

NutriFreez[®] D10 Cryopreservation Medium

Discover Performance

Simplifying Progress

SARTORIUS

Table of Content

NutriFreez® D10 Cryopreservation Medium.....	3
Human Mesenchymal Stromal Cells.....	4
The Ottawa Hospital Research Institute	10
Human Pluripotent Stem Cells	12
WiCell Research Institute	16
Primary Cells & Various Cell Lines	18



NutriFreez® D10 Cryopreservation Medium



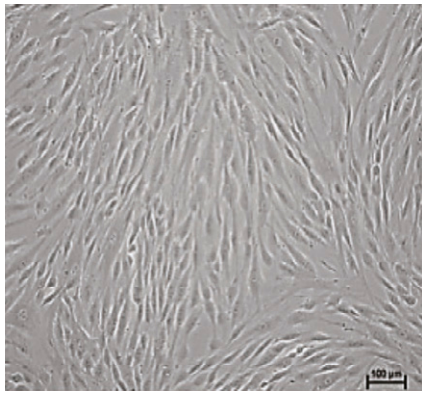
✓ Chemically defined
and animal
component-free

✓ Manufactured
under cGMP
conditions

✓ FDA Drug Master File
(DMF) available

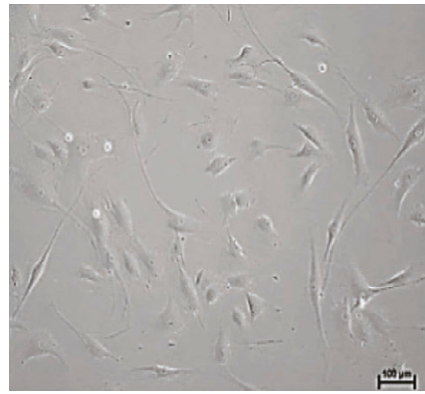
✓ Contains:
Methylcellulose
and 10% DMSO

Proliferation and Morphology Comparison Post Cryopreservation of Human Mesenchymal Stromal Cells in NutriFreez® D10 Medium.



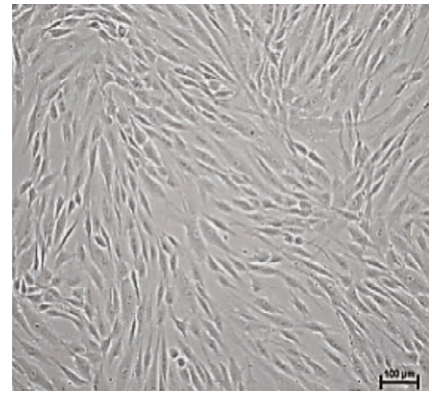
NutriFreez® D10 Medium

38,000 cells/cm²
Normal morphology



Cryostor® CS10

~4,000 cells/cm²
Abnormal morphology



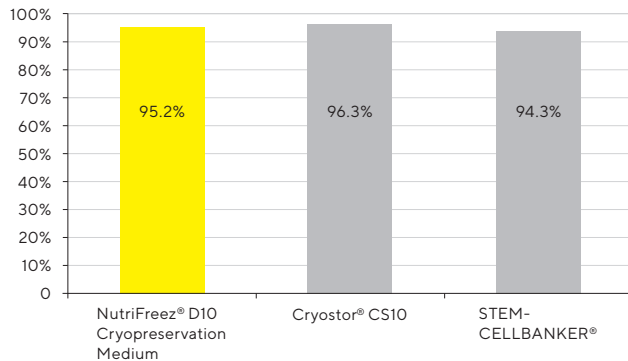
STEM-CELLBANKER®

29,000 cells/cm²
Normal morphology

Viability and Recovery Comparison of Human Mesenchymal Stromal Cells Following Cryopreservation in NutriFreez® D10 Medium.

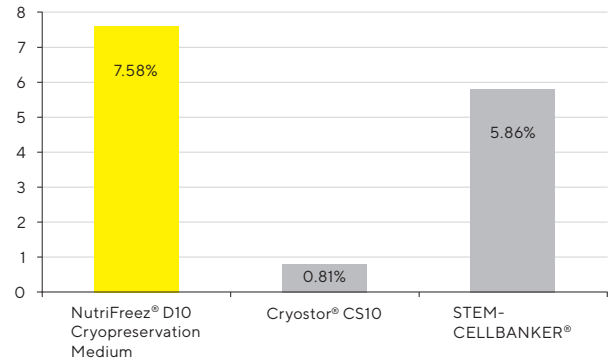
High Viability

≥ 95% viability when compared to other commercial serum-free solutions direct post-thaw



Superior Recovery

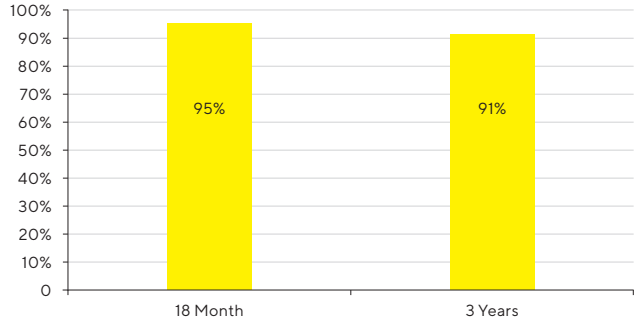
More cells in less time at 3 days post-thaw with a >7-fold cell increase



Viability Comparison of Human Mesenchymal Stem Cells Following Long-Term Cryopreservation in NutriFreez® D10 Medium.

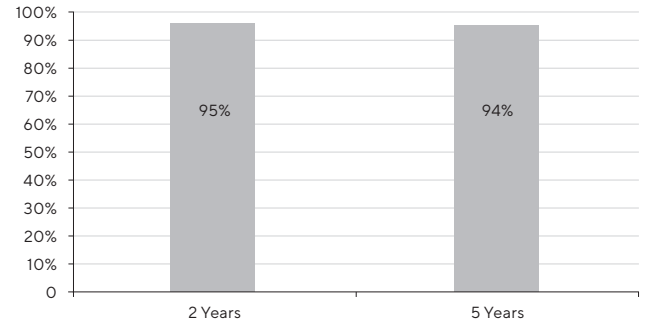
High Viability (Long-Term)

hMSC-BM show ≥ 91% viability after 3-years of cryopreservation



High Viability (Long-Term)

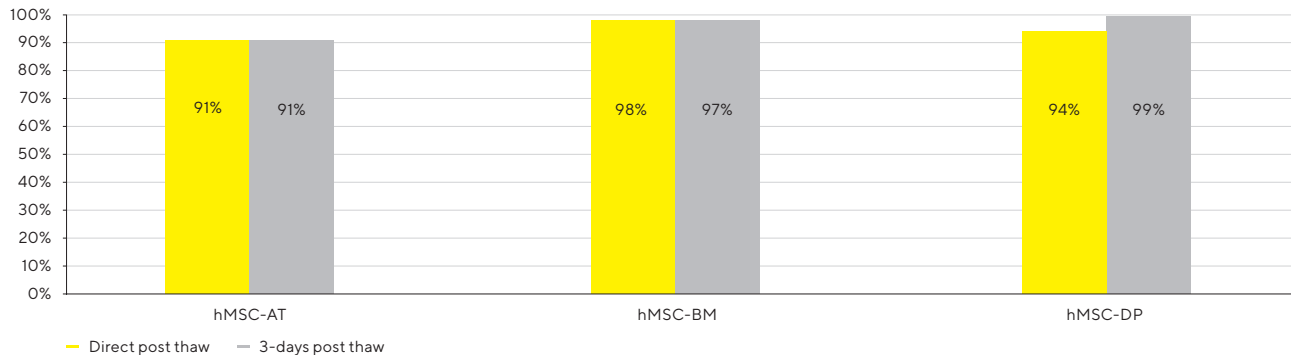
hMSC-AT show ≥ 94% viability after 5-years of cryopreservation



Viability Comparison of Various Human Mesenchymal Stromal Cells Post Cryopreservation in NutriFreez® D10 Medium.

High Viability

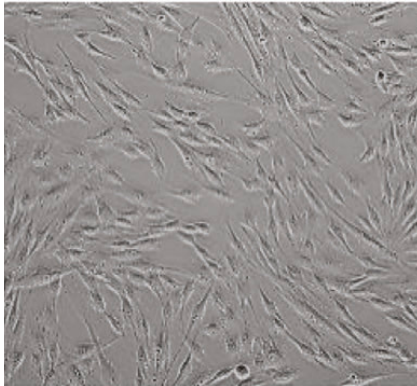
MSCs derived from adipose tissue (AT), bone marrow (BM), and dental pulp (DP) show $\geq 91\%$ viability after 3 days post thaw compared to direct post thaw



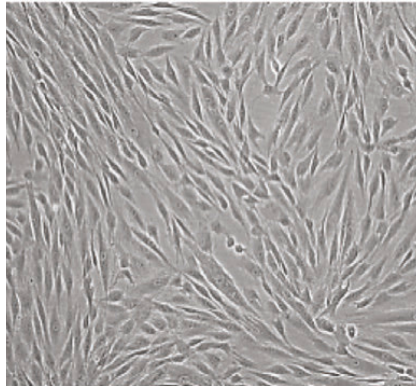
Morphology Comparison of Various Human Mesenchymal Stromal Cells Post Cryopreservation in NutriFreez® D10 Medium.

Normal Morphology

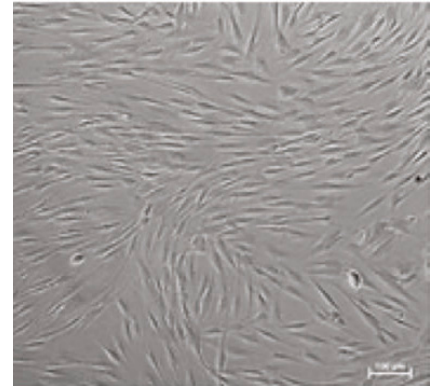
MSCs derived from adipose tissue (AT), bone marrow (BM), and dental pulp (DP) exhibit normal morphology after 3 days post thaw compared to direct post thaw



hMSC-AT

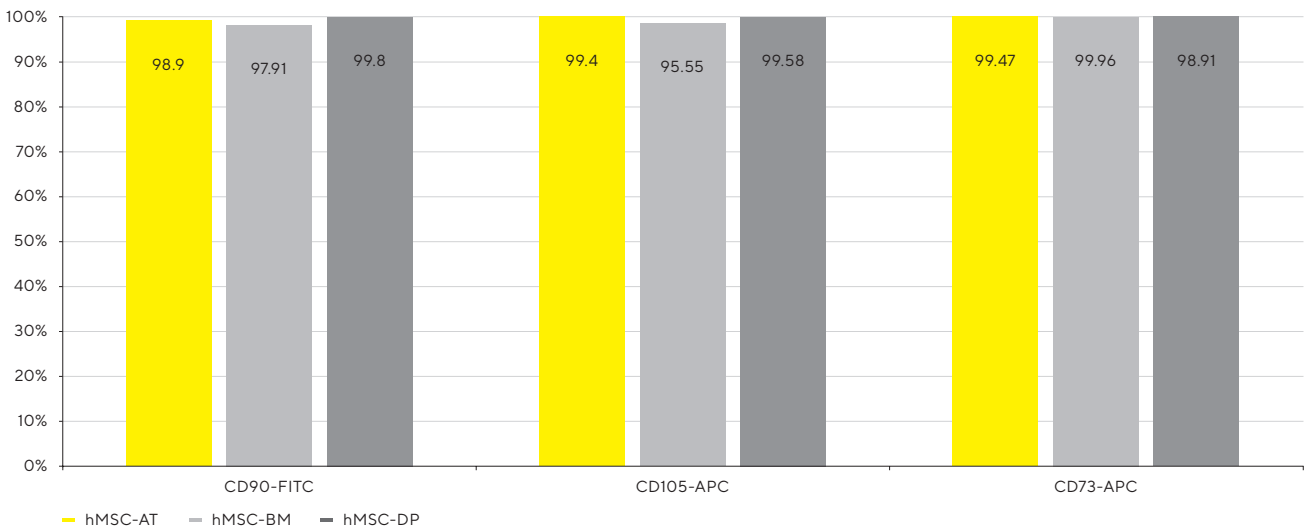


hMSC-BM



hMSC-DP

Various Human Mesenchymal Stromal Cells Maintain Multipotency Marker Expression via FACS Analysis Following Cryopreservation in NutriFreez® D10 Medium.



Clinical Applications

The Ottawa Hospital Research Institute, Canada



The Study:

Clinical trials for Septic Shock Patients

The Results:

When compared to homebrew and serum-free competitor freezing solutions, primary human mesenchymal stem cells (from healthy donors) cryopreserved in NutriFreez® D10 Cryopreservation Medium exhibited the best post-thaw viability and recovery rates in addition to increased cell attachment and growth performance.

Data Acknowledgment:

Thank you to Prof. Shirley H.J. Mei and research team Yuan Tan and Mahmoud Salkhordeh, Regenerative Medicine Program, Ottawa Hospital Research Institute.

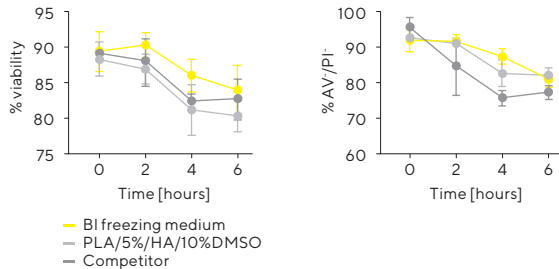
Clinical Applications

The Ottawa Hospital Research Institute, Canada



Superior Viability

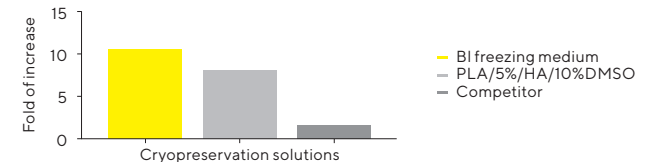
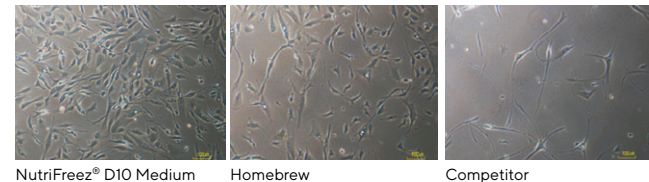
Comparison of cell viability over homebrew and competitor freezing solutions by Trypan blue exclusion and Annexin V/PI staining FACS analysis (direct post thaw)



Reference: Salkhordeh, et. al. May 2018. Evaluation of different cryopreservation agents for mesenchymal stem cell as final study product. Cytotherapy.

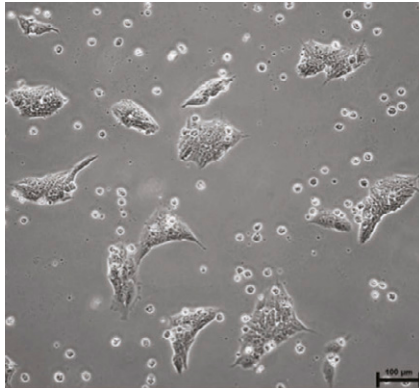
Superior Recovery

Comparison of cell recovery over homebrew and competitor freezing solutions at 6 days post thaw

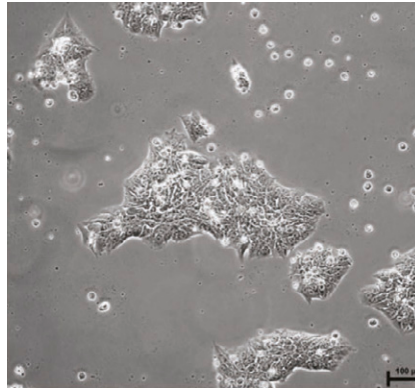


Reference: Salkhordeh, et. al. May 2018. Evaluation of different cryopreservation agents for mesenchymal stem cell as final study product. Cytotherapy.

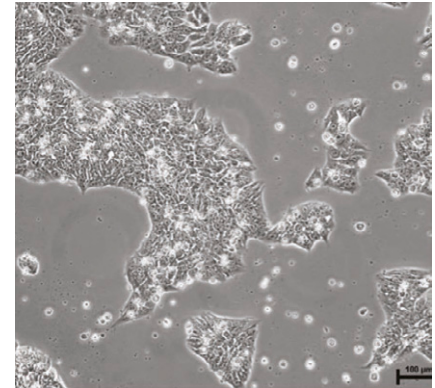
Human Embryonic Stem Cells Exhibit Superior Recovery and Morphology Post Cryopreservation in NutriFreez® D10 Medium.



Day 1

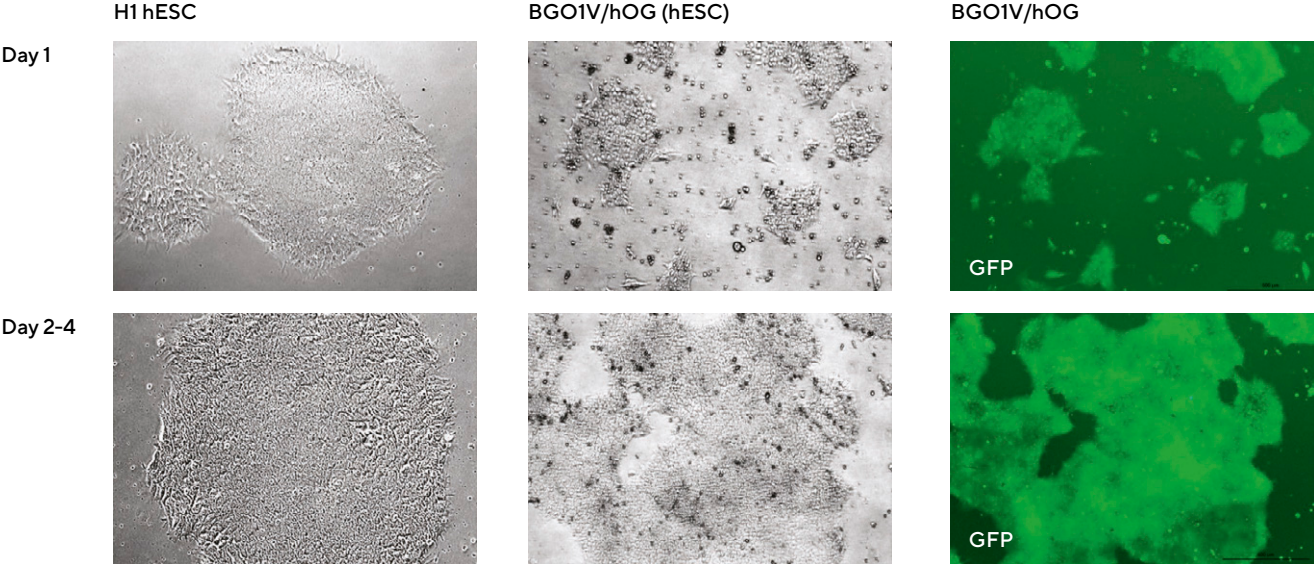


Day 3



Day 4

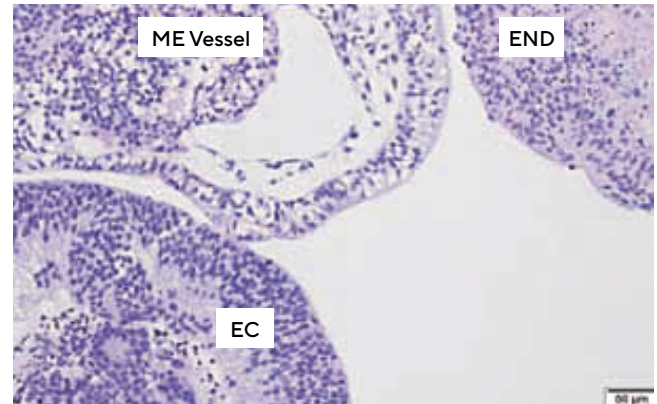
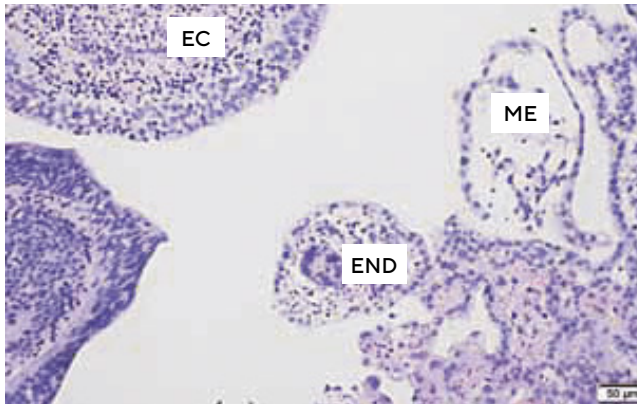
Human Embryonic Stem Cells Exhibit Superior Recovery and Morphology Post Cryopreservation of Cell Colonies in NutriFreez® D10 Medium.



Human Embryonic Stem Cells Maintain Trilineage Differentiation Potential Post Cryopreservation in NutriFreez® D10 Medium.

High Viability

H1 hESC identified by analysis of embryoid bodies spontaneously formed for 18 days, histological sections stained with H&E

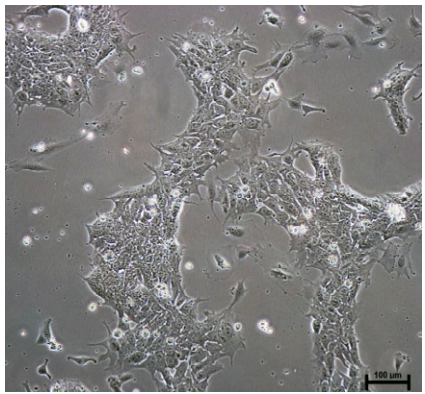


EC=neural rosettes, ME=primitive vessels, END=primitive parenchyma (100X)

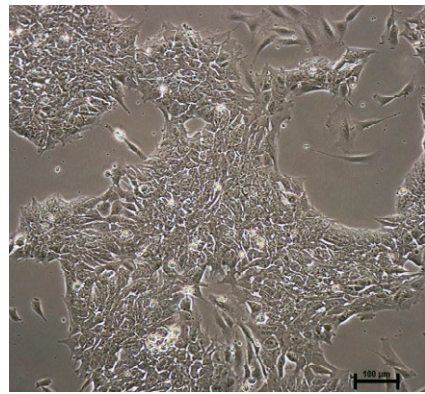
Single Cell Recovery, Morphology, and Attachment of Human Pluripotent Stem Cells Post Cryopreservation in NutriFreez® D10 Medium.

High Recovery

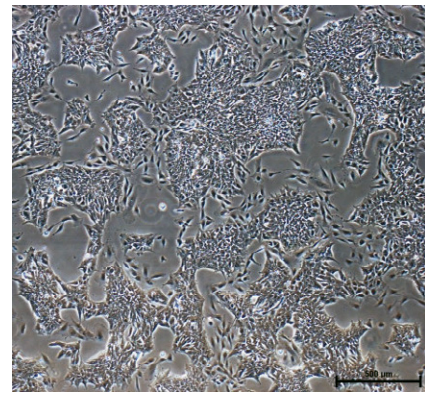
ACS-1019 cells demonstrate high recovery and attachment



Day 2



Day 3



Day 4

Third-Party Validation Studies

WiCell Research Institute, USA



The Study:

Validation study testing the ability to appropriately cryopreserve hPSCs without affecting the undifferentiated state and expansion rate of hPSCs post thaw.

The Results:

Study confirmed no negative effect on cell proliferation, differentiation, morphology, or karyotype was noted for human pluripotent cells cryopreserved using NutriFreez® D10 Medium* (lot 1617350). NutriFreez® D10 Medium was noted to have met all WiCell requirements for quality and when used as directed, is appropriate for use in pluripotent cell culture cryopreservation.

* Please note that this test was conducted under the product brand name CryoStem™ Freezing Medium. The NutriFreez® brand name replaces CryoStem™ and is the same formulation depicted here in this study.

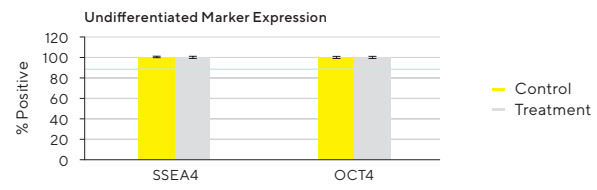
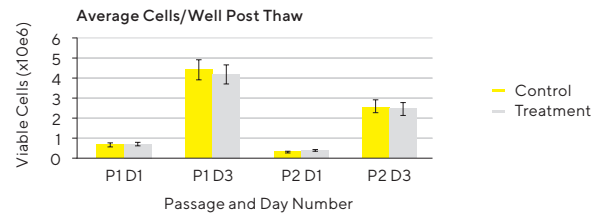
Third-Party Validation Studies

WiCell Research Institute, USA



Positive Cell Proliferation and Expression

Oct3/4 and SSEA4 marker expression exceeds $\geq 85\%$ positive for undifferentiated PSCs.



Reference: WiCell Research Institute Lot Qualification Report. January 2017. bioind.com.

Normal Karyotype

No clonal abnormalities were detected at the band resolution of 500-550. This is a normal karyotype.

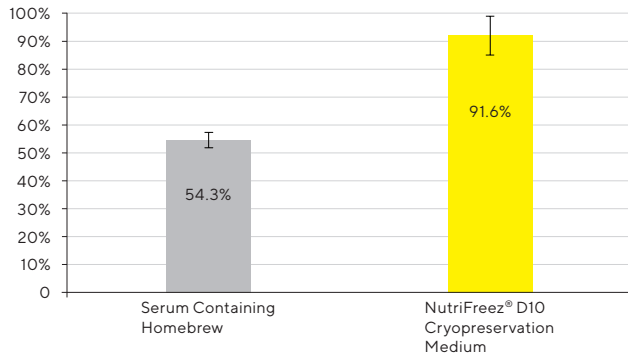


Reference: WiCell Research Institute Lot Qualification Report. January 2017. bioind.com.

Viability Comparison of Human Peripheral Blood Mononuclear Cells Following Cryopreservation in NutriFreez® D10 Medium.

High Viability

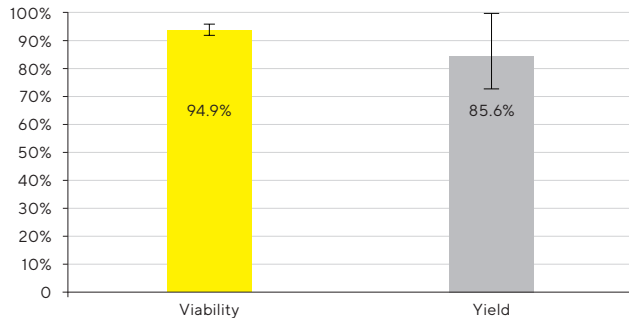
PBMCs show $\geq 91\%$ viability when compared to cells cryopreserved in homebrew freezing solutions



Viability and Morphology of Human Umbilical Vein Endothelial Cells Following Cryopreservation in NutriFreez® D10 Medium.

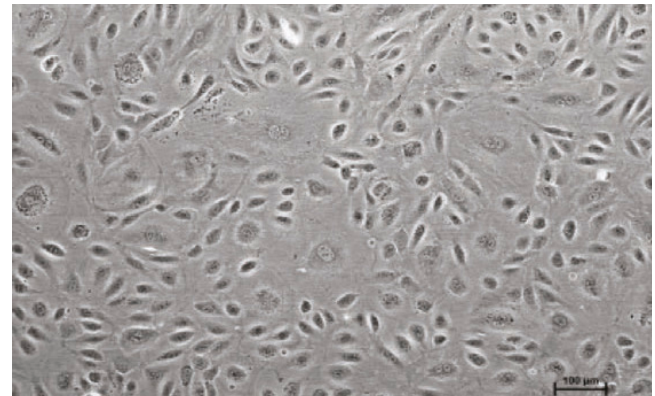
High Viability and Yield

HUVECs show $\geq 94\%$ viability and high cell yield post thaw



Normal Morphology

Normal morphology of HUVECs 4 days post thaw;
cells cultured in EndoGo™ XF Medium

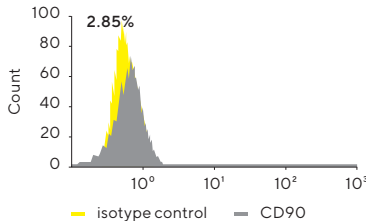
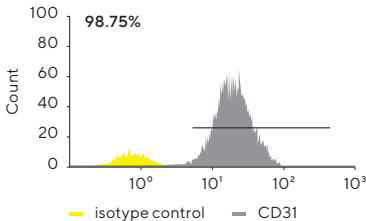
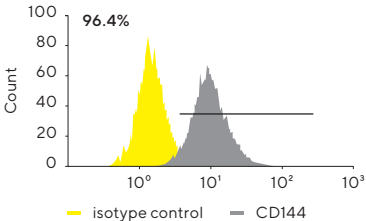
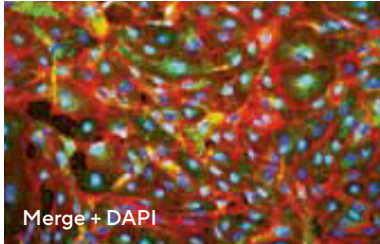
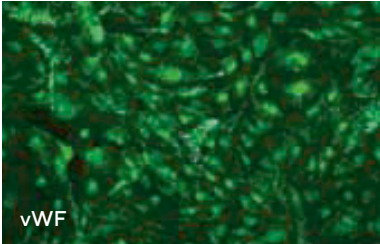
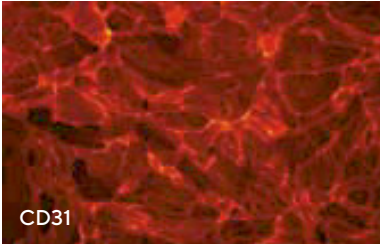


100X

Human Umbilical Vein Endothelial Cells Maintain Surface Markers via FACS Analysis Post Cryopreservation in NutriFreez[®] D10 Medium.

Typical Markers

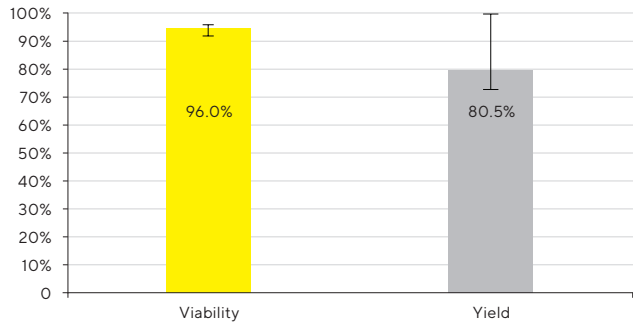
HUVECs were harvested and labeled with antibodies against endothelial cell surface markers CD31, CD144 and CD90



Viability and Morphology of Human Dermal Microvascular Endothelial Cells Following Cryopreservation in NutriFreez® D10 Medium.

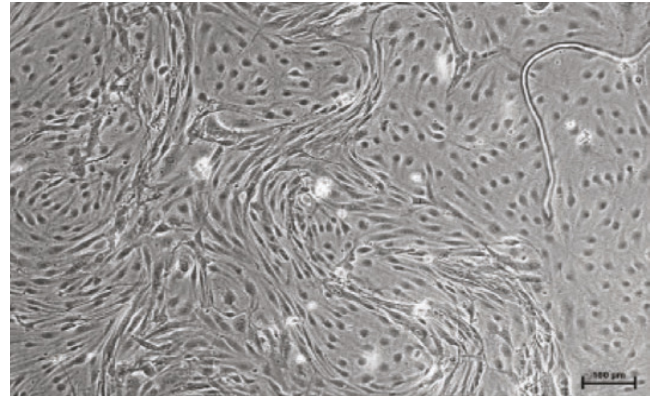
High Viability and Yield

HDMECs ≥ 96% viability and high cell yields post thaw



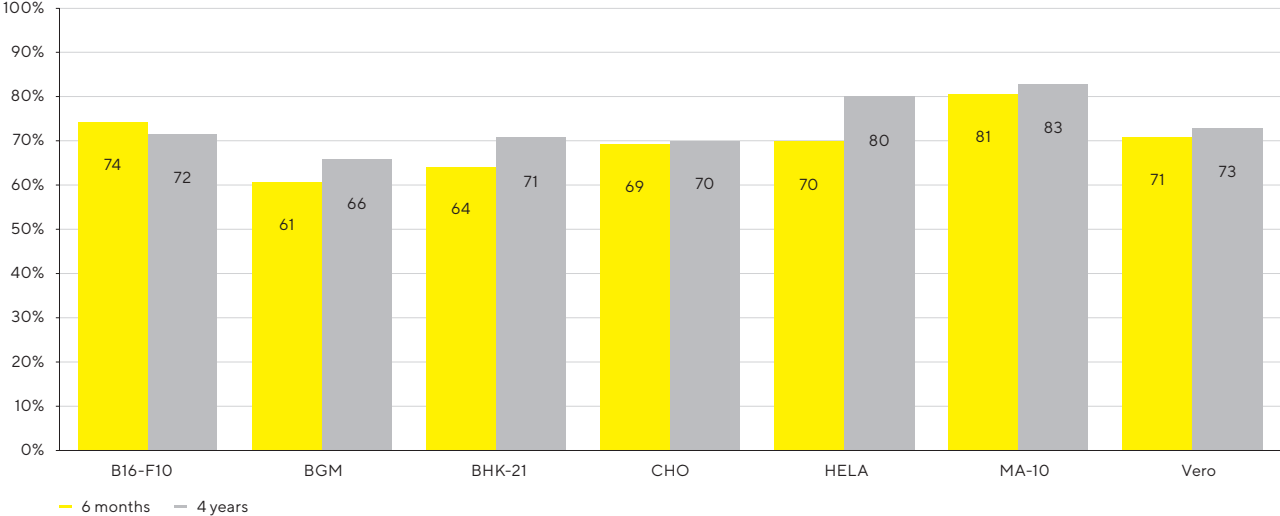
Normal Morphology

Normal morphology of HDMECs 4 days post thaw;
cells cultured in EndoGo™ XF Medium



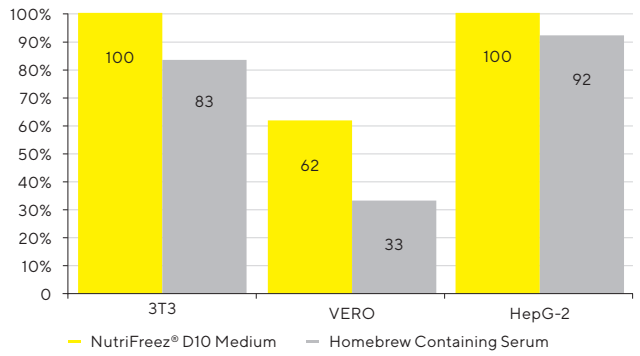
100X

Viability Comparison of Various Cell Lines Following Long-Term Cryopreservation in NutriFreez® D10 Medium.

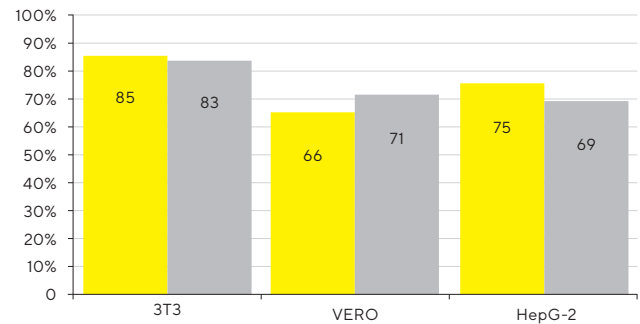


Attachment and Viability Comparison of Various Cell Lines Following Cryopreservation in NutriFreez® D10 Medium.

Superior Attachment



High Viability



Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Israel

Biological Industries Israel
Beit Haemek Ltd.
2511500 Kibbutz Beit Haemek
Phone: 972 4 9960595



For further contacts, visit
www.sartorius.com

Specifications subject to change without notice.

© 2021 Biological Industries Israel Beit Haemek Ltd., 2511500 Kibbutz Beit Haemek, Israel