biocision®

Standardizing Samples™

Blood, cord blood, serum, cells, stem cells, T cells, B cells, dendritic cells, bacteria, virus, sputum, follicle, tooth, bones, biopsy, tumor, tissue, urine, saliva, spinal fluid, skin……..

CoolCell Overview

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Biozym®
SCIENCE IS OUR BUSINESS

Lack of Standardization

"The lack of standardized, high-quality biospecimens has been widely recognized as one of the most significant roadblocks to the progress of cancer research." NIH / NCI / OBBR

Worldwide efforts to develop new standards for collecting, handling, and processing pre-analytical samples (blood, tissue, tumor, other biological specimens or sample materials)

- **EU:** http://www.devicelink.com/ivdt/archive/09/04/003.html
- **NCI/NIH:** http://biospecimens.cancer.gov/practices/default.asp
- **NIH Office of Biorepositories and Biospecimen Research 2010 Symposium:** http://www.brnsymposium.com/meeting/brnsymposium/2010/
- **BioCoR:** http://biocor.umn.edu/
- **Duke:** http://web.genome.duke.edu/cores/biorepository/protocols/
- **Molecular Epidemiology Workshop,** Shanghai, 2008

CoolCell®

Standardized Controlled-Rate Cell Freezing

-1°C/min Controlled-Rate Cell Freezing

Highest reproducibility vs. other methods

- -1°C/minute freeze rate in -80°C freezer
- Identical freezing profiles every time
- Format: 1ml – 5ml cryo vials or injectable vials

Highest cell viability

- Cell lines, PBMC, primary cells, stem cells, yeast, algae

Alcohol-free rate control

- Requires no isopropanol, no fluids
- No on-going expense
- No toxic waste
- No alcohol-induced variability

Controlled vs. Uncontrolled Freezing

	Controlled (-1°C/min)	Uncontrolled/ Inconsistent
Post-thaw viability	Controlled freezing reproducibly minimizes cell disruption and improves viability	Allows variation in cell viability and function with unpredictable result batch to batch
Freezing artifacts	Reduces freeze selection of sub-populations of cells and/ or biomarkers	Selects for sub-population of cells and/or function that can best survive the freezing conditions
Gene/biomarker expression & function	Ensures cells will be recovered in a predictable & consistent state (advantage when working with precious and small samples)	Cells will be recovered with an unpredictable and inconsistent state
Cell therapy & diagnostic	Essential to cell therapy and diagnostics	Roadblock to consistent diagnostic & clinical outcome

Multiple Formats – Same Function

Consistent -1°C/min freezing

1ml and 2ml cryo vials
12 wells

1ml and 2ml cryo vials
30 wells
- Vial module can be easily transferred to cryo storage box

4ml and 5ml cryo vials
12 wells

2ml serum vials
12 wells
10ml serum vials
6 wells

What is CoolCell?

Passive Controlled Cell Freezing*

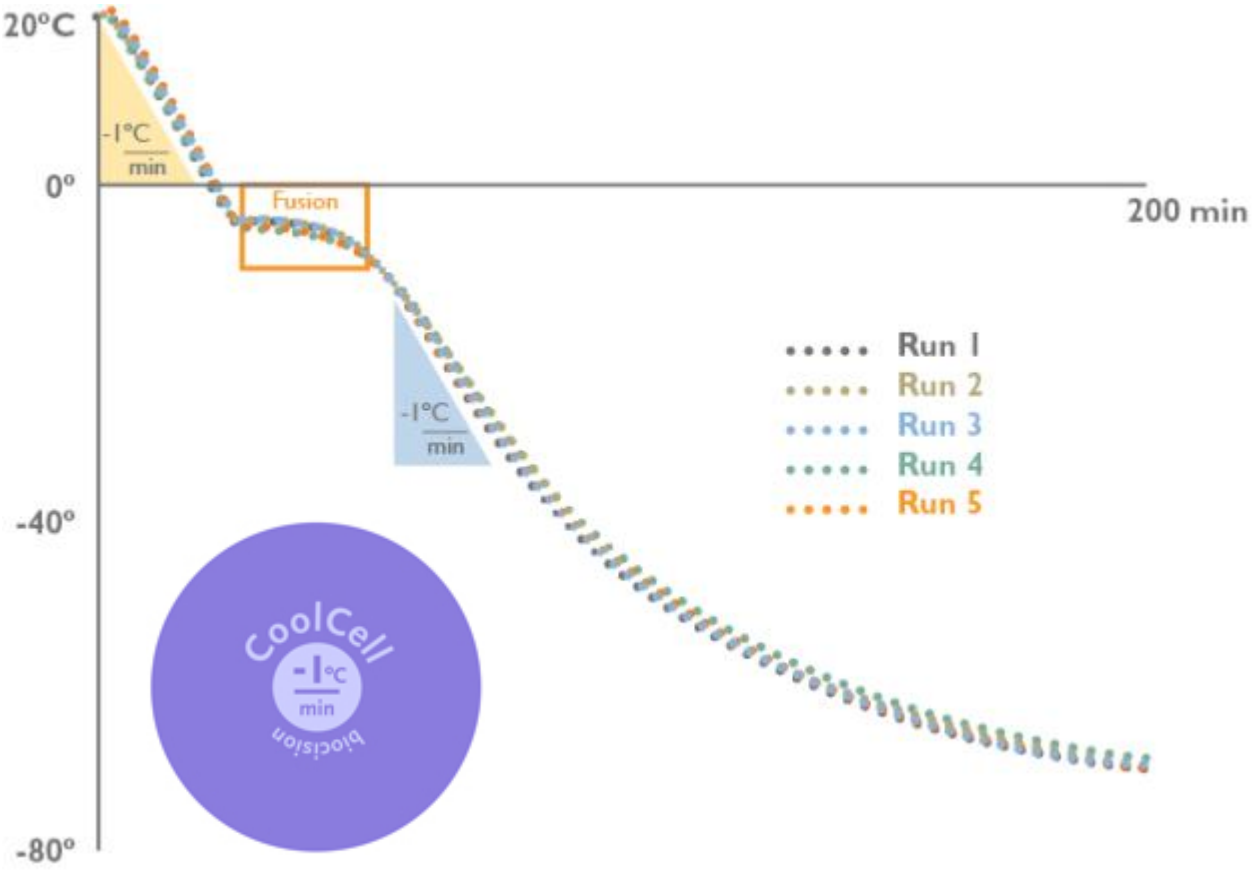
- Radially-symmetric thermal exchange to ensure uniform cooling profiles to all vial positions
- HD Materials used in aerospace industry for lightweight & insulative properties
- Highly Thermo-conductive core
- Conduction and/or micro-convection air control (FTS30)

* Patents Pending

CoolCell: Thermo-Dynamic Consistency

Consistent freezing, reproducible results

Five consecutive freeze cycles in -80ºC freezer using CoolCell (12-well, 2ml cryo vials). Cooling profiles and phase change are identical every time.

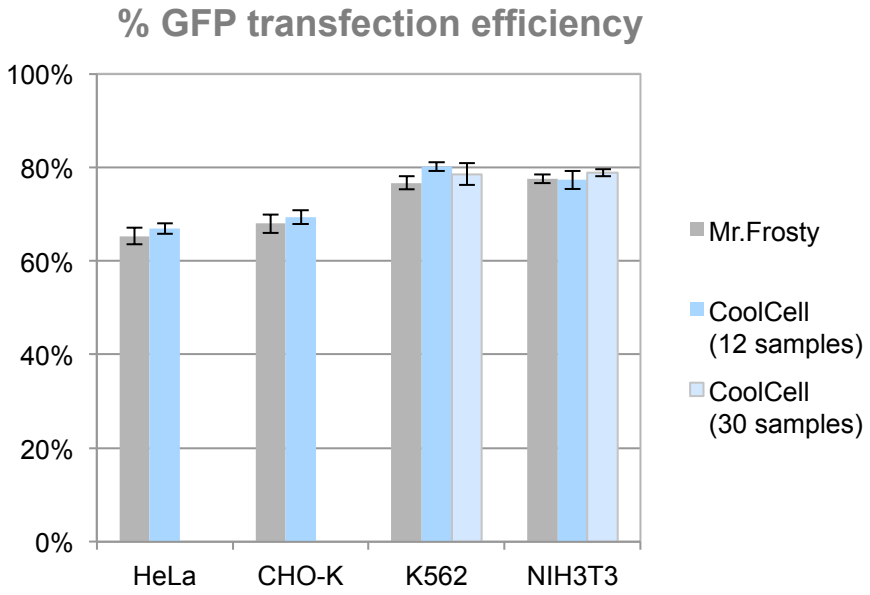


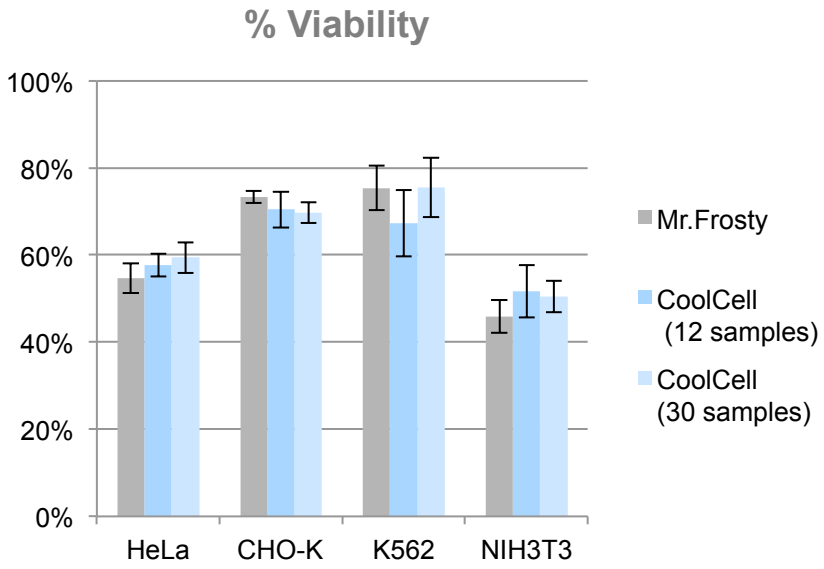
Advantage CoolCell

CoolCell	Isopropanol Container
No IPA/No maintenance •No fluids •No pre-cooling required •No hazardous waste	**100% Isopropanol** •On-going purchase, maintenance, hazardous waste due to IPA degradation •Pre-cool hazardous alcohol in refrigerator
No variability •All vials have uniform freeze rate *every time* •Radially symmetric design ensures consistency to vials and freeze runs	**Inconsistent freeze rate** •Each run is slightly different due to IPA degradation •Two circles of wells – uneven heat removal from vials
No hidden cost •*Once you buy it, you're done paying for it!!*	**~$350/year maintenance *per unit*** •Change IPA every five uses + hazardous waste disposal **•10 units = $3,500 maintenance cost *EVERY YEAR*** 
Lid opens easily •Cap comes off easily when frozen •Not cold to the touch, shatter-proof	**Screw cap very difficult to remove when frozen** •Protective glove required when handling frozen unit •Unit is cold, slippery, breakable
Highest post-thaw cell viability & function	
Ready to use again in 5 minutes	**Takes >1 hour for device to return to room temp**
Low impact on freezer •1/3 the heat impact on freezer compared to alcohol-filled units	**Large thermal mass impacts local freezer area** •Large heat capacity removed from alcohol impacts nearby samples

Cell Line Performance Comparison I

CoolCell vs. “Mr. Frosty”



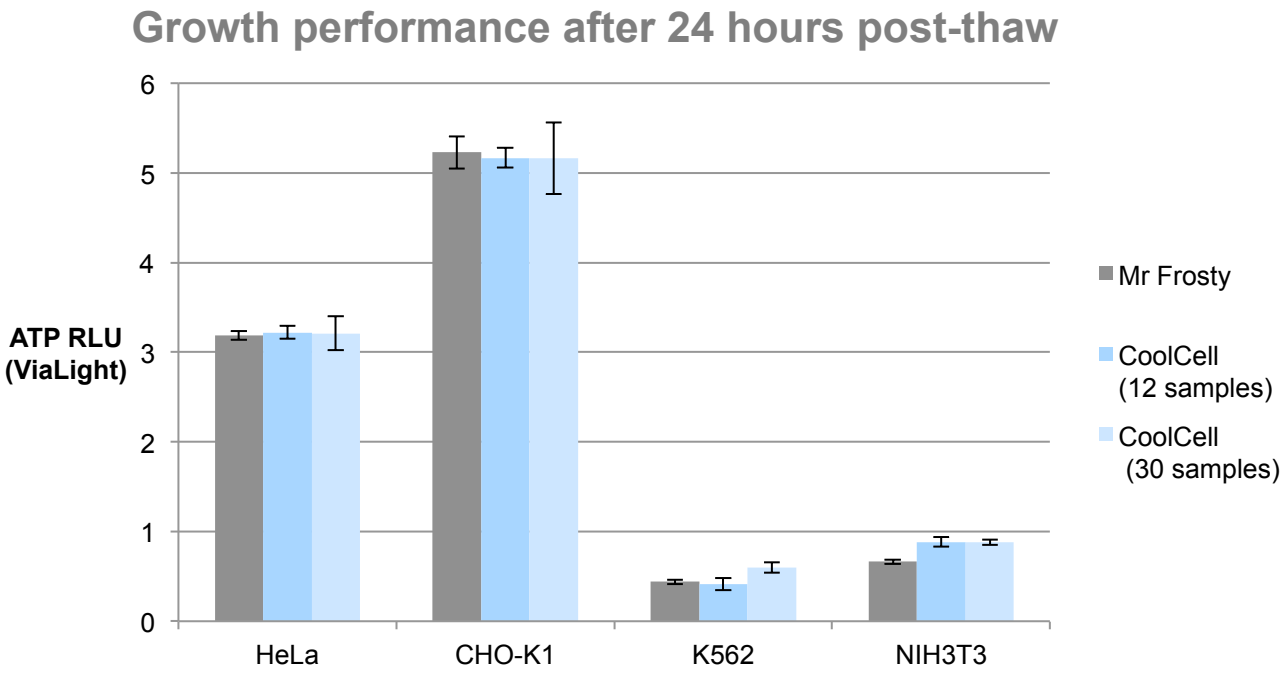


Independent Performance Analysis by LONZA/Germany

- Identical transfection efficiencies and viabilities after freezing & thawing of cells
- CoolCell is more durable than Mr. Frosty (i.e. CoolCell is shatter-proof)

Cell Line Performance Comparison II

CoolCell vs. “Mr. Frosty”



Independent Performance Analysis by LONZA/Germany

- Identical growth of cells observed 24 hours after thawing
- Freezing and thawing is easier and more convenient with CoolCell (no isopropanol)

Primary Cells Performance Comparison

CoolCell vs. “Mr. Frosty”

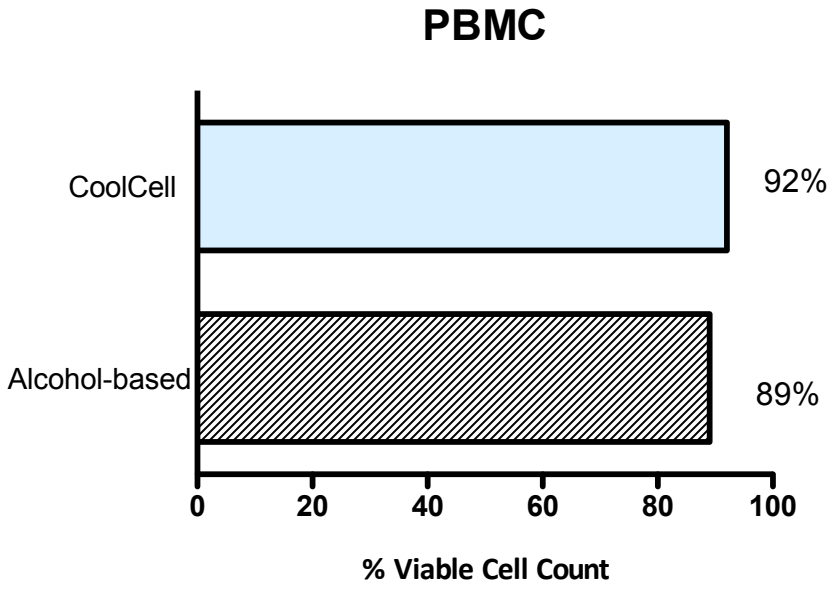


Figure 1. Post freeze viability, PBMC. $2x10^7$ PBMC cells total were isolated and split into two vials, at $1x10^7$ cells per vial, in Human Sera with 10% DMSO. One vial was frozen overnight using a BioCision CoolCell and one in a commercial IPA-based freezing system (“Mr. Frosty”). Following long term storage in LN2, cells were thawed and viability counts for each vial were obtained by the trypan blue stain method.

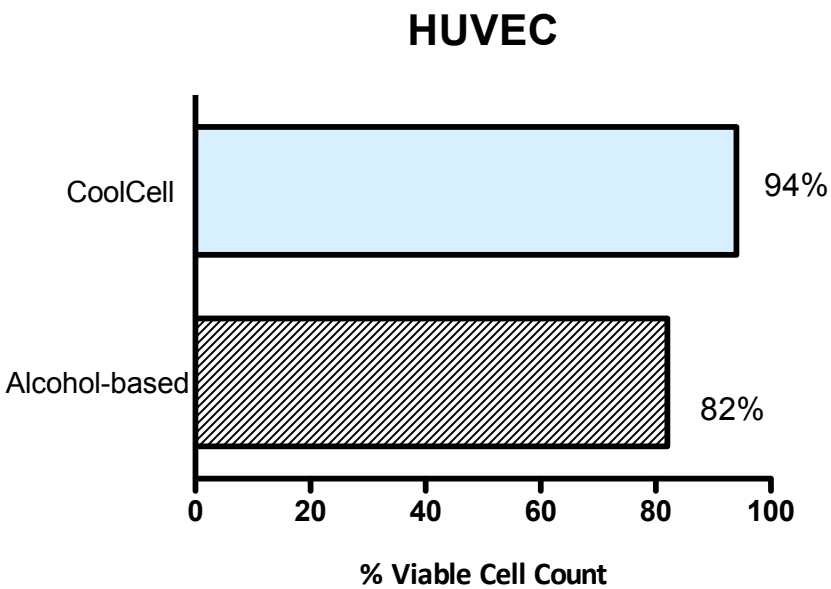


Figure 2. Post freeze viable cell count, HUVEC (n=5). Cells were resuspended in freezing medium at a concentration of $2x10^6$ cells per ml. 1ml aliquots were proportioned into 1.8ml cryo vials and frozen at -1°C/ minute in either a BioCision CoolCell or in an alcohol-filled freezing unit. Five vials frozen by either method were rapidly thawed and resuspended in growth media. Live cell counts were obtained by the trypan blue exclusion method.

Independent Performance Analysis

- In primary human PBMC and HUVEC, freezing with CoolCell produces >90% viable cell count post-thaw without the use of isopropanol (a flammable, hazardous solvent) which is used in the alcohol-based method

Stem Cells

CoolCell vs. other common methods

Common un-standardized methods of stem cell cryopreservation

- Styrofoam box
- Paper towel insulation

Performance comparison with CoolCell

- Independent evaluation of CoolCell cyropreservation system initiated by Roslin Cellab, a leading stem cell research institute
- Determine effect of different freeze/thaw methods on critical stem cell growth parameters (viability)

Dead Cells after Freezing

CoolCell vs. other common methods

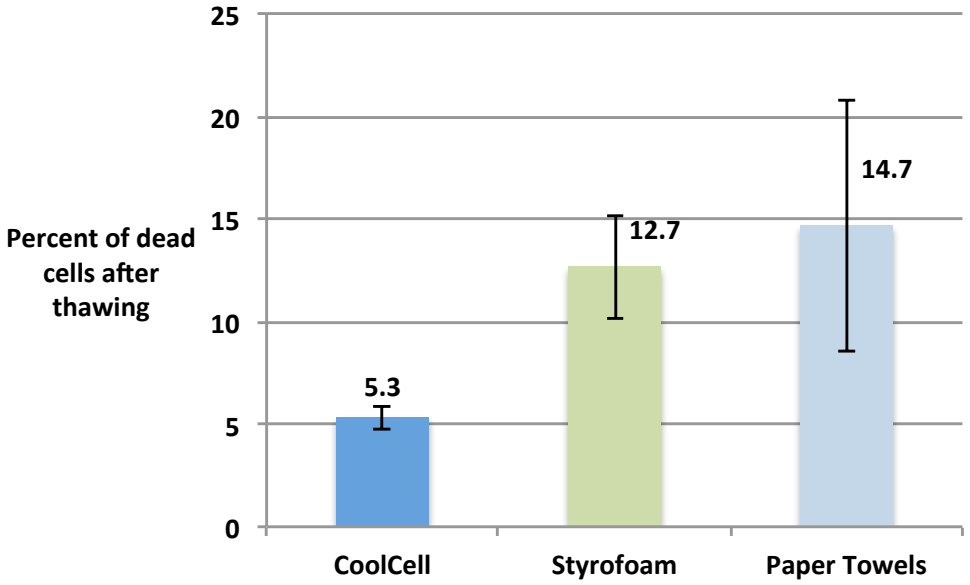


Figure 1. Determination of amount of dead RC10 cells (human embryonic stem cell) using Trypan Blue after thawing. Comparison of 3 difference freezing methods (n=3)

Independent Performance Analysis by Roslin Cellab

- Cryopreservation with CoolCell results in less cell death upon thaw ($P<0.05$)
- CoolCell ensures less variability when freezing multiple vials.

Stem Cell Performance Comparison

CoolCell vs. other common methods morphology data

CoolCell | **Styrofoam Box** | **Paper Towel**

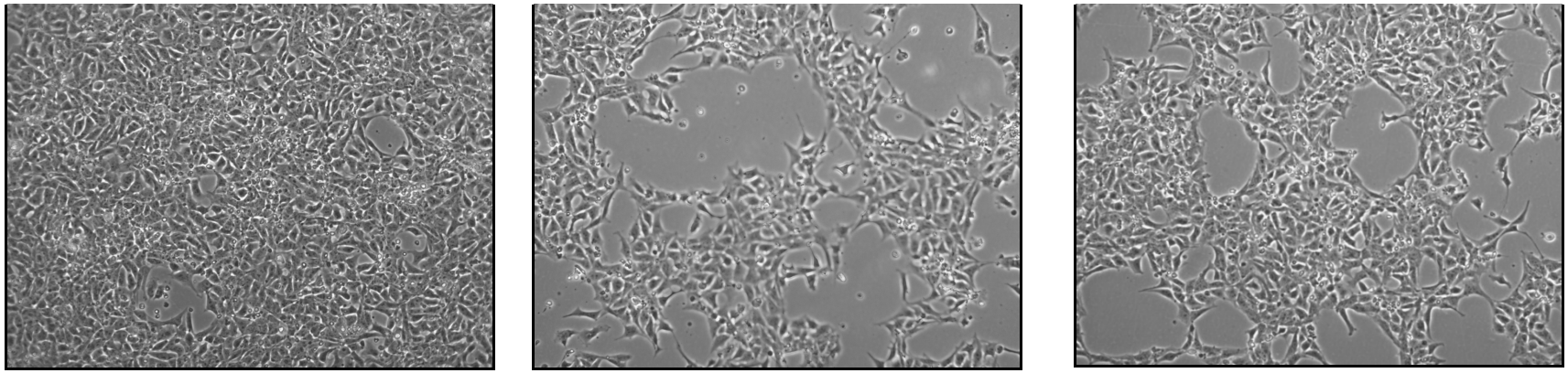


Figure 2. Human embryonic stem cell line RC10 was observed at day 1 post resuscitation under x10magnification using three different freezing methods.

Independent Performance Review – Roslin Cellab

- Stem cell morphology was visually improved when using the CoolCell compared to other methods. This complements the data showing the higher viability and proliferative capacity results, as seen with CoolCell.

Stem Cell Proliferation

CoolCell vs. other common methods

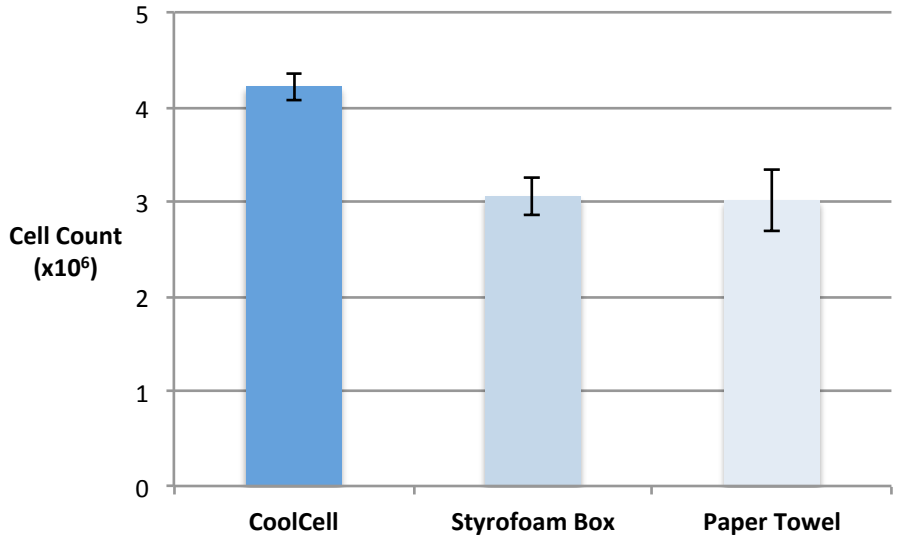


Figure 3. RC10 cells count using Trypan Blue at 3 days post-thaw using three different freezing methods (n=3)

Independent Performance Analysis by Roslin Cellab

- Significant increase in viable cell count using a CoolCell ($P<0.001$)

1 Million cells (33%) more than other methods

- Direct impact in various experimental results (i.e. detection of proteins secreted in low levels)
- Decrease in patient sample size required for diagnostics, treatment or research

Stem Cell Proliferation over Time

CoolCell vs. other common methods

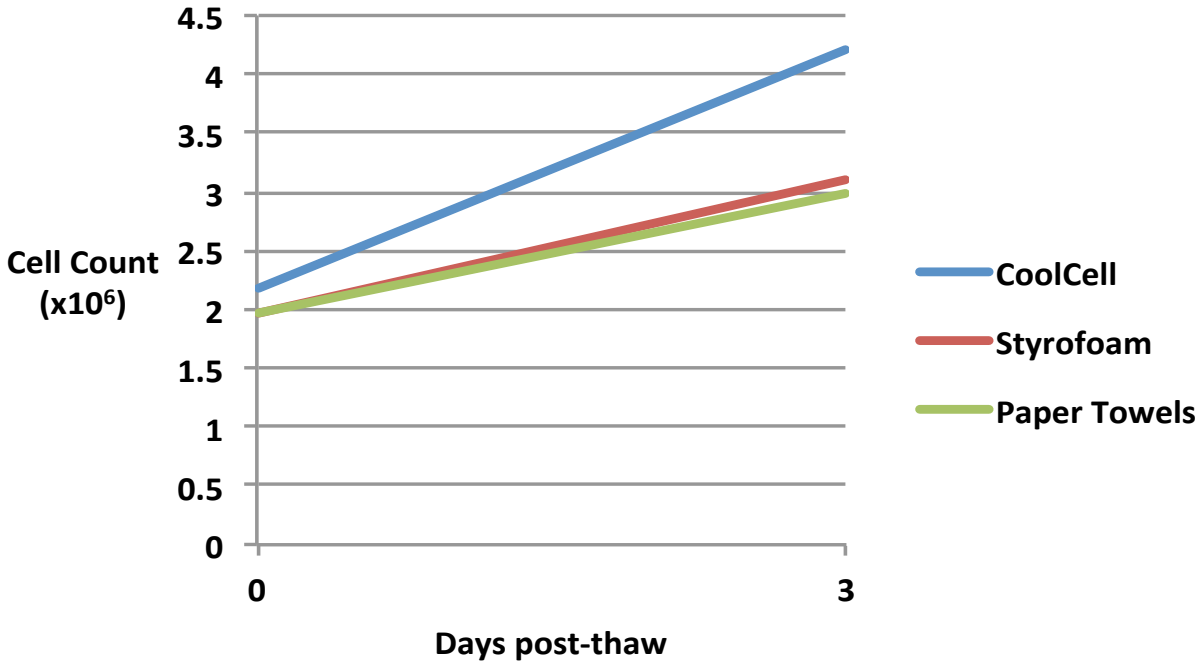


Figure 4. RC10 cells count using Trypan Blue at days 0 and 3 post-thaw using three different freezing methods. Average of 3 samples.

Independent Performance Analysis by Roslin Cellab

- Stem cells cryopreserved with a standardized and temperature controlled CoolCell recover better and their proliferation rate is much higher

CoolSystem® CRYO

Kit for ice-free vial preparation and alcohol-free cell freezing

CoolSystem CRYO (Item #BCS-142)

Includes:

CoolBox CFT30 for ice-free vial cooling during preparation in the biosafety cabinet

CoolCell 12-place 2ml vial controlled-rate freezing container

TruCool™ Cryogenic Tubes

Leak-proof caps, individually barcoded

- Thermally-fused gasket prevents leakage and O-ring contamination
- Sterile, medical-grade polypropylene – will not discolor upon autoclaving
- Individually barcoded – no external labeling required (*Barcodes are Code 128 and 10 characters*)
- Internal- and external-threading options

Customer Testimonials

John Gardner – Roslin Cellab
"This is by far and away the best benchtop technology to enter the field of cell cryopreservation for decades."

Rohit G. - Stanford University
"We run a registry, in which large amounts of PBMCs are processed for long-term cyropreservation…….Overall, the CoolCell has proven to us to set a new bar in cryopreservation."

Sam Knight - Ceramisphere (Australia)
"I spent nearly a year getting terrible viability-recovery on cell freezing with dry ice (using an alcohol device) followed by storage in LN2, and this on HeLa cells which aren't exactly hard to store. I then bought a CoolCell and now I can just use standard cryo-preservation techniques (DMSO, 20% NBS) and get nearly 100% viability on thawing."

CoolCell Summary

- Controlled cryopreservation results in
  - greater cell recovery (less dead cells)
  - better “functional” recovery (higher rate of proliferation)
- Highest reproducibility between samples
- Less selection of cell subsets
- Smaller sample size (patient sample/biopsy, hard to transfect cells, flow cytometry sorted cells, generation of primary cells)
- Easy to use, no toxic waste, easy transport to freezer farms

Summary BioCision
Pre-Analytical Sample Standardization

Sample handling has become increasingly important

- Growing number of multi-national companies, labs, studies
- Increasing use of biomarkers and highly sensitive analytical methods

BioCision = Consistency. Reproducibility. Standardization.

- Test to test
- Researcher to researcher
- Lab to lab
- Site to site

Watch the VIDEO

Genentech Amgen Pfizer Roche Harvard MIT
CalTech UCSF Glaxo Bristol Myers Squibb Novartis
Translational Genomics Gilead Sciences Merck
MedImmune ATCC Genpharm Elan Pharmaceuticals
Centocor Boehringer Ingelheim Oncomed NIH/NCI
Takeda Pharmaceuticals CDC Covance XOMA
Quest Diagnostics Ortho-Clinical Pharmaceuticals
Salk Institute Burnham Institute Scripps Institute
Stanford University Yale Genzyme EMD Serono
Shire HGT Alnylam Wyeth Cell-Systems
Jackson Labs Beth Israel Deaconess Hospital HHMI
Whitehead Institute Harvard Systems Biology
Exelixis Charles River Labs Sanofi Pasteur Penn State
Lawrence Berkeley Lab Batelle UC Berkeley
Cedars Sinai Hospital Cleveland Clinic IDEXX Biogen
Perkin Elmer Genetics Blood Systems Research Inst.

Thank you