AN OPTIMIZED AND VERSATILE SMALL MOLECULE-BASED SUPPLEMENT MITIGATES STRESS AND IMPROVES SURVIVAL OF PSCS AND PSC-DERIVED CELLS

Michael A Derr, Kurt Laha, Chris Yankaskas, Michael Akenhead, David Kuninger, Soojung Shin
Thermo Fisher Scientific, 7335 Executive Way, Frederick, MD, USA 21704

ThermoFisher SCIENTIFIC

Abstract

Advancements in cell culture media development have led to improved survival, expansion and maintenance of PSCs and PSC-derived cells; however, poor cell survival and recovery following stress-inducing culture applications remain a significant challenge. The use of ROCK inhibitors (RI) provides moderate improvement in recovery of PSCs in some routine applications (single-cell passaging and cryopreservation), but RIs show little improvement in survival of differentiated cells. Existing commercially available cell recovery supplements offer improved performance compared to RI's yet are only recommended for a limited number of applications. Here we present Gibco™ CultureCEPT™ Supplement, a new and highly versatile cell recovery reagent. The CultureCEPT Supplement is offered at 1000x concentration and the formulation is based on advancements at the NIH's National Center for Advancing Translational Sciences (NCATS). This study demonstrates the versatility and superior performance of CultureCEPT compared to existing recovery reagents in: (1) Recovery of PSCs from single-cell passaging (monolayer and spheroid) and cryopreservation, (2) Recovery of PSC-derived cells (neurons) from dissociation and cryopreservation, (3) Recovery of organoid models (lung) from cryopreservation. For many of these applications CultureCEPT improves survival by 25-50% compared to the RI Y-27632. CultureCEPT Supplement reduces cellular stress and improves viability of stem cells, differentiated cells, and primary cells during a wide variety of handling and processing steps where cell damage and death can limit the success of a workflow.

Introduction

Gibco CultureCEPT Supplement (1000x) reduces cellular stress and improves viability of stem cells, differentiated cells, and primary cells during a wide variety of handling and processing steps where cell damage and death can limit the success of a cell culture workflow. It is a unique formulation that provides superior cell protection compared to traditional ROCK inhibitors, such as Y-27632, and other commercially available cell culture reagents. Here we show the utility of CultureCEPT Supplement in a wide range of applications including PSCs (monolayer and suspension culture), PSC derived cells, and a lung organoid model.

Materials and methods

In each experiment presented here, cells were treated with CultureCEPT for 24 hours. After 24 hours, medium was changed with appropriate medium without CultureCEPT supplementation.

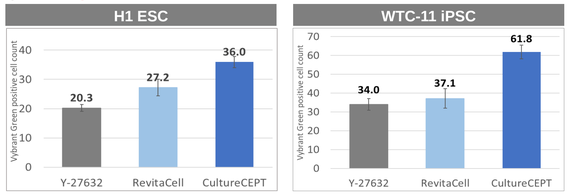
CultureCEPT Supplement (1000x) Cat. No. A56799 0.5mL, A56800 0.1mL

Figure 1:
Essential 8™ medium, Cat. No. A1517001
RevitaCell™ Supplement, Cat. No. A2644501
Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix, Cat. No. A1413301
Figure 2:
StemScale™ PSC Suspension Medium, Cat. No. A4965001
StemFlex™ Medium, Cat. No. A3349401
Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit, Cat. No. A24881
Figure 4:
StemScale™ PSC Suspension Medium, Cat. No. A4965001
Nunclon™ Sphera™96-well U-bottom microplate
Figure 5:
B27 Supplement, Cat. No. 17504044
Poly-D-Lysine, Cat. No. A3890401
Laminin Mouse Protein, Cat No. 23017015
Figure 6:
A Simplified protocol for the development of 3D lung organoids.
thermofisher.com/stemscale

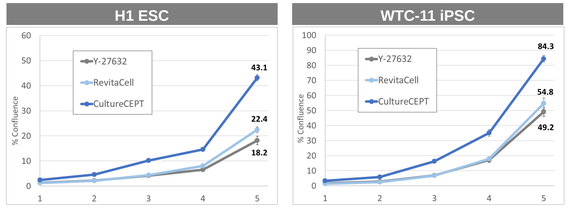
Results

Figure 1. CultureCEPT™ Supplement improves PSC recovery from cryopreservation

a) 24-hour post-thaw survival of PSCs: live cell count



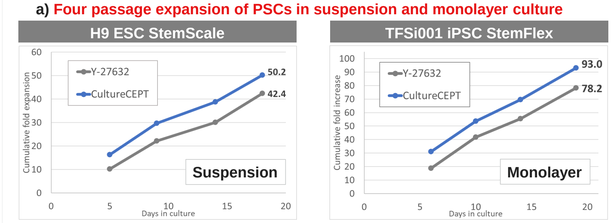
b) Percent Confluence over 5 days in culture post-thaw

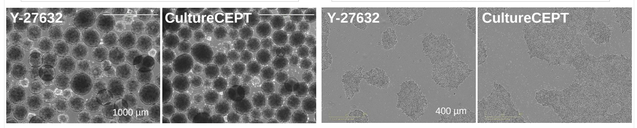


a) CultureCEPT improved 24hr post-thaw survival of PSCs by as much as 82% compared to Y-27632 and 66% vs RevitaCell™.
b) Improved post-thaw recovery and cell-attachment led to higher % Confluence after 5 days in culture for the CultureCEPT treated cells.

Figure 2. CultureCEPT™ Supplement increases PSC expansion yields while maintaining pluripotency markers

a) Four passage expansion of PSCs in suspension and monolayer culture





b) CultureCEPT treated PSCs maintain pluripotency markers after 4 passages: %OCt4 positive cells

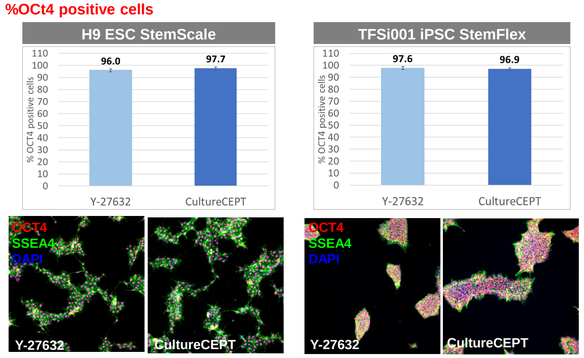
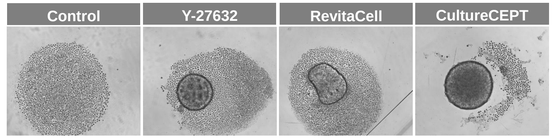
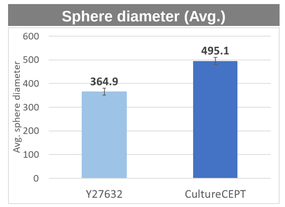


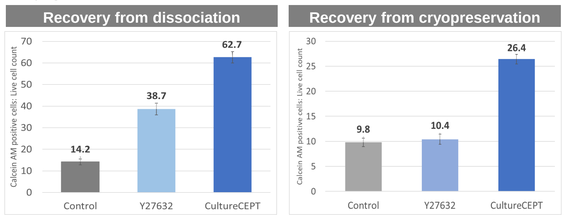
Figure 3. CultureCEPT™ Supplement enables efficient PSC sphere formation

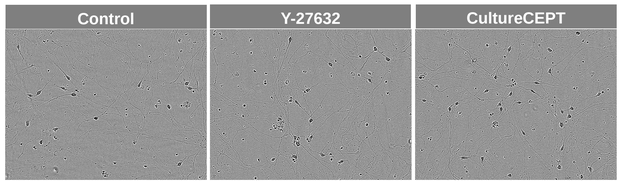




WA01 ESC were thawed in StemScale™ medium and seeded in Nunclon™ Sphera U-bottom 96 well plates. Cells were treated with indicated cell survival reagents. Images were captured at day 4 and sphere formation and size was assessed CultureCEPT treated cells resulted in highly efficient and uniform sphere formation and low levels of surrounding dead cells. CultureCEPT treated sphere diameter was 35.7% larger than Y-27632 spheres. RevitaCell™ treated cells did not form uniform spheres and is not recommended for 3D applications.

Figure 4. CultureCEPT™ Supplement enhances recovery of dissociated and cryopreserved induced neurons



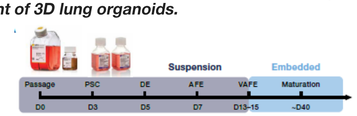


Induced neurons were generated from ngn2 inducible PSC line. Following three days of NGN2 induction the cells were Accutase dissociated. The dissociated cells were either replated for maturation or cryopreserved for one week, followed by thawing and plating for maturation. CultureCEPT treatment improved survival of neurons post-dissociation and cryopreservation compared to Y-27632.

Figure 5. CultureCEPT™ Supplement improves the recovery and re-formation of lung organoids following cryopreservation

a) Lung Organoid protocol background

Lung organoids were generated following a protocol available on the Thermo Fisher Scientific website at **thermofisher.com/stemscale: *A Simplified protocol for the development of 3D lung organoids.***



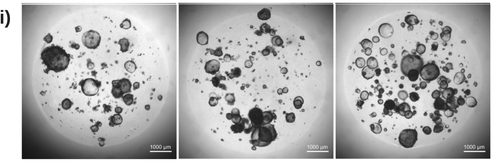
For this study, mature lung organoids were dissociated and cryopreserved at day 56. Post-thaw recovery and re-formation of lung organoids was assessed.

b) Summary of experiment: Recovery of dissociated and cryopreserved lung organoids

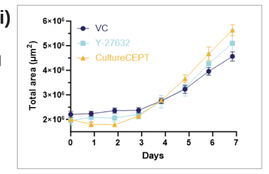
- Lung organoids were thawed and embedded in Geltrex domes.
- Cells were cultured for 24 hours in lung organoid maturation medium containing either Y-27632, CultureCEPT, or maturation medium only control.
- After 24 hours the medium was changed to maturation medium only and cultured for one week.
- After one week, organoid recovery and formation was assessed

c) Recovery and re-formation of lung organoids from cryopreservation

i)

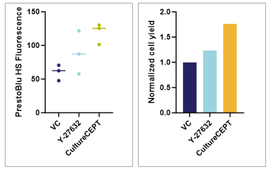


ii)



i) Representative images of lung organoids at D7 **(top)**
ii) Images were captured and analyzed over 7 days and comparison of total area was plotted **(right)**
iii) Live cell quantitation was performed in two ways:
- PrestoBlu cell viability reagent **(bottom left)**
- Dissociation and counting of live cells **(bottom right)**

iii)



- CultureCEPT treatment improved survival, recovery, and re-formation of lung organoids post-thaw from cryopreservation.

Conclusions

Our studies demonstrate the utility of CultureCEPT Supplement for a broad range of applications. CultureCEPT effectively mitigates cell stress and improves cell survival following stressful cell culture procedures and manipulations. Improving cell survival can dramatically increase work-flow success rates, save time, and may enable the implementation of workflows where poor cell survival is a limiting feature.

References

1. Chen, Y. et al.: Nat. Methods, 18(5), 528 (2021).
A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells.
2. Tristan, C.A. et al.: Stem Cell Reports, 16(12), 3076 (2021).
Robotic high-throughput biomanufacturing and functional differentiation of human pluripotent stem cells.
3. Tristan, C.A. et al.: Nat. Protoc., 18(1), 58 (2022).
Efficient and safe single-cell cloning of human pluripotent stem cells using the CEPT cocktail.
4. Deng, T. et al.: Stem Cell Reports, 18(4), 1030 (2023).
Scalable generation of sensory neurons from human pluripotent stem cells.
5. ***A Simplified protocol for the development of 3D lung organoids.***
thermofisher.com/stemscale

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.