (A431) Engineered tissue
- Renal Cells (RPTEC, MDCK) Organ / Tissue
- Fibroblasts (NHDF, 3T3) Vascular Tissue
- Transfected Cells Hair Follicle
- HEK Dendritic Cells

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**Figure 1.** Cells were assayed for metabolic activity following 24 hours recovery post-preservation.

**Figure 2.** Cells were assayed for metabolic activity following 24 hours recovery post-preservation.
Sericin

Sericin is a protein derived from the silkworm cocoon that has a number of beneficial characteristics for culturing mammalian cells. It has cryoprotective properties that allow for replacement of FBS in cryopreservation media. Figures 1, 2 and 3 clearly show that 1% sericin (w/v) along with 0.5% (w/v) maltose, 0.3% (w/v) proline, 0.3% (w/v) glutamine and 10% DMSO is comparable to 90% FBS and 10% DMSO. Furthermore, sericin can act as a FBS replacement in cell culture and stimulate cell growth\(^2,3\) and in certain situations\(^4\), inhibit cell death.

Sericin Sigma Cat. No. SS201 Coming Soon! Check sigma-aldrich.com for availability.
Figure 3. PC12, NHDF, HEK or insect Sf-9 cells (1×10⁶) in 1 ml of freezing medium were frozen at –80 °C for 1 day. The vials were then thawed and the viability of the cells was determined. Freezing media: FBS supplemented with 10% DMSO (red bars); PBS supplemented with 1% sericin, 0.5% maltose, 0.3% proline, 0.3% glutamine and 10% DMSO (tan bars); and PBS supplemented with 10% DMSO (gray bars). The error bars indicate S.D. (n=3). *P<0.05.

References

Cell Freezing Media and Reagents

**Dimethyl sulfoxide**

DMSO, Methyl sulfoxide

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tbody>
<tr>
<td>mp</td>
<td>16 to 19 °C</td>
</tr>
<tr>
<td>vd</td>
<td>2.7 (vs air)</td>
</tr>
<tr>
<td>bp</td>
<td>189 °C</td>
</tr>
<tr>
<td>vp</td>
<td>0.42 mmHg (20 °C)</td>
</tr>
<tr>
<td>density</td>
<td>1.10 g/mL</td>
</tr>
<tr>
<td>n20/D</td>
<td>1.479</td>
</tr>
<tr>
<td>(lit (63 °F))</td>
<td>0.42 mmHg (20 °C)</td>
</tr>
</tbody>
</table>

≥99.7%, Hybri-Max™, sterile-filtered, hybridoma tested

DMSO is a polar aprotic solvent used in chemical reactions, in polymerase chain reactions (PCR) and as a cryoprotectant vitrification agent for the preservation of cells, tissues and organs. DMSO is used in cell freezing media to protect cells from ice crystal induced mechanical injury. It is used for frozen storage of primary, sub-cultured, and recombinant heteroploid and hybridoma cell lines; embryonic stem cells (ESC), and hematopoietic stem cells. DMSO is frequently used in the combinations with BSA or fetal bovine serum (FBS). 5 mL and 10 mL in flame sealed ampules, 100 mL in amber bottle. Endotoxin tested.

<table>
<thead>
<tr>
<th>Product</th>
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<tbody>
<tr>
<td>D2650-5X5ML</td>
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<tr>
<td>D2650-5X10ML</td>
<td>5 x 10 mL</td>
</tr>
<tr>
<td>D2650-100ML</td>
<td>100 mL</td>
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</tbody>
</table>

Biotechnology Performance Certified, sterile-filtered, hybridoma tested, meets USP testing specifications

Human and animal cell lines grown in culture are generally stored frozen. Freezing protects the cell line from changes due to genetic drift and minimizes risk of contamination. Liquid nitrogen used in conjunction with a cryoprotective agent such as DMSO is a widely used method for preserving cells. Without the presence of a cryoprotective agent, freezing is lethal to most mammalian cells. Damage is caused by mechanical injury by ice crystals, concentration of electrolytes, pH changes, and denaturation of proteins. These lethal effects are minimized by adding a cryoprotective agent which lowers the freezing point and allows for a slower cooling rate. BPC-tested DMSO also meets the requirements of biotechnology and tissue engineering applications.

**Features and Benefits**


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<tr>
<td>D2438-50ML</td>
<td>50 mL</td>
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</tbody>
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**Glycerol**

1,2,3-Propanetriol; Glycerin

FW 92.09

Glycerol is used both in sample preparation and gel formation for polyacrylamide gel electrophoresis. Glycerol (5 –10%) increases the density of a sample so that the sample will layer at the bottom of a gel’s sample well. Glycerol is also used to aid in casting gradient gels and as a protein stabilizer and storage buffer component.

mp.............................................20 °C
vd.............................................3.1 (vs air)
bp..................................................182 °C/20 mmHg
vp..................................................<1 mmHg (20 °C)
density........................................1.25 g/mL
n20/D...........................................1.474

≥99% (GC)

<table>
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<tbody>
<tr>
<td>G2025-100ML</td>
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<tr>
<td>G2025-500ML</td>
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**D-(+)-Trehalose dihydrate**

α-D-Glucopyranosyl-β-D-fructofuranosyl-α-D-glucopyranosyl [57-50-1] C12H22O11 · 2H2O FW 378.33

mp.........................................185 to 187 °C

Use as a cryoprotectant in a variety of cell freezing media.

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<tr>
<td>T0167-25G</td>
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**Sucrose**

α-D-Glucopyranosyl-β-D-fructofuranosyl-α-D-glucopyranosyl [57-50-1] C12H22O11 · 2H2O FW 378.33

mp.........................................185 to 187 °C

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<tr>
<td>S1888-5KG</td>
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Meets USP testing specifications

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<td>5 kg</td>
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<tr>
<td>S3929-10KG</td>
<td>10 kg</td>
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Insect cell culture tested

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<th>Weight</th>
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</thead>
<tbody>
<tr>
<td>S9031</td>
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</tr>
</tbody>
</table>
o-Sorbitol

**o-Glucitol**

[50-70-4] C₇H₁₄O₆ FW 182.17

May be used for washing spheroplasts² and in isoelectric focusing to minimize endoosmotic flow in agarose gels². May be used to induce osmotic stress.

mp.................................................98 to 100 °C
vp.................................................<0.1 mmHg (25 °C)
vd.................................................<1 (vs air)

>≥99.5% (HPLC)

Allelic variation of the Tas1r3 gene affects behavioral taste responses to this sugar alcohol, suggesting that it is a T1R3 receptor ligand.


Hydroxyethyl starch

[9005-27-0]

Degree of substitution, Average of 1 OH group per 10 glucose units converted to -OCH₂CH₂OH.

Prepared from corn starch

**Mₐ ~500,000**

May contain greater parts of lower molecular weight.

09184-10G-F 10 g
09184-50G-F 50 g
09184-250G-F 250 g

**DEXTRAN 500**

**Mₐ ~100,000**

May contain greater parts of lower molecular weight.

31392-10G 10 g
31392-50G 50 g
31392-250G 250 g

**Mₐ ~40,000**

narrow molecular weight distribution

68084-25G 25 g
68084-100G 100 g
68084-500G 500 g

Hydroxyectoine

(C₆H₁₀N₂O₃)ₙ

*Mr ~15,000–25,000*

**Ectoine**

Thi(β)-2-Methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid [96702-03-3] C$_{6}$H$_{10}$N$_{2}$O$_{2}$ FW 142.16

An osmoprotectant in a wide variety of microorganisms including heterotrophic, halophilic bacteria and non-halophilic bacteria such as *Streptomyces* species and *E. coli*. It also exerts protective effects in *E. coli* during drying and storage.

- **81619-1G-F**: 1 g
- **81619-10G-F**: 10 g
- **81619-100G-F**: 100 g

**Betaine**

Oxyurene, (Carboxymethyl)trimethylammonium inner salt [107-43-7] (CH$_{3}$)$_{2}$NCOO

- anhydrous, ≥98.0% (NT)
- water…………………………………………………………..≤1%
- mp…………………………………………………………..301 to 305 °C (dec.)

Polyvinylpyrrolidone

PVP, Polyvidone, Povidone [9003-39-8] (C$_{6}$H$_{9}$NO)$_{n}$

Polyvinylpyrrolidone is a component of Denhardt’s Solution and is included at a concentration of 1% (w/v) in the standard 50X stock solution.

- mouse embryo tested average mol. wt. 40,000
- endotoxin…………………………………………………tested

**Cell Freezing Medium-DMSO Serum free 1x**

- sterile-filtered, cell culture tested
- Complete ready-to-use medium containing Minimum Essential Medium, dimethyl sulfoxide (8.7%) and methyl cellulose. Prepared according to the formulation of Waymouth.
- Recommended for the cryopreservation of cells.
- endotoxin…………………………………………………tested
- store at −20 °C

**Cell Freezing Medium-DMSO Serum free 1x**

- sterile-filtered, cell culture tested, endotoxin tested
- Complete ready-to-use medium. Proprietary formulation containing Minimum Essential Medium, glycerol, calf serum and fetal bovine serum.
- Recommended for the cryopreservation of cells.
- store at −20 °C

**Cell Freezing Medium-Glycerol 1x**

- sterile-filtered, cell culture tested
- Complete ready-to-use medium. Proprietary formulation with Minimum Essential Medium, dimethyl sulfoxide, calf serum and fetal bovine serum.
- Recommended for the cryopreservation of cells.
- endotoxin…………………………………………………tested
- store at −20°C

**Cell Freezing Medium-Serum-free 1×**

- sterile-filtered, cell culture tested
- Ready-to-use formula that does not contain dimethyl sulfoxide or serum.
- For use with cells grown in serum-free conditions.
- store at 2–8 °C
ECACC Cell Lines

Sigma® and ECACC have partnered to bring you a great selection of high quality cell lines. We are expanding our offering of stocked cell lines to the list below in the U.S. and plan to expand the offering to up to 1500 cell lines later this year. Check our website often for updates. Sigma now supplies ECACC cell lines to Europe, China, Australia, New Zealand, U.S. and Canada. The following is a sampling of the products that Sigma can offer from ECACC.

### ECACC Cell Lines

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Package Size</th>
<th>Cell Line Name</th>
<th>Cell Line Description</th>
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<tr>
<td>01051619</td>
<td>1 vial</td>
<td>1301</td>
<td>Human T-cell leukemia</td>
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<tr>
<td>86050803</td>
<td>1 vial</td>
<td>A7r5</td>
<td>Rat BDIX smooth muscle embryonic aorta</td>
<td>Rat</td>
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<tr>
<td>85120602</td>
<td>1 vial</td>
<td>293 (also known as HEK 293)</td>
<td>Human Embryo Kidney</td>
<td>Human</td>
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<tr>
<td>86110401</td>
<td>1 vial</td>
<td>3T3 clone A31</td>
<td>Mouse BALB/c embryo</td>
<td>Mouse</td>
</tr>
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<td>86052701</td>
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<td>3T3 L1</td>
<td>Mouse Embryo</td>
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<td>A549</td>
<td>Human Caucasian lung carcinoma</td>
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<td>Rat BDIX smooth muscle embryonic aorta</td>
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<tr>
<td>89090402</td>
<td>1 vial</td>
<td>AG5</td>
<td>Human Caucasian gastric adenocarcinoma</td>
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<tr>
<td>94042254</td>
<td>1 vial</td>
<td>B16 melanoma 4A5</td>
<td>Mouse melanoma, melanin producing</td>
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</tr>
<tr>
<td>92101204</td>
<td>1 vial</td>
<td>B16-F0</td>
<td>Mouse melanoma, producing melanin</td>
<td>Mouse</td>
</tr>
<tr>
<td>92101203</td>
<td>1 vial</td>
<td>B16-F1</td>
<td>Mouse melanoma, producing melanin</td>
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<td>96080128</td>
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<td>Mouse 8 cell hybridoma</td>
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<td>B95-8</td>
<td>Monkey Marmoset peripheral blood lymphocyte</td>
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<tr>
<td>95102433</td>
<td>1 vial</td>
<td>BEAS-2B</td>
<td>Human bronchial epithelium, normal</td>
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<tr>
<td>86082803</td>
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<td>BeWo</td>
<td>Human choriocarcinoma</td>
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<td>Rat glial tumor</td>
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<td>1 vial</td>
<td>CHO</td>
<td>Hamster Chinese ovary</td>
<td>Hamster</td>
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<td>00102307</td>
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<td>CHO (PROTEIN FREE)</td>
<td>Chinese hamster ovary</td>
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Contact your local Sigma-Aldrich® office for price and availability.
Protocol for the Cryopreservation of Cell Lines

Aim

This protocol is recommended by the European Collection of Cell Cultures (ECACC) for the cryopreservation of its cell lines. This protocol employs the use of passive methods involving an electric –80 °C freezer for the cryopreservation of cell cultures. ECACC routinely uses a programmable rate controlled freezer (Planer Series Two) from Planer Products. This is the most reliable and reproducible way to freeze cells but as the cost of such equipment is beyond the majority of research laboratories the methods below are described in detail. If large numbers of cell cultures are regularly being frozen then a programmable rate controlled freezer is recommended.

Materials

- Freeze medium (commonly 70% basal medium, 20% FBS, 10% DMSO (Cat. No. D2650) or glycerol, check ECACC data sheets for details).
- 70% ethanol in water (Cat. No. R8382)
- PBS without Ca2+ Mg2+ (Cat. No. D8537)
- 0.25% trypsin/EDTA in HBSS, without Ca2+/Mg2+ (Cat. No. T4049)
- DMSO (Cat. No. D2650)
- Trypsin/EDTA (Cat. No. T4049)
- HL60 (Cat. No. 98070106-1vl)

Equipment

- Personal protective equipment (sterile gloves, laboratory coat)
- Full-face protective mask/visor
- Waterbath set to 37 °C
- Microbiological safety cabinet at appropriate containment level
- Centrifuge
- Haemocytometer (Sigma Bright-Line™ Cat. No. Z359629, Improved Neubauer-Camlab CCH.AC1)
- Pre labeled ampules/cryotubes
- Cell Freezing Device (e.g. Nalgene® Mr. Frosty Cat. No. C1562)

Procedure

View cultures using an inverted microscope to assess the degree of cell density and confirm the absence of bacterial and fungal contaminants.

Bring adherent and semi adherent cells into suspension using trypsin/EDTA (Cat. No. T4049) as above and re-suspend in a volume of fresh medium at least equivalent to the volume of trypsin. Suspension cell lines can be used directly. Remove a small aliquot of cells (100–200 µl) and perform a cell count. Ideally the cell viability should be in excess of 90% in order to achieve a good recovery after freezing. Centrifuge the remaining culture at 150 g for 5 minutes.

Re-suspend cells at a concentration of 2–4x10⁶ cells per ml in freeze medium. Pipette 1ml aliquots of cells into cryoprotective ampules that have been labeled with the cell line name, passage number, cell concentration and date.

Place ampules inside a passive freezer e.g. Nalgene Mr. Frosty (Cat. No. C1562). Fill freezer with isopropyl alcohol and place at –80 °C overnight. Frozen ampules should be transferred to the vapor phase of a liquid nitrogen storage vessel and the locations recorded.

Key Points

The most commonly used cryoprotectant is dimethyl sulphoxide (DMSO Cat. No. D2650); however, this is not appropriate for all cell lines e.g. HL60 (Cat. No. 98070106-1vl) where DMSO is used to induce differentiation. In such cases an alternative such as glycerol should be used. ECACC freeze medium recommended above has been shown to be a good universal medium for most cell types. Another commonly used freeze medium formulation is 70% basal medium, 20% FBS, 10% DMSO but this may not be suitable for all cell types. Check if it works for your cells before using on a regular basis (Cat. No. C6164).

It is essential that cultures are healthy and in the log phase of growth. This can be achieved by using pre-confluent cultures (cultures that are below their maximum cell density) and by changing the culture medium 24 hours before freezing. The rate of cooling may vary but as a general guide a rate of between –1 °C and –3 °C per minute will prove suitable for the majority of cell cultures.

An alternative to the Mr. Frosty system is the Taylor Wharton passive freezer where ampules are held in liquid nitrogen vapor in the neck of Dewar. The system allows the ampules to be gradually lowered thereby reducing the temperature. Rate controlled freezers are also available and are particularly useful if large numbers of ampules are frozen on a regular basis. As a last resort if no other devices are available ampules may be placed inside a well insulated box (such as a polystyrene box with sides that are at least 1cm thick) and placed at –80 °C overnight. It is important to ensure that the box remains upright throughout the freezing process. Once frozen, ampules should be transferred to the vapor phase of a liquid nitrogen storage vessel and the locations recorded.

If using a freezing method involving a –80 °C freezer it is important to have an allocated section for cell line freezing so that samples are not inadvertently removed. If this happens at a crucial part of the freezing, cell viability will be compromised.

Problem Solving Suggestions

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Corning Incorporated, Life Sciences
Acton, MA 01720

Viability problems associated with cryogenic storage are usually noticed soon after cultures are thawed and plated. There are four major areas where problems occur:

1. During harvesting and processing of the cells. Problems may be caused by excessive exposure of the cells to dissociating agents; using a cryoprotective agent that is toxic;
or allowing high density cell suspensions to remain too long at room temperature or at a pH that is too basic.

2. During the cooling (freezing) process. Excessive cell damage and reduced culture viability often result from using a cooling rate that is too fast or too slow, or when the cooling process is temporarily interrupted. Not using a suitable cryoprotective agent at an appropriate concentration will also result in viability problems.

3. During cryogenic storage. Culture viability is often reduced when vials are allowed to warm up during transfer to the freezer, or if the repository temperature is not consistently maintained at appropriate cryogenic temperatures.

4. During thawing and recovery. Problems arise when the thawing process is too slow or the cryoprotectants are improperly removed.

These viability problems can often be corrected by using the following technique to identify the stage in the freezing process where the problem originates. Harvest enough cells to prepare at least four vials. Then remove a sample of cell suspension, equivalent in cell number to that which will be placed into the vials, and immediately place it into a culture vessel with an appropriate amount of medium and incubate. This culture will be used as a control to compare with the cultures set up in the remaining steps.

Next, add the cryoprotective agent to the remaining cells and divide among three vials. Place one vial at 4 °C for one hour. Then remove the cells from the vial, process as though they had just been thawed from the freezer, and plate in medium as above. This culture will be compared with the control culture to determine if there are any problems associated with the cryoprotective agent.

Meanwhile, process the remaining vials through the slow cooling process as usual. One vial is then immediately thawed and processed as above. This culture will be compared with the control culture to determine if there are any problems associated with the slow cooling process.

The remaining vial is then transferred to the cryogenic freezer and stored overnight before being thawed and processed as above. This culture will be compared with the control culture to determine if there are any problems associated with the cryogenic storage conditions. If additional vials of cells are available, several different recovery techniques should be used to determine if the recovery technique is the source of the problem.

By comparing all of the cultures to the original culture, it should then be possible to determine at which stage of the freezing process the problem occurred. Once this is known, the information presented in this guide and its references should be enough to eliminate the problem.

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2,200 new Prestige Antibodies are now available! Developed through the Human Protein Atlas Program, Prestige Antibodies are standardized in universal protocols to enhance the efficiency and effectiveness of your research. Each antibody is supported with over 700 immunohistochemistry, immunofluorescence and Western blot images that are all publicly available on the Human Protein Atlas website.

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Cryoware

Cryovial Color Coders

**Color Coders for Nalgene® cryogenic vials**

Colored disks fit into the recessed tops of Nalgene cryogenic vials. The system allows color-coding of vials for quick visual identification. Cut out top facilitates handling of vials with forceps. Flat surface permits writing.

- **polystyrene**
  - blue
    - C0812-100EA 100 ea
  - green
    - C0937-100EA 100 ea
  - red
    - C1062-100EA 100 ea
  - white
    - C1187-100EA 100 ea
  - yellow
    - C1312-100EA 100 ea

**Cryo Containers**

- **Nalgene® CryoBox™**
  For ultra-low temperature storage of cryogenic vials in mechanical or liquid nitrogen freezers. Usable temperature range: −196 to +121 °C. Numbers molded into grid system in box correspond to numbers printed on lid. Lid is keyed to box so markings will always match. Boxes accept writing with markers.
  - polycarbonate
    - 9 × 9 Array for 1.2 and 2.0 mL vials
      - L × W × H ........................................................ 5 ¼ in. × 5 ¼ in. × 3 ¾ in.
      - R0888-4EA 4 ea
    - 9 × 9 Array for 5.0 mL vials
      - L × W × H ..................................................5 ¾ in. × 5 ¾ in. × 3 ¾ in.
      - R1013-4EA 4 ea
    - CryoBox 100, 10 × 10 array
      For ultra-compact storage of 1.0 mL and 1.5 mL System 100 vials or other similar-sized vials. Box fits most existing vertical racks with 2 in. shelf spacing.
      - L × W × H .................................................5 ¼ in. × 5 ¼ in. × 2 ⅛ in.
      - Z359017-10EA 10 ea

- **CryoCane™ aluminum canes**
  Firmly holds 1.2 or 2.0 mL Nalgene cryogenic vials for storage in Dewar-type, liquid nitrogen freezers.
  - **capacity:** 6 vials
    - overall L. .............................................................300 mm
      - C9936-12EA 12 ea

- **CryoSleeve™ clear PVC cane sleeve**
  Will not become brittle when frozen. Encloses a Nalgene CryoCane™ for extra security during handling and storage. Easy to install and remove.
  - C1437-100EA 100 ea

- **Freezing container, Nalgene® Mr. Frosty**
  Polycarbonate container, blue high-density polyethylene closure, white high-density polyethylene vial holder, and foam insert. Provides the critical, repeatable, 1 °C/min cooling rate required for successful cryopreservation of cells. Easy to use in any mechanical freezer. Container is imprinted with graphic instructions. Requires only isopropyl alcohol. Rigid vial holder keeps vials from contacting alcohol, preventing contamination by wicking. Vial holder floats to allow thawing in a water bath. Numbers molded for identification. All components withstand repeated freeze/thaw cycles. Stackable.
  - Holds 18 1.2 and 2.0 mL cryovials
    - H × diam ..................................................86 mm × 117 mm
      - C1562-1EA 1 ea
Online Metabolomics Resource

Use the IUBMB–Sigma-Aldrich Interactive Metabolic Pathway Chart to find the Metabolite Standards you need.

The Metabolic Pathways Map contains over 500 hyperlinks to Sigma product listings. Just click on the metabolite name or the enzyme’s E.C. number to access product information.

You can access the chart at sigma-aldrich.com/metpath
Note: Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen unless correctly sealed in Nunc CryoFlex™ tubing. Such use may cause entrapment of liquefied nitrogen inside the vial and lead to pressure build-up resulting in possible explosion or biohazard release.

polypropylene vials
high-density polypropylene closures

1.2 mL, sterile
package = 25 vials 20
V4757-500EA 500 ea

1.2 mL, sterile, pkg = (Bulk packed)
V4882-1000EA 1000 ea

2.0 mL, sterile
package = 25 vials 20
V5007-500EA 500 ea

2.0 mL, sterile, pkg = (Bulk packed)
V5132-1000EA 1000 ea

5.0 mL, sterile
package = 10 vials 25
V5257-250EA 250 ea

1.2 mL, non-sterile
Non-sterile vials and closures are packed separately. Vials have no printing or graduations.
V4507-1000EA 1000 ea

2.0 mL, non-sterile
Non-sterile vials and closures are packed separately. Vials have no printing or graduations.
V4632-1000EA 1000 ea

Nalgene® cryogenic vials, System 100
Innovative vials suitable for long-term sample storage in vapor-phase liquid nitrogen freezers. Gasket allows leakproof use in microcentrifuge up to 8,000 × g. Vial is externally threaded and closure has deep skirt to facilitate aseptic transfers. Vials have slightly reduced diameter to fit ultra-compact System 100 CryoBox. Self-standing skirt at bottom has grooves that mate with holder, facilitating one-hand manipulation.
sterile (certified)
polypropylene vials
capsile gasket
closure
capacity 1.0 mL
Z359025-500EA 500 ea

capacity 1.5 mL
Z359033-500EA 500 ea

Nalgene® cryogenic vials, System 100
Innovative vials suitable for long-term sample storage in vapor-phase liquid nitrogen freezers. Gasket allows leakproof use in microcentrifuge up to 8,000 × g. Vial is externally threaded and closure has deep skirt to facilitate aseptic transfers. Vials have slightly reduced diameter to fit ultra-compact System 100 CryoBox. Self-standing skirt at bottom has grooves that mate with holder, facilitating one-hand manipulation.
sterile (certified)
polypropylene vials
capsile gasket
closure
capacity 1.5 mL
Z359033-500EA 500 ea

Nalgene® specimen vial
capacity 15 mL
Vials of polypropylene, linerless screw closures of high-density polyethylene. For storage of solid specimens. Use in mechanical freezers only. Wide-mouth, shoulderless vial allows easy access to sample. Flat bottom permits self-standing on flat surface. Certified sterile, non-cytotoxic and non-pyrogenic.
sterile
75 vials per package

Rack for Nalgene® microcentrifuge tubes
Bottom of white polycarbonate, lid of clear polycarbonate, permanent foam insert.
For safe storage of samples in microcentrifuge tubes from −135 °C to +121 °C. Not for use in liquid-phase liquid nitrogen. May be repeatedly autoclaved. Holes in foam are placed diagonally and spaced to avoid overlapping of tube closures. Lid accepts writing with marker.

size × H............................................ 5 ¼ in. × 5 ¼ in. × 2 in.

capacity: 81 0.5-mL microcentrifuge tubes
R1138-4EA 4 ea

capacity: 64 1.5-mL microcentrifuge tubes
R1263-4EA 4 ea

Nalgene® cryogenic vials
Vials designed for exceptional ease of use and sample integrity in ultra-low temperature storage. Closure seals without gasket or O-ring, minimizing risk of contamination. The deep skirt of the closure allows single-handed aseptic technique without exposing fingers to vial contents. Ribs around the bottom of the vial interlock with wells in the Cryovial Racks so the vial will not turn when opening or closing. Bottoms of vials are skirted; vial stands without holder.
Sterile vials and closures are pre-assembled and radiation-sterilized. A white marking area, fill line and graduations are printed on the side. Certified sterile, non-cytotoxic and non-pyrogenic.
**Benchtop Coolers**

**Nalgene® Labtop cooler**
Molded of durable polycarbonate and filled with non-toxic freezable gel. A convenient alternative to ice buckets for keeping reagents and enzymes cool. Also protects critical samples from temperature fluctuations in the freezer. Holes accommodate tubes:
- 1.5 mL microtubes or cryogenic vials (with adapters to hold 0.5 mL tubes)
- 12–13 mm diameter tubes
- 16–17 mm tubes

Domed lid permits storage of taller screw cap microtubes. Lids have gridded write-on surfaces for sample identification. Stackable. Usable to −135 °C.

- −20 °C Labtop cooler, gel-filled lid
  Maintains 32 microtubes below −15 °C for up to 2 hr at room temperature.
  Z359068-1EA 1 ea

- 0 °C Labtop cooler, gel-filled lid
  Maintains 32 microtubes below 1 °C for up to 5 hr at room temperature.
  Z359084-1EA 1 ea

- −20 °C Labtop cooler, Jr., transparent lid
  Maintains 12 microtubes below −15 °C for up to 1 hr at room temperature.
  C2312-1EA 1 ea

- 0 °C Labtop cooler, Jr., transparent lid
  Maintains 12 microtubes below 1 °C for up to 3 1/2 hr at room temperature.
  Z359076-1EA 1 ea

- 0 °C Labtop cooler, clear lid
  Maintains twelve 12–13 mm diameter tubes below 1 °C for up to 5 hr at room temperature.
  Z370509-1EA 1 ea

- 0 °C Labtop cooler, clear lid
  Maintains twelve 16–17 mm diameter tubes below 2 °C for up to 4 hr at room temperature.
  Z370517-1EA 1 ea

**Quick Chill™ cooler**
A reusable, economical alternative to dry ice-solvent baths for quickly chilling specimens. The unit is molded of durable polycarbonate and filled with a non-toxic solution. After thorough cooling in an ultra-cold freezer, unit can be used on benchtop for up to 45 min to bring alcohol solutions to −20 °C in 3 min or less; aqueous solutions to 0 °C in 4 min or less. There is no direct contact between sample tube and chilling solution, so labels and markings stay on. Unit holds 12 x 1.5 mL microcentrifuge tubes; adapters are included to modify holes for 0.5 mL tubes. Non-skid rubber feet keep the unit from sliding on the benchtop.

**Cryo Accessories**

**Dewar 35L accessories**

- **aluminum cane without tube stops, diam. 1.2 cm x L 29 cm**
  Holds tubes totalling approx. 28 cm in height.
  Z3296

- **Plastic storage box**
  Rugged alternative to cardboard storage boxes; numbered grid printed on top
  Z7397-8EA 8 ea

- **aluminum cane with tube stops**
  Holds 5 tubes, each up to approx. 5 cm in height.
  C3171-50EA 50 ea

**Cryoclaw**
Remote manipulator designed specifically for removing vials and boxes that have fallen to the bottom of cryogenic vessels. Heavy-duty stainless steel with rubber gripper cups, the Cryoclaw is long enough to reach the bottom of any common Dewar and narrow enough to work through a 2 in. (5 cm) necktube. Replacement parts sold separately.

**Aldrich® wide-mouth dewar flasks**

- **capacity 350 mL, aluminum housing**
  ID: 7.0 cm
  D: 11.3 cm
  Z120758-1EA 1 ea

- **capacity 665 mL, aluminum housing**
  ID: 19.5 cm
  D: 7.0 cm
  Z120774-1EA 1 ea

- **capacity 1,000 mL, aluminum housing**
  ID: 7.0 cm
  D: 30.2 cm
  Z120790-1EA 1 ea
| Capacity       | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    | Diameter   | Height   | ID    |
|---------------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|------------|----------|-------|
| 1,900 mL      | 12.0 cm    |         |       |            |          |       | 19.8 cm    |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 4,300 mL      | 14.3 cm    |         |       |            |          |       | 30.0 cm    |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 350 mL        | 7.0 cm     | 12.5 cm  |       |            |          |       | 7.0 cm     | 19.5 cm  |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 665 mL        | 7.0 cm     | 12.5 cm  |       |            |          |       | 7.0 cm     | 19.5 cm  |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 1,000 mL      | 7.0 cm     | 30.2 cm  |       |            |          |       | 7.0 cm     | 30.2 cm  |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 1,900 mL      | 12.0 cm    | 19.8 cm  |       |            |          |       | 12.0 cm    |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |
| 665 mL        | 7.0 cm     | 19.5 cm  |       |            |          |       | 7.0 cm     | 19.5 cm  |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |            |          |       |

**Barnstead/Thermolyne® Dewars, Biocane™ Series**

A can and cane cryopreservation system with features to meet the requirements of almost any research lab: available in five sizes for small- to large-scale sample storage; unsurpassed temperature uniformity with low liquid nitrogen consumption rates; secure locking hasp prevents unauthorized access; each Biocane vessel includes six stainless steel canisters that accommodate Nalgene CryoCanes or similar storage systems. CryoCanes not included.

<table>
<thead>
<tr>
<th>Biocane, 11</th>
<th>Product of Barnstead CK509X1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>10.2 in. (26 cm) × 21.6 in. (55 cm)</td>
</tr>
<tr>
<td>Height</td>
<td>21.6 in. (55 cm)</td>
</tr>
<tr>
<td>Capacity</td>
<td>11 L</td>
</tr>
<tr>
<td>Neck size</td>
<td>2 in. (5 cm)</td>
</tr>
<tr>
<td>Static liquid nitrogen</td>
<td>0.14 L/day (average consumption)</td>
</tr>
<tr>
<td>Storage</td>
<td>180 vials</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biocane, 20</th>
<th>Product of Barnstead CK509X2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>14 ½ in. (37 cm) × 25.7 in. (65 cm)</td>
</tr>
<tr>
<td>Height</td>
<td></td>
</tr>
<tr>
<td>Capacity</td>
<td>20 L</td>
</tr>
<tr>
<td>Neck size</td>
<td>2 in. (5 cm)</td>
</tr>
<tr>
<td>Static liquid nitrogen</td>
<td>0.09 L/day (average consumption)</td>
</tr>
<tr>
<td>Storage</td>
<td>180 vials</td>
</tr>
</tbody>
</table>

**Corning® Cell Culture**

Making your research possibilities real

- **HYPERFlask®** — Grow enough cells in one 1,720 cm² HYPERFlask Cell Culture Vessel to seed 2,000 microplates.
- **Epic System** — The Corning Epic System, a new high-throughput, label-free detection technology, allows researchers to bring drugs to market faster.

For more information on our Corning product offerings, visit [sigma-aldrich.com/corning](http://sigma-aldrich.com/corning)
Biocane, 34
product of Barnstead CK509X3
capacity ........................................ 34 L
diam. × H ........................................ 18.2 in. (46 cm) × 26.6 in. (68 cm)
neck size ........................................ 3 ½ in. (9 cm)
static liquid nitrogen ........................................ 0.17 L/day (average consumption)
storage ............................................................. 720 vials
F1536-1EA 1 ea

Biocane, 47
product of Barnstead CK509X4
neck size ........................................ 5 in. (12.7 cm)
capacity ........................................ 47 L
diam. × H ........................................ 20 in. (51 cm) × 26 ½ in. (67 cm)
static liquid nitrogen ........................................ 0.37 L/day (average consumption)
storage ............................................................. 1512 vials
F1661-1EA 1 ea

text

Cart for transporting Dewars safely
Accepts Biocane 20, 34, and 47
product of Barnstead AYS09X9
Z363642

Barnstead/Thermolyne® Dewars, Locator® Series

Barnstead/Thermolyne® Dewars, Locator® series
For ultra-cold storage of biological specimens in liquid nitrogen. Not to be used for transportation of samples.

Features:
• Three sizes fit the needs of almost any research lab
• Advanced vacuum insulation provides a maximum –190 °C throughout flask with as little as 2 inches of liquid nitrogen, and delivers improved liquid nitrogen consumption rates
• Secure locking clamp prevents unauthorized access
• Each Locator vessel includes stainless steel racks that accommodate Nalgene Cryoboxes for 2 mL freezing vials. Cryoboxes not included

Locator Jr., 50L flask
product of Barnstead International CYS0925
diam. × H ........................................ 22 in. (56 cm) × 26 in. (66 cm)
neck size ........................................ 8 ½ in. (21.6 cm)
static liquid nitrogen ........................................ 0.65 L/day (avg.)
storage ............................................................. 1296 vials
F0911-1EA 1 ea

Locator 4, 110L flask
product of Barnstead International CYS0935
diam. × H ........................................ 22 in. (56 cm) × 36 in. (91 cm)
static liquid nitrogen ........................................ 0.65 L/day (avg.)
storage ............................................................. 2916 vials
F1036-1EA 1 ea

Locator 8, 110L flask
product of Barnstead International CYS0945
diam. × H ........................................ 22 in. (56 cm) × 36 in. (91 cm)
static liquid nitrogen ........................................ 0.4 L/day (avg.)
storage ............................................................. 1800 vials
F1161-1EA 1 ea

Four-wheel roller base
product of Barnstead International AYS09X1
R9640-1EA 1 ea

Nalgene® Dewars

Nalgene® Dewars
Shatterproof. Safe for short-term storage of ice water, dry ice/solvent baths, and liquid nitrogen; can also be used as a hot bath. Temperature range: –196 to +100 °C. Cover is vented. Polyethylene-coated handle on 1, 2, and 4 L sizes only.
Style 4150. Dimensions are inside depth × I. D.
urethane foam-filled high-density polyethylene

capacity 1 L
polyethylene handle
size ........................................ 7 ½ in. (194 mm) × 3 ½ in. (95 mm)
F9401-1EA 1 ea

capacity 2 L
polyethylene handle
size ........................................ 8 ½ in. (225 mm) × 4 ½ in. (121 mm)
F9526-1EA 1 ea

capacity 4 L
polyethylene handle
size ........................................ 11 ½ in. (287 mm) × 5 ½ in. (146 mm)
F9651-1EA 1 ea

capacity 10 L
no handle
size ........................................ 15 ½ in. (394 mm) × 7 ½ in. (197 mm)
F9776-1EA 1 ea

Taylor-Wharton Dewars

Taylor-Wharton Dewars
For storage of small quantities of liquid nitrogen. High-strength aluminum shell is both durable and light-weight. Super insulation provides advanced thermal protection. Neck tube joins inner and outer container, providing maximum durability and minimum nitrogen consumption. Cap and neck tube core provide positive closure. Accessories sold separately.
Wide-mouth Dewars with Silvered Glass Liner

Wide-mouth Dewars with silvered glass liner

Straight sides, a swing-over carrying handle, and no neck constriction; suitable for benchtop immersion applications. Vented insulating lids clamp to the body of the flask.

1 L, stainless steel jacket and vented cap
outside dimensions D x H......4.6 in. (116 mm) x 9 in. (228 mm)
inside dimensions D x H......3.3 in. (85 mm) x 7.3 in. (185 mm)
D3170-1EA 1 ea

2 L, stainless steel jacket and vented cap
inside dimensions D x H......4.2 in. (106 mm) x 8.1 in. (205 mm)
outside dimensions D x H......5.6 in. (142 mm) x 10.5 in. (268 mm)
D3295-1EA 1 ea

4.5 L, stainless steel jacket and vented cap
inside dimensions D x H......5.9 in. (150 mm) x 11 in. (280 mm)
outside dimensions D x H......7.2 in. (182 mm) x 10.5 in. (350 mm)
D3420-1EA 1 ea

1 L, Blue enamelled steel jacket and vented cap
outside dimensions D x H......4.6 in. (116 mm) x 9 in. (228 mm)
inside dimensions D x H......3.3 in. (85 mm) x 7.3 in. (185 mm)
D3545-1EA 1 ea

2 L, Blue enamelled steel jacket and vented cap
outside dimensions D x H......4.2 in. (106 mm) x 8.1 in. (205 mm)
outside dimensions D x H......5.6 in. (142 mm) x 10.5 in. (268 mm)
D3670-1EA 1 ea

4.5 L, Blue enamelled steel jacket and vented cap
inside dimensions D x H......5.9 in. (150 mm) x 11 in. (280 mm)
outside dimensions D x H......7.2 in. (182 mm) x 10.5 in. (350 mm)
D3795-1EA 1 ea

Detachable handle and lid (lids do not fasten to the body of the flask).

stainless steel
For over 25 years, Sigma Life Science has been a primary manufacturer of quality cell culture media, sera and reagents. During that time, a lot has changed. The breadth of application-tested cell culture products we offer has increased substantially, as have the speed and simplicity with which they’re delivered to you. Some things, however, we’ve intentionally left alone. Like our unwavering commitment to dependability and quality, and our dedication to delivering world-class chemicals in that square bottle you’ve come to know and love. After all, it’s what’s on the inside that counts.

sigma.com/cellculture