Improved Cryopreservation & Recovery Solutions for Pluripotent Stem Cells & Difficult-to-Preserve Primary Cells

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ABSTRACT

Pluripotent stem cells (PSCs) and primary cells are foundational tools for basic research and applied applications including regenerative therapy, drug discovery, and toxicological assessment. While stem cells have a tremendous proliferative capacity, long term culture of these cells has been shown to cause an accumulation of mutations that result in genetic instability, increasing tumorigenicity and thus limiting their usefulness in research and clinical applications. Improved solutions for cryopreservation of early passage cells that minimize loss of viability, maximize post-thaw recovery, and minimize unwanted differentiation are essential components to PSC, as well as primary cell, workflows. While many cryopreservation reagents afford high viability immediately post-thaw, significant apoptosis and necrosis is often observed during the first 24 hours post-thaw, decreasing the effective viability, reducing cell numbers and adding additional stress and selective pressure to cultures. Further, this extends the time post-thaw cells must be cultured prior to use in downstream experiments. Using a series of Design of Experiments (DOE) and mathematical modeling methods, we describe the development of a xeno-free cryomedium for use in cryopreservation of PSCs and ESCs, and a chemically defined recovery supplement for use in recovery of PSCs, ESCs, as well as difficult-to-preserve primary cells. When used together, we show this system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. As a result of increased post-thaw survival rate, cells recover faster and are ready to passage sooner than with current solutions, while maintaining pluripotency and normal karyotype over 10 passages. Additionally, the chemically defined recovery supplement was tested in combination with other cryopreservation reagents which lead to markedly improved 24 hour post-thaw viability of difficult-to-preserve primary cells, including primary cortical neurons and human corneal epithelial cells.

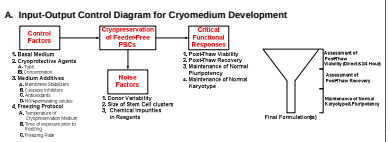
INTRODUCTION

The goals of this research were to (1) provide improved, cost-effective solutions for cryopreservation and recovery of PSCs, (2) identify applicability of solutions to additional difficult-to-preserve cell types, as well as, (3) assess alternative applications for the developed recovery supplement.

Life Technologies currently offers two ready-to-use cryopreservation media: Synth-a-Freeze® and Recovery™ Cell Culture Freezing Medium. During our development, these media were tested for their applicability for cryopreservation of PSCs in comparison with the currently recommended protocol of use of Essential 8™ + 10% DMSO, as well as optimized solutions.

Six Sigma Design Excellence principles were used to guide generation of improved cryopreservation and recovery systems with an initial focus on the PSC workflow. Broader applicability of solutions to primary cell types was subsequently assessed.

Figure 1. Experimental Workflow for Optimizing PSC Cryomedium

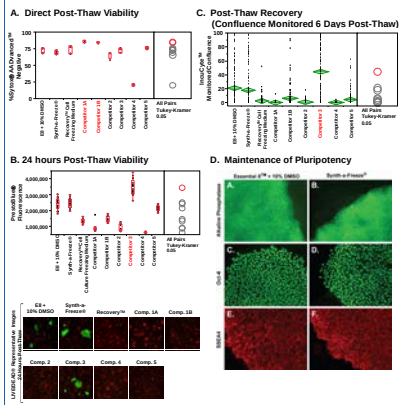


•Process workflows were used in optimization of cryomedium and recovery medium solutions

• Assays fit for purpose were determined and used in development of reagents using a series of screens, iterative DOEs, and titrations

RESULTS

Figure 2. Analysis of Existing Solutions for PSC Cryopreservation



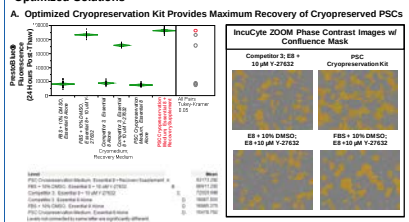
• Competitor 3 was shown to be "Best-in-Class"

• While several commercial cryopreservation solutions provide high viability (>70%) post-thaw, these measurements are overestimates of effective iPSC survival, as cell death from apoptosis and necrosis following cryopreservation can take days to be realized [1-2].

• Viability 24 hours post-thaw is predictive of post-thaw recovery

•There was shown to be significant room for improvement

Figure 3. Feeder-Free iPSC Cryopreservation & Recovery Using Optimized Solutions

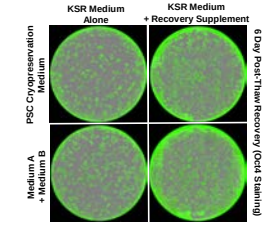


• Our improved xeno-free cryomedium coupled with chemically defined recovery supplement provides optimum post-thaw recovery of iPSCs

• When used together with the chemically defined recovery supplement being applied for 18-24 hours post passaging at each passage, iPSCs are shown to maintain normal morphology, pluripotency, and karyotype

• Use of the recovery supplement in conjunction with various cryomedia provides significant improvement over that observed for the traditional ROCK inhibitor, Y-27632

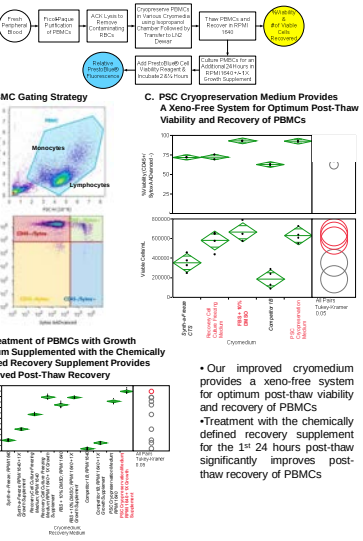
Figure 4. Feeder-Dependent iPSC Cryopreservation & Recovery Using Optimized Solutions



• The PSC Cryopreservation Kit shows utility for cryopreservation & recovery of feeder-dependent cultures

• Chemically defined recovery supplement provides significant boost in post-thaw recovery.

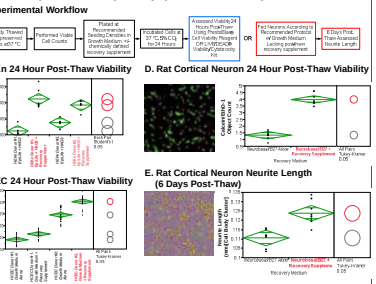
Figure 5. PBMC Cryopreservation/Recovery Using Optimized Solutions



• Our improved cryomedium provides a xeno-free system for optimum post-thaw viability and recovery of PBMCs

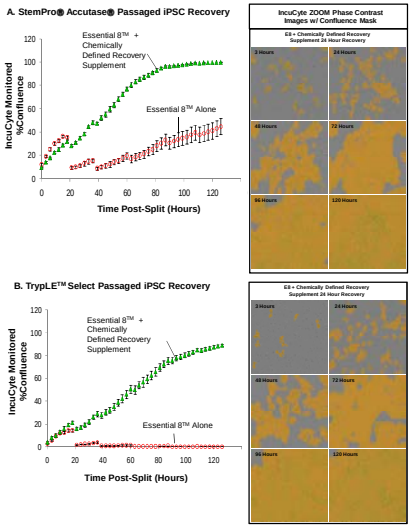
•Treatment with the chemically defined recovery supplement for the 1st 24 hours post-thaw significantly improves post-thaw recovery of PBMCs

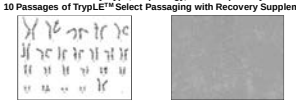
Figure 6. Chemically Defined Recovery Supplement Demonstrates Applicability for Recovery of Cryopreserved Primary Cells



• Our chemically defined recovery supplement shows utility in recovery from cryopreservation of a variety of primary cell types, including human epidermal keratinocytes, neonatal (HEKn), human corneal epithelial cells (HCECs), and rat cortical neurons

Figure 7. Chemically Defined Recovery Supplement Demonstrates Applicability for Survival of PSCs Following Single Cell Passaging in Essential 8™ System





• Use of the chemically defined recovery supplement for 18-24 hours post-single cell passaging via StemPro® Accutase® or TrypLE™ Select passaging provides vast improvement of cell survival compared to recovery in Essential 8™ Alone.

• Normal karyotype, morphology, and pluripotency maintained over 10 passages

CONCLUSIONS

• Measurement Systems Analyses (MSAs) were used to define assays fit for purpose for determining cryomedia which maximize post-thaw viability & recovery

• DOEs and mathematical modeling were used to improve Life Technologies' solutions and associated protocols for cryopreservation and recovery of PSCs

• PSC Cryopreservation Medium & chemically defined recovery supplement show utility for use in PBMC cryopreservation and recovery

• Additional utility of post-thaw recovery supplement is demonstrated for recovery of additional cryopreserved cell types, as well as for single cell passaging of PSCs

• Prototypes are available of the PSC Cryopreservation Kit (Cat #A24439SA) containing 50 mLs of PSC Cryopreservation Medium and 5 mLs of the chemically defined recovery supplement (aka, Growth Supplement (100X)). The chemically defined recovery supplement (aka, Growth Supplement) is also available as a standalone product (Cat #A24422SA).

• For Research Use Only. Not for use in diagnostic procedures.

• Please contact Rhonda Newman (rhonda.newman@lifetech.com) or Wendy Bray (wendy.bray@lifetech.com) for more information.

REFERENCES

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TRADEMARKS/LICENSING