

CoolCell®

Controlled-Rate, Alcohol-Free Cell Freezing



BIOGENERICA S.R.L.
 Via Pescara 7 Int. 23 - 95030 Mascali (CT) - Italia
 Tel. +39 095914678 - Fax +39 0952246205 - info@biogenerica.it
 www.biogenerica.it



CoolCell® alcohol-free cell freezing containers ensure standardized controlled-rate $-1^{\circ}\text{C}/\text{minute}$ cell freezing in a -80°C freezer - *without alcohol or any fluids*. Proven for use with a variety of cell types including stem cells, primary cells, PBMC cell lines, insect cells, yeast and others. The patent-pending CoolCell technology utilizes a thermo-conductive alloy core and highly-insulative outer material to control the rate of heat removal and provide reproducible cell cryopreservation. CoolCell units are easy to use and deliver comparable results to expensive programmable freezers.

CoolCell® is proven to work with many cell types including:

Stem Cells

Human Embryonic Stem
 Preadipocytes
 Breast Cancer Stem
 Colon Cancer Stem
 Glioblastoma Stem
 Mouse Embryonic Stem
 Human Endothelial Progenitor

Primary Cells

Neonatal Keratinocytes
 Human WBCs
 Mouse
 WBCs
 Human CD34+
 Muscle
 Human Tendon Fibroblasts
 Melanoma Tumor
 Human Cardiac Ventricular
 Human Cardiac Atrial

Cell Lines

CHO
 LnCap
 HTB77
 A549
 HeLa

CoolCell® Container



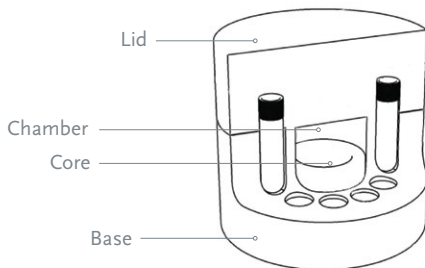
- No alcohol**
 - No fluids
 - No pre-cooling
 - Saves 12L/unit of IPA per year
- No variability**
 - All vials have uniform freeze rate
 - Radially symmetric design ensures vial consistency
- No on-going cost**
 - No alcohol purchase or disposal
- No stuck lids**
 - Ergonomic lid comes off easily when frozen
 - Not cold to the touch when removing from the -80°C freezer
- Quick re-use time**
 - Ready to use again after five minutes

Isopropanol (IPA) Container

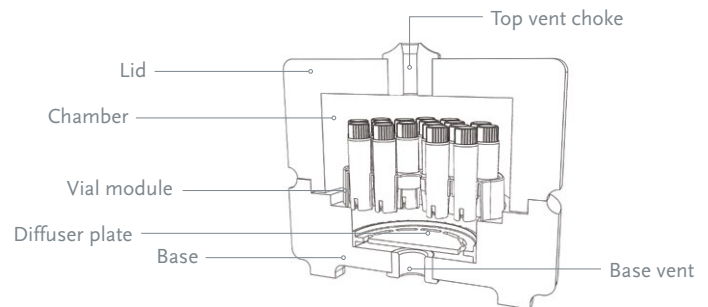


- Requires isopropanol**
 - Replace alcohol every 5 uses
 - Track number of uses
 - Pre-cool alcohol in refrigerator
- Inconsistent freeze rate**
 - Alcohol degradation induces variability
 - Two circles of wells; two freeze rates
- Approximately \$350/year**
 - Change alcohol weekly
 - Dispose of hazardous waste
- Difficult to handle and open**
 - Screw cap difficult to remove when frozen
 - Frozen unit is slippery and cold to touch
- Wait between runs**
 - Takes >1 hr for alcohol to warm-up

How It Works

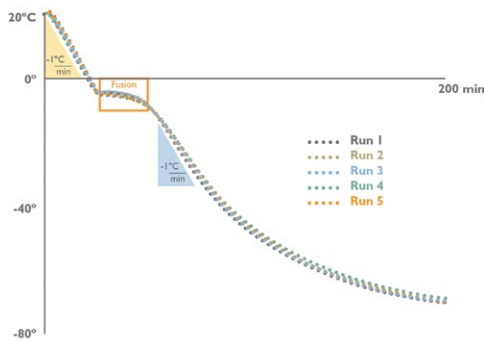


CoolCell® LX, in combination with a -80°C freezer, will provide the freezing rate of $-1^{\circ}\text{C}/\text{minute}$ that is ideal for cryopreservation of most cells and cell lines. Using a combination of uniform-density cross-linked polyethylene foam, a solid state core, and radial vial symmetry, freezing profiles are consistent and reproducible. It is important to fully load CoolCell containers prior to freezing. Foam is non-absorbent and will impose negligible change in the freezer environment; thereby protecting nearby frozen samples. The low heat content also ensures that CoolCell LX containers will rapidly return to room temperature when removed from freezer.



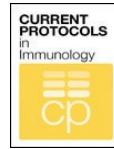
CoolCell® FTS30 utilizes a solid state core and controlled micro-convection technology to evenly draw in -80°C freezer air through a bottom base vent, uniformly disperse the cold air around each vial in the central chamber and then release the thermal load from the vials through a top vent choke. The inner vial module holds 30 cryogenic vials and can be removed in one step. Each vial achieves a uniform and reproducible $-1^{\circ}\text{C}/\text{minute}$ freezing profile and thermal profiles are highly reproducible. Due to the low thermal mass of the uniform-density cross-linked polyethylene foam container, freezing can be conducted without a rise in local freezer temperature, thereby protecting nearby samples.

CoolCell® Reproducibility



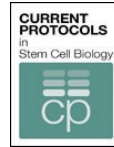
Performance test: A temperature probe was placed into a 2.0 mL cryogenic vial containing 1.0 mL of water and the tube was inserted into a room temperature CoolCell®. The CoolCell was placed into a -80°C freezer and the temperature rate and profile was recorded over a 3 hour period. The test was repeated 5 consecutive times. **Conclusion:** CoolCell generated identical fusion time and cooling profiles over five consecutive freeze cycles.

CoolCell® Protocols



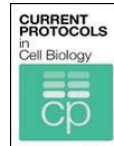
Cryopreservation and Thawing of Cells

Wayne M. Yokoyama, Maria L. Thompson, Rolf O. Ehrhardt
University of California School of Medicine, San Francisco, CA
BioCision LLC, Larkspur, California
Curr. Protoc. Immunology. 2012 Nov; 99 Appendix 3G



Standardized Cryopreservation of Pluripotent Stem Cells

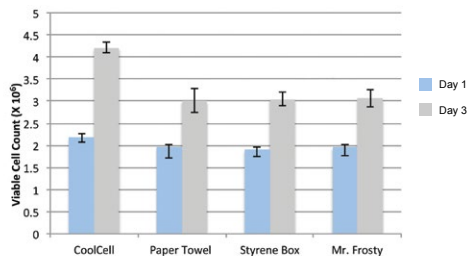
Rick I. Cohen, Maria L. Thompson, Brian Schryver, Rolf O. Ehrhardt
Rutgers University, Piscataway, New Jersey
BioCision LLC, Larkspur, California
Curr. Protoc. Stem Cell Biology. 2014 Feb; 28 Unit 1C.14



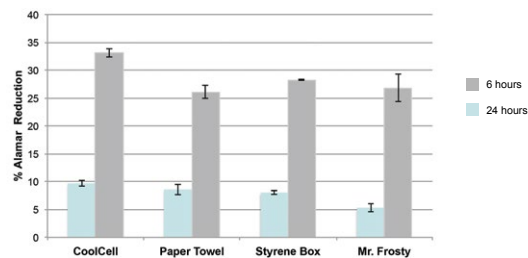
Standardized Cryopreservation of Human Primary Cells

Thomas V. Ramos, Aby J. Mathew, Maria L. Thompson, Rolf O. Ehrhardt
HemaCare Corporation, Van Nuys, California, BioLife Solutions, Bothell, Washington, BioCision, Larkspur, California
Curr. Protoc. Cell Biology. 2014 Sept; 64 Appendix 3I.

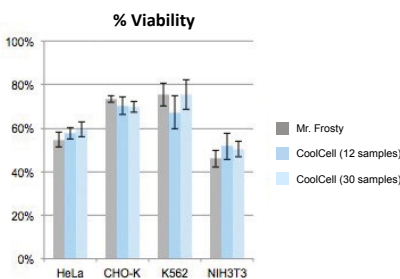
CoolCell® Performance vs. IPA Container



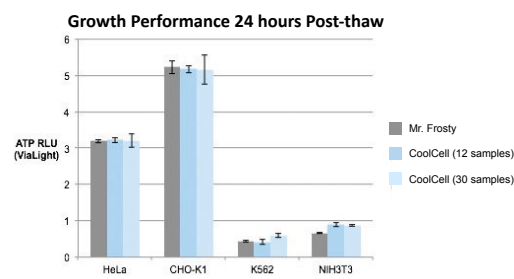
Human embryonic stem cells, RC-10 were frozen using the technique indicated, thawed after 2 weeks in LN₂, and counted immediately (Day 1) or after 3 days of growth (Day 3).



Alamar blue reduction assay for proliferation assessment showed cells frozen in CoolCell® grew more quickly, leading to more total cells.

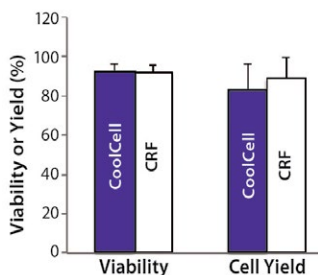


HeLa, CHO-K, K562, NIH3T3. CoolCell® 12-well, CoolCell FTS30 30-well or "Mr. Frosty" freezing containers were used to freeze all four cell lines. Identical transfection efficiencies and viabilities were observed after thawing.

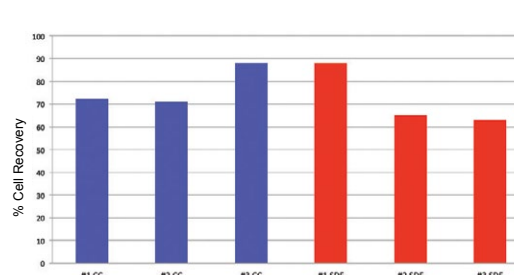


Identical growth of cells was observed 24 hours post-thaw.

CoolCell® Performance vs. Programmable Freezer



Ag-Tregs. Effects of freezing on antigen-specific Treg (Ag-Treg) cell therapy products; Ag-Tregs ($n = 6$) were frozen at concentration of 1 to 10×10^6 cells/mL using the CoolCell freezing device or programmable freezer (freezing rate of $-1^\circ\text{C}/\text{min}$). Viability and absolute viable cell count of thawed Ag-Treg cell therapy products were evaluated by flow cytometry.



Comparison of freezing methods. Graph comparing % of cell recovery after freezing with the CoolCell freezing device (blue) versus freezing using a programmable step-down freezer (red) in 3 different samples at high cell concentration.

- Data performed by UCSF Diabetes Center

For 1.0 mL or 2.0 mL Cryogenic Vials



CoolCell® LX

For 12 standard 1.0 mL to 2.0 mL cryogenic vials, 1.0 mL fill per vial. Radially symmetric for uniform vial freezing. Numbered wells for easy sample identification. Beveled lid for secure gripping and easy opening. Exposed vial tops when lid is open for quick, organized removal of frozen samples.

Item No.	Description	External Dimensions	Well Diam.
BCS-405	● CoolCell LX, purple	Diam 11.7 x H 9.9 cm	12.7 mm
BCS-405G	● CoolCell LX, green		
BCS-405O	● CoolCell LX, orange		
BCS-405PK	● CoolCell LX, pink		



CoolCell® FTS30

For 30 standard 1.0 mL to 2.0 mL cryogenic vials, 1.0 mL fill per vial. Controlled micro-convection for uniform freezing of 30 vials. Removable vial tray for one-step transfer of samples into and out of freezing chamber.

Item No.	Description	External Dimensions	Well Diam.
BCS-170	● CoolCell FTS30, purple	Diam 16.5 x H 11.5 cm	12.3 mm
BCS-170G	● CoolCell FTS30, green		
BCS-170O	● CoolCell FTS30, orange		
BCS-170PK	● CoolCell FTS30, pink		

For 3.5 mL to 5.0 mL Cryogenic Vials



CoolCell® 5mL LX

For 12 standard 3.5 mL to 5.0 mL fill cryogenic vials, 3.5 to 5.0 fill per vial. Radially symmetric for uniform vial freezing. Numbered wells for easy sample identification. Beveled lid for secure gripping and easy opening. Exposed vial tops when lid is open for quick, organized removal of frozen samples.

Item No.	Description	External Dimensions	Well Diam.
BCS-406	● CoolCell 5ml LX, purple	Diam 9.5 x H 14.5 cm	15.2 mm

For Injectable Cell Therapy Ampoules



CoolCell® SV2 and CoolCell® SV10

For 12 standard 2.0 mL injectable ampoules, 1.0 mL fill per ampule (SV2). For 6 standard 10.0 mL injectable ampoules, 5.0 mL fill per ampule (SV10). Radially symmetric for uniform freezing of injectable ampoules. Easy open lid. Exposed vial tops when lid is open for quick, organized removal of frozen samples.

Item No.	Description	External Dimensions	Well Diam.
BCS-172	● CoolCell SV2, purple	Diam 13.9 x H 10.5 cm	14.7 mm
BCS-262	● CoolCell SV10, purple	Diam 12.1 x H 9.8 cm	23.6 mm



CoolCell® Filler Vials

To ensure cell freezing rate consistency and uniform results when using CoolCell containers, insert a CoolCell Filler Vial into empty wells when freezing less than a full load. Suitable for repeated use and compatible with CoolCell LX, CoolCell FTS30 and CoolCell 5mL LX containers. 6 per pack.

Item No.	Description	For Use With
BCS-3105	CoolCell Filler Vial, 2ml	CoolCell LX, CoolCell FTS30
BCS-3106	CoolCell Filler Vial, 5ml	CoolCell 5mL LX



CoolCell® FTS30 Vial Module

FTS30 Vial Module is a holder for 30 1.0 mL or 2.0 mL cryogenic vials that allows one-step insertion and removal of all 30 vials at once. Fits into a standard 5.0 x 5.0 x 2.0 inch cryostorage box. Compatible with dry ice and liquid nitrogen.

Item No.	Description	For Use With
BCS-210	Removable cryogenic vial module for CoolCell FTS30	CoolCell FTS30

Note: For optimal freezing it is important to fully load each CoolCell container prior to freezing. CoolCell Filler Vials are recommended for filling any empty wells.