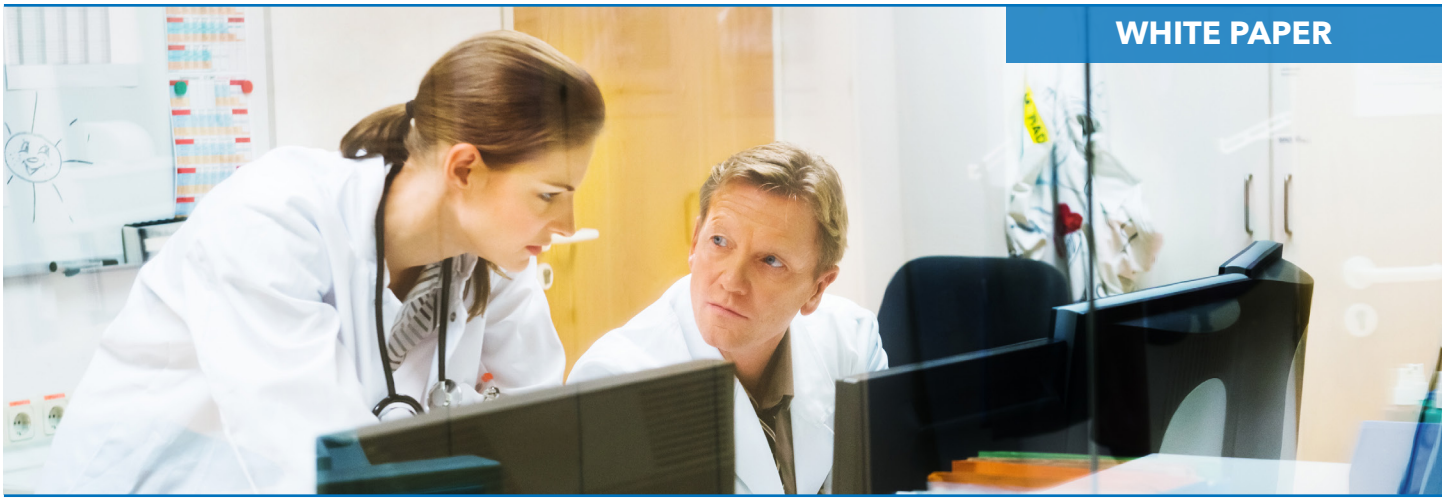


WHITE PAPER

# ThawSTAR® Automated Cell Thawing System

## Standardized thawing using breakthrough solid-state technology

### Introduction

Advances in cryopreservation techniques over the last fifty years[1,2] have helped enable progress in a wide range of fields including cell biology research, drug discovery, biobanking, assisted reproduction, plant and animal conservation, cellular therapy, and regenerative medicine. While cryopreservation techniques have improved markedly, downstream thawing techniques have been largely overlooked, even though many reports show that non-standardized thawing methods can have deleterious effects on cryopreserved products[3-5]. The success of high promise fields such as cellular therapy and regenerative medicine will require reproducible and standardized handling of the therapeutic cells[6,7], including thawing during manufacturing and prior to patient administration to ensure effective patient responses.

This white paper describes in detail BioLife Solutions' ThawSTAR® Automated Cell Thawing System (Figure 1), a first-of-its-kind automated thawing instrument that is designed to de-risk thawing of live cell therapeutics. ThawSTAR® rapidly thaws the live biological contents of a cryogenic vial with high reproducibility and minimal risk of contamination, thereby bringing standardization to this critical step in the process. Topics covered in this white paper include features and utilization of the instrument, as well as performance data.

### Standardizing Cell Thawing is Critical

Cryopreservation has become an invaluable technique within the biological sciences where cells and tissues are routinely handled. Stem cells, genetically modified immune cells, tumor cells and cell lines, bone marrow and cord blood-derived cells are examples of cell types regularly cryopreserved by clinicians. Optimal thawing of these cells is critical to successful downstream research. A broad range of applications such as cell therapy, cell-based drug discovery, biobanking reproduction and other cell-based assays can benefit from standardized cell thawing to increase their productivity and assist in fulfilling their missions to improve human health.

**Figure 1: ThawSTAR® Cell Thawing System**

Specifications

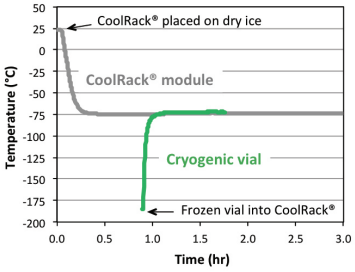
| | |
|---|---|
| Dimensions | 5.7″ (height), 4.3″ (base diameter) |
| Vial Size | 1.8–2 mL (round, flat, skirted) |
| Vials Thawed | 1 per cycle |
| Thawable Volume | 0.8–1.4 mL |
| Thaw Time | ~160 seconds |
| Final Vial Temp | < 10°C (same as water bath) |

As these industries continue to expand their collections of cryopreserved samples, the need for minimizing variability in sample handling through automation and standardization becomes essential[8-10]. Improving cryopreservation techniques, including thawing, to maximize viability and function of cryosensitive cells such as stem cells, hepatocytes[11] or neurons[12] is vital to regenerative transplant medicine. The success of the cell therapy industry is likewise dependent on having high quality, consistent products. Cryopreserved cells, whether they are being used as a primary treatment, or a reagent for cell-based discovery assays, drive the need for optimized cell thawing and resuscitation methods[13]. For controlled animal breeding, optimization of freezing and thawing protocols for semen[14] has enabled more efficient insemination as a result of reduced spermatozoa damage.



BIOLIFE SOLUTIONS
cell and gene therapy tools

**Figure 2: ThawSTAR® CFT Transporter**




Specifications

| | |
|---|---|
| Dimensions | 5.9" (height), 4.5" (diameter) |
| Vial Capacity | 5 total (1.8–2 mL) |
| Holding Temp | < 70°C |
| Holding Time | > 1 hr |
| Dry Ice Used | ~150 g |

The ThawSTAR® CFT Transporter is engineered to hold cryogenic vials at near dry ice temperatures prior to thawing them in ThawSTAR® System. Dry ice is placed in the foam holder below the metal CFT2 Core. To verify the holding temperature, a cryotube filled with cryopreservative solution with a centrally-located thermocouple was frozen in LN2 then transferred to the pre-equilibrated Transporter. The temperature profile shows that the vial warms quickly (< 10 min) to the holding temperature and remains stable for > 1 hr.

As part of a strategic vision to develop products that improve standardization of the entire cryopreservation workflow, BioLife Solutions has identified cell thawing as one of the most critical points in the process. As described below, the ThawSTAR® Automated Cell Thawing System was engineered to provide an intuitive and reproducible solution for thawing cryogenic vials in research, manufacturing and clinical settings.

## Current Thawing Methods

*Biophysics and Biology of Cell Thawing.* Cryopreservation of cells and tissues has been studied extensively for decades. In its most basic form, effective cryopreservation (not accomplished by direct vitrification) requires controlled-rate cooling of the cells generally in the presence of cryoprotectant to allow (a) minimization of intracellular ice crystal formation during the liquid-to-solid phase transition by effective cell dehydration, (b) control of osmotic gradients across the cell membrane as extracellular solute concentrations increase, and (c) minimization of extracellular ice crystal size. The microscopic processes occurring upon the thawing of cryopreserved cells and tissues are almost mirror images of those that occur during freezing: warming of the sample from cryogenic temperatures toward the solid-to-liquid phase transition, melting of extracellular ice crystals to form liquid water, rehydration of the cells, and reformation of an extracellular salt and protein solution.

During a thaw, it is critical to minimize both osmotic shock to the rehydrating cells and overall ice recrystallization in the thawing mixture[1,4,5,15-17]. Ice recrystallization during thawing is a commonly observed phenomenon where small ice crystals generated during the freezing process can reform into larger crystals at sub-freezing temperatures and act as nucleation sites for the liquid water formed at higher temperatures[5,15,16]. The result of either process is progressively larger ice crystal formation that can be injurious to cells. Temperature fluctuations or slow warming of a frozen sample will increase ice crystal size. The extent of damage from ice recrystallization during cell thawing can range from very minor to significant depending on the thaw procedure[16-19]. Decades of empirical results have demonstrated that rapid, controlled thawing of cryopreserved samples provides optimal post-thaw viability for the majority of cells and tissues by limiting ice recrystallization and rehydrating cells as rapidly as possible. Ideally, thawing rate and temperature should also be optimized for cell size and volume, cell type, and choice of cryopreservative.

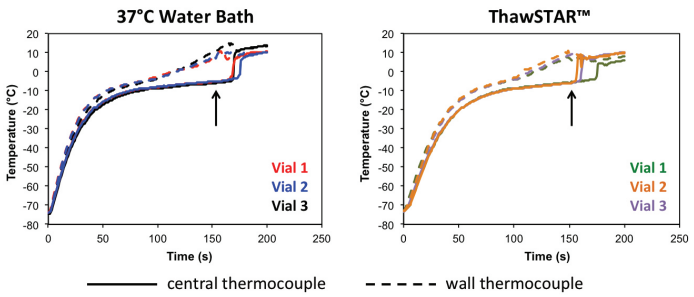
**Current Methods for Cell Thawing.** To achieve rapid thawing rates, biologists routinely plunge frozen samples into water of varying temperatures (from 37°C up to 60°C) for seconds to minutes. By far, the most common and well-accepted method for rapidly thawing cryopreserved cell samples is partial submersion of the vial in a 37°C water bath. There are several reasons for using this approach: water baths are relatively cheap and easily available, they allow efficient heat transfer from the water to the vial due to the high heat capacity and thermal conductivity of liquid water, and there is little danger of "over cooking" the cells since the maximum temperature the vial can achieve is 37°C.

However, there are significant disadvantages to using a water bath for thawing, particularly in a clinical environment. These disadvantages include: (1) lack of scalability post-manufacturing, (2) user-to-user variability in subjectively determining thaw times, final vial temperature, and end point, (3) no data management or chain-of-custody connectivity, (4) high risk of contamination of vial contents through wicking of water into the cap threads and seal in a poorly maintained, and often communal, water bath, (5) inability to use a water bath as part of a sterile process inside a cell culture hood, (6) restrictions in using a water bath in a GMP or clinical environment. To overcome some of the limitations of using a water bath for thawing, researchers and process engineers have explored other options such as dry bead baths or heat blocks[20,21]. Unfortunately, these solutions have very inefficient thermal contact, resulting in reduced heat influx, and can take 2-3 times longer (~7 minutes in a dry bead bath vs. ~2.5 minutes in a 37°C water bath) to thaw samples, which can increase the risk of ice recrystallization damage.
As demonstrated below, the ThawSTAR® Automated Cell Thawing System is designed to optimize and de-risk cell thawing at point-of-care. ThawSTAR® provides an innovative solution for standardized, reliable, and highly reproducible cell thawing that is equivalent or superior in performance to an ideal water bath-based thaw and can be integrated into processes within research, GMP, and clinical settings.

## ThawSTAR® Thawing Platform

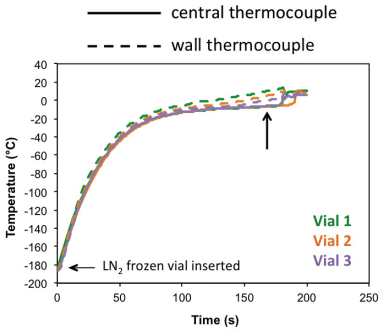
ThawSTAR® Automated Cell Thawing System (Figure 1) is a compact instrument that uses solid-state heating blocks with a pliant conductive material interface to maximize contact

## Figure 3: Thermal Profile of Vials Thawed in a Water Bath or ThawSTAR® System




1.8 mL cryogenic vials filled with 1 mL of cryopreservation medium (10% DMSO/20% FBS/70% DMEM) were fitted with an interior wall thermocouple (< 0.5 mm from internal wall surface) and a central axis thermocouple, frozen at -80°C, and maintained at -75°C in a CFT2 Core on dry ice as in Figure 2. Three vials were thawed in a 37°C water bath (left panel) or in the ThawSTAR® System (right panel). The temperature profiles recorded by both thermocouples were very similar for both the water bath thaw and the ThawSTAR® thaw. For the water bath thaw, the vials were removed from the bath when a pea-sized ice chunk remained (arrow) and then gently tapped to melt the chunk. Similarly, ThawSTAR ejected the vial at the point where a pea-sized ice chunk remained (arrow) and after mixing the final vial temperature is ~5-10°C. Note that the sharp rise in temperature seen with the central thermocouple is indicative of the ice chunk breaking away from the thermocouple.

## Figure 4: Thawing Vials Directly from $LN_2$ Storage



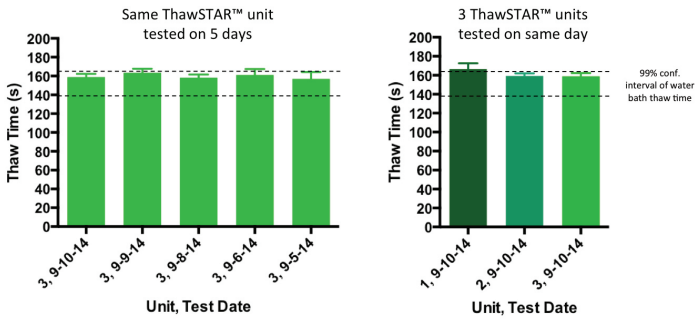

1.8 mL cryogenic vials filled with 1 mL of cryopreservation medium (10% DMSO/20% FBS/70% DMEM) were fitted with an interior wall thermocouple (< 0.5 mm from internal wall surface) and a central axis thermocouple and frozen at -196°C. The vials were removed from LN2 and immediate placed in the ThawSTAR® instrument. The ThawSTAR® ejected the vial at the point where a pea-sized ice chunk remained (arrow). Note that the sharp rise in temperature seen with the axial thermocouple is indicative of the ice chunk breaking away from the thermocouple. Final vial temperature is ~5-10°C, comparable to a water bath thaw.

and heat transfer to vials being thawed. The patent-pending STAR™ sensing technology monitors vial temperature and utilizes software algorithms to detect the solid-to-liquid phase change. BioLife Solutions recognizes that cell type, cell size and volume, and choice of cryopreservative all affect optimal thawing rate and temperature. Each ThawSTAR system can be programmed with a customized thawing algorithm, specific to a given cell therapy product or cell type, in order to preserve optimal function. The result is a reproducible and standardized thaw for vials taken directly from $LN_2$ storage, a -80°C freezer, or those equilibrated and held at to dry ice temperature (-78.5°C) in the ThawSTAR® CFT Transporter (Figure 2). The ThawSTAR® Automated Cell Thawing System is designed to thaw cryogenic vials similarly to a water bath in terms of heat influx rate and final temperature achieved, resulting in cell viability and recovery rates that are statistically identical to or superior to those achieved with a water bath. Furthermore, ThawSTAR® Automated Cell Thawing System eliminates the risk of water borne contamination, and the variability in thawing times and endpoints associated with using a water bath.

**Breakthrough Technology for Solid-State Thawing.** The ThawSTAR® solidstate technology platform is engineered to provide a heating profile and final vial temperature similar to that achieved when thawing in a 37°C water bath. This is achieved by using conductive heating blocks that are coupled to the vial to be thawed through an inert, malleable, conductive material that conforms to any irregularities in the vial wall, thus providing a uniform heat transfer surface. This coupling solves the problem of inefficient heat transfer commonly seen with other solid state thawing processes such as dry bead baths or bare metal heating blocks. The thermal profile of vials thawed in a ThawSTAR® System is virtually identical to that seen in a water bath (Figure 3) including heating rate and final vial temperature.

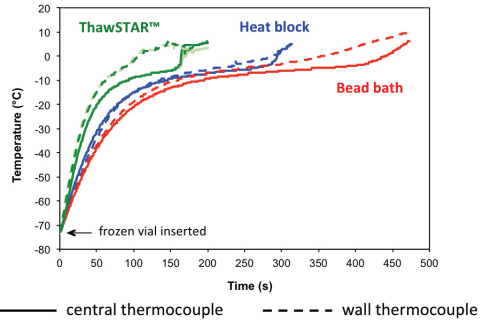
The ThawSTAR® technology monitors the vial temperature during initial heating and detects the point at which the contents initiate phase change from solid to liquid. Labels or writing on the cryogenic vial do not affect the performance of the unit. This unique feature of the ThawSTAR® System ensures active detection of the phase change initiation, enabling successful vial thawing from $LN_2$ temperatures

## Figure 5: Thaw Time Reproducibility of the ThawSTAR®




Left panel: The average time for the same ThawSTAR® System to thaw > 5 frozen vials each day for 5 days. Right panel: The average thaw time for three different ThawSTAR® Systems was measured using > 5 frozen vials per unit on one day. No significant differences were identified for either scenario at $p<0.05$ (2-way ANOVA with post hoc Sidak test). For comparison, six vials were thawed in a 37°C water bath. The average thaw time in the water bath was 151 seconds, with a 99% confidence interval of 139-164 s (range shown as dotted lines).

**Figure 6: Rapid Vial Thawing with ThawSTAR Compared to Dry Bead Bath or Heat Block**




1.8 mL cryogenic vials filled with 1 mL of cryopreservation medium (10% DMSO/20% FBS/70% DMEM) were fitted with a wall thermocouple (< 1 mm from internal wall surface) and a central axis thermocouple, frozen at -80°C, and maintained at -75°C in a CFT2 Core on dry ice as in Figure 2. Vials were thawed in either a ThawSTAR® System (green traces), a 37°C bead bath (Lab Armor beads; red traces), or an aluminum heat block equilibrated to 37°C (blue traces). The ThawSTAR® System thaw time is 2-3X faster than these other dry thawing methods.

(Figure 4) in addition to dry ice temperatures. In addition, the ThawSTAR® System can thaw samples stored at -20°C, enabling controlled thawing of antibody, enzyme, or other biospecimen aliquots.

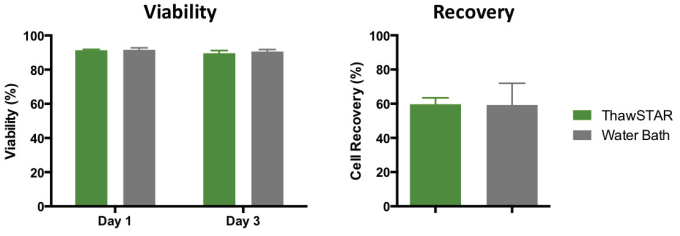
The performance of the ThawSTAR® System's hardware and software algorithms are highly reliable, enabling the System to be a powerful standardization tool. Figure 5 demonstrates the high run-to-run reproducibility of a single unit when multiple vials were thawed on different days.

Additionally, high unit-to-unit reproducibility was also demonstrated by testing multiple units on the same day. The average thawing time of 1.0 mL of frozen cell suspension with a ThawSTAR® System was 160 s; statistically equivalent to the average thaw time of 151 s obtained when using a water bath. The ThawSTAR® System thaws a vial 2-3X faster than other commonly used dry thawing methods (Figure 6), thus minimizing the risk of ice recrystallization damage to the cells.

**Instrument Validation by Effective Thawing of Multiple Cell Types.** The ability of the ThawSTAR® System to effectively thaw cryopreserved cells was analyzed by multiple independent investigators in a head-to-head comparison with the standard 37°C water bath thawing method (Figures 7 and 8). In each case, 6 vials of the same lot of frozen cells were removed from $LN_2$ stores, randomly assigned to either a "37°C water bath" or "ThawSTAR®" group, equilibrated to dry ice temperatures in a CFT2 Core placed in dry ice, then thawed by either method. In each case, cell count and viability (or recovery of viable cells) were assessed immediately post thaw (day 1) and after 3 days of growth or recovery (day 3) as described in the figure legends, and the results compared statistically.

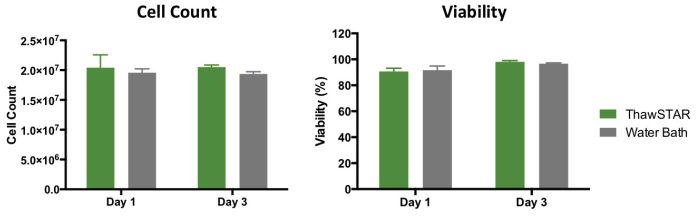
The ThawSTAR® System showed statistically equivalent results compared to a water bath for all cell types tested including peripheral blood mononuclear cells (PBMC; Figure 7), the K562 erythromyeloblastoid cell line (Figure 8), as well as human splenocytes, stimulated CD8+ central memory T cells, and antibody producing mouse hybridoma cells (data not shown). Studies also showed significantly higher post-rest recovery in PBMCs thawed with the ThawSTAR® system as compared to those thawed by water bath (Figure 9). This data is significant because it has been demonstrated that apoptosis and necrosis arising from the stress of the thawing procedure reaches its peak 24 hours post-thaw[2]. It is therefore likely that the ThawSTAR® System subjects PBMCs to less stress during the thawing procedure than water bath thawing. Taken together, these results demonstrate that the ThawSTAR® Automated Cell Thawing System can achieve identical or superior cell recovery and viability compared to the standard 37°C water bath thawing method.

**Figure 7: Thawing of Peripheral Blood Mononuclear Cells**



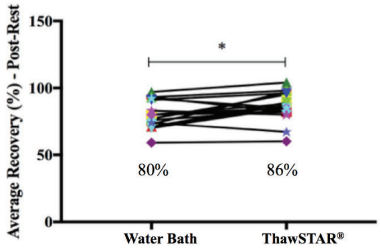

Cell viability (left panel) and recovery (right panel) of PBMC were measured using trypan blue exclusion on a hemacytometer. Recovery is the number of viable cells post-thaw as a percentage of the pre-freeze viable cells. No statistical difference in viability (2-way ANOVA with post hoc Sidak test) or recovery (unpaired two-tailed t-test) at $p<0.05$ were found. Data courtesy of Dr. Mars Stone at the Blood Systems Research Institute.

**Figure 8: Thawing of K562 Erythromyeloblastoid Cells**




Cell count (left panel) and viability (right panel) of K562 cells were measured using trypan blue exclusion on a hemacytometer. No statistical difference in viability or cell count (each tested with 2-way ANOVA with post hoc Sidak test) at $p<0.05$ were found. Data courtesy of Helen Huls at the MD Anderson Cancer Center.

**Figure 9: Higher Post-Rest Average Recovery**




Average recovery post-rest (16-24 hrs). The post-rest recovery is the ratio of the total number of viable cells postrest and the total number of viable cells post-thaw multiplied by 100%. For each donor, the data is expressed as the average of the post-rest recovery triplicates. The percentages written on the figures represent the mean of the average recovery for all donors. The results are based on 6 independent runs (n = 17 vials for water bath and n = 17 vials for ThawSTAR® instrument). * $p \leq 0.05$ ($p = 0.0333$). Data courtesy of Caprion BioSciences and McGill University.

## Summary

ThawSTAR® Automated Cell Thawing System is an intuitive, fully automated system designed to de-risk and standardize clinical cell thawing. It is engineered to reproducibly thaw the live biological contents of a cryogenic vial in a similar manner to the commonly used 37°C water bath but without the subjectivity or risk of contamination found with water bath usage. The intuitive operation of the ThawSTAR® System can be readily integrated into most current workflows with the added advantage of easy adoption into sterile procedures performed inside a cell culture hood, GMP or clinical setting. The ThawSTAR® Automated Cell Thawing System standardizes the "last mile" in the cryopreservation workflow and enables future cell-based discoveries and therapies.

## About BioLife Solutions

BioLife Solutions develops and commercializes standardization and automation technologies for vital preclinical and clinical sample handling. An industry first, the ThawSTAR® Automated Cell Thawing System replaces uncontrolled and highly variable manual methods with a customizable algorithm for each unique cell therapy product. BioLife Solutions' global customers include pharmaceutical, medical, stem cell and other GMP facilities where consistent and repeatable outcomes are paramount.

## References

1. Mazur, P., *et al.* (1970) Interactions of cooling rate, warming rate, and protective additive on the survival of frozen mammalian cells. Ciba Foundation Symposium on the Frozen Cell. J&A Churchill Publication, London, UK.
2. Bissoyi, A., *et al.* (2014) Targeting cryopreservation-induced cell death: a review. *Biopreserv Biobank*. 12(1):23-34.
3. http://www.bioprocessintl.com/wp-content/uploads/bpicontent/BPI_A_110904AR11_O_145195a.pdf
4. Fowler, A. and Toner, M. (2005) Cryo-injury and biopreservation. *Ann N Y Acad Sci*. 1066:119-135.
5. Chaytor, J.L., *et al.* (2012) Inhibiting ice recrystallization and optimization of cell viability after cryopreservation. *Glycobiology*. 22(1):123-133.
6. Yang, H., *et al.* (2014) Effects of interruptions of controlled-rate freezing on the viability of umbilical cord blood stem cells. *Transfusion*. doi: 10.1111/trf.12774.
7. Li, Y., and Ma, T. (2012) Bioprocessing of cryopreservation for large-scale banking of human pluripotent stem cells. *BioResearch Open Access*. 1(5):205–214.
8. Peakman, T., *et al.* (2010) Current standards for the storage of human samples in biobanks. *Genome Medicine*. 2:72.
9. Querol, S., *et al.* (2010) Quality rather than quantity: the cord blood bank dilemma. *Bone Marrow Transplantation*. 45:970-978.
10. Cuhadar, S., *et al.* (2013) The effect of storage time and freezethaw cycles on the stability of serum samples. *Biochemia Medica*. 23(1):70-7.
11. Terry, C., *et al.* (2010) Optimization of the cryopreservation and thawing protocol for human hepatocytes for use in cell transplantation. *Liver Transplantation*. 16:229-237.
12. Shi, Y. *et al.* (2012) Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nature Protocols*. 7(10):1836-46.
13. Guido, J.R., *et al.* (2007) Cryopreserved cells facilitate cell-based drug discovery. *Drug Discovery Today*. 12:13-14.
14. Bradford, L.L. and Buhr, M.M. (2002) Function of cryopreserved horse semen is improved by optimized thawing rates. *J Equine Vet Sci*. 22(12):546-550.
15. Cao, E. *et al.* (2003) Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. *Biotechnol Bioeng*. 82(6):684-90.
16. Carpenter, J.F. and Hansen, T.N. (1992) Antifreeze protein modulates cell survival during cryopreservation: Mediation through influence on ice crystal growth. *Proc Natl Acad Sci USA*. 89:8953-8957.
17. Deller, R.C., *et al.* (2014) Synthetic polymers enable non-vitreous cellular cryopreservation by reducing ice crystal growth during thawing. *Nature Communications*. 5:3244.
18. Norkus, M. *et al*. (2013) The effect of temperature elevation on cryopreserved mesenchymal stem cells. *Cryoletters*. 34(4):349-359.
19. Olson, W.C., *et al.* (2011) Shipping blood to a central laboratory in multicenter clinical trials: Effect of ambient temperature on specimen temperature, and effects of temperature on mononuclear cell yield, viability and immunologic function. *J Trans Med*. 9(26):1-11.
20. Röllig, C., *et al.* (2002) Thawing of cryopreserved mobilized peripheral blood--comparison between waterbath and dry warming device. *Cytotherapy*. 4(6):551-5.
21. Triana, E., *et al.* (2013) Thawing of cryopreserved hematopoietic progenitor cells from apheresis with a new dry-warming device. *Transfusion*. 53(1):85-90.

We would like to thank the following contributors: Dr. Mars Stone, Blood Systems Research Institute, Helen Huls, MD Anderson Cancer Center and Caprion BioSciences and McGill University.

BIOLIFE SOLUTIONS
cell and gene therapy tools

**info@biolifesolutions.com | biolifesolutions.com**





WP-AST-19018R01