



NutriFreez™ D10 Cryopreservation Medium

DISCOVER PERFORMANCE

B I O L O G I C A L I N D U S T R I E S

NutriFreez™ D10 Cryopreservation Medium

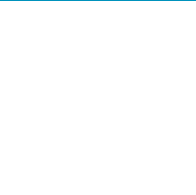
**OBTAIN MORE
CELLS IN LESS TIME**

**ATTAIN SUPERIOR
VIABILITY AND RECOVERY**

**MINIMIZE CELLULAR IMPACT
FOR LONG-TERM VIABILITY**



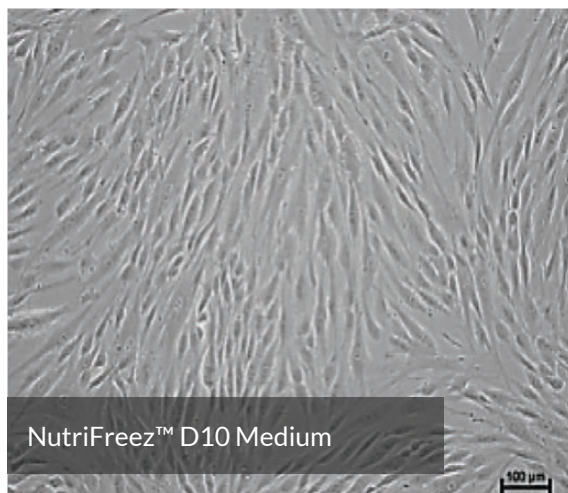
- ✓ Chemically defined and animal component-free
- ✓ Manufactured under cGMP conditions
- ✓ FDA Drug Master File (DMF) submitted
- ✓ Contains: Methylcellulose and 10% DMSO



Human Mesenchymal Stem Cells

DISCOVER PERFORMANCE

Proliferation and morphology comparison post cryopreservation of Human Mesenchymal Stem Cells in NutriFreez™ D10 Medium.



38,000 cells/cm²
Normal morphology



~4,000 cells/cm²
Abnormal morphology

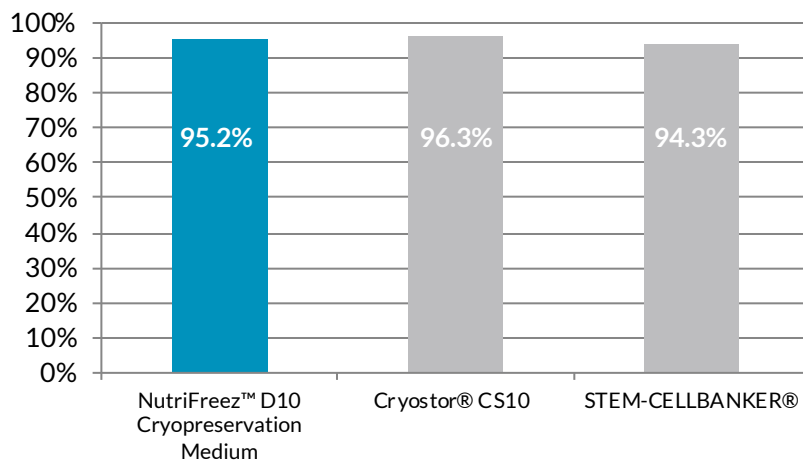


29,000 cells/cm²
Normal morphology

Viability and recovery comparison of Human Mesenchymal Stem Cells following cryopreservation in NutriFreez™ D10 Medium.

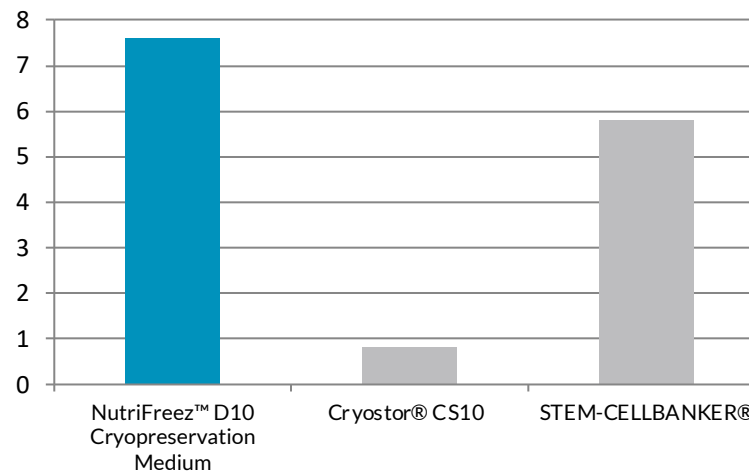
High Viability

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



Superior Recovery

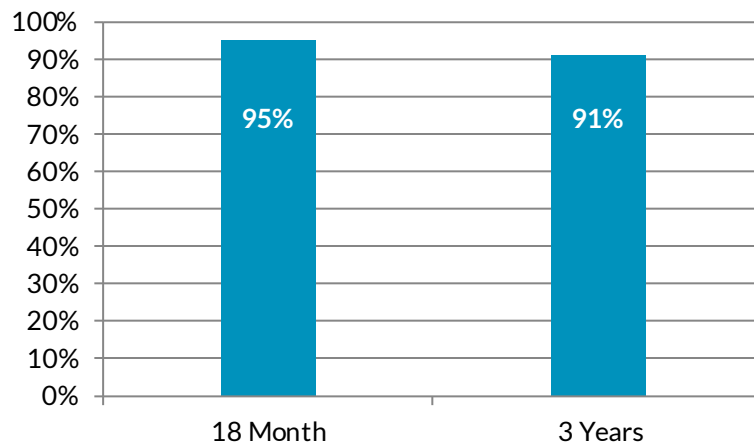
More cells in less time at 3 days post-thaw with a >7-fold cell increase



Viability comparison of Human Mesenchymal Stem Cells following long-term cryopreservation in NutriFreez™ D10 Medium.

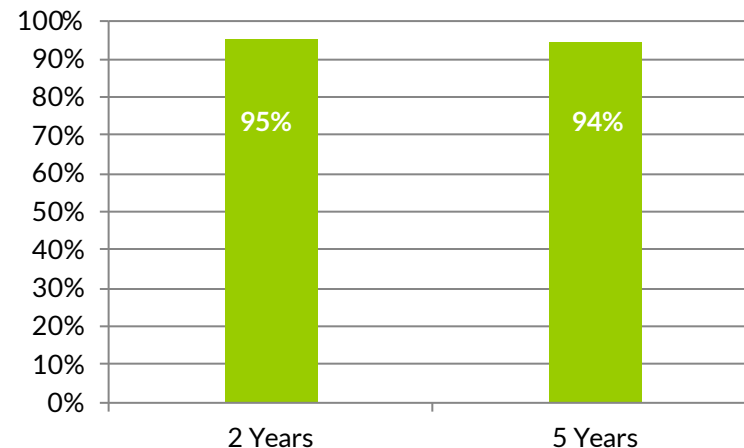
High Viability (long-term)

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



High Viability (long-term)

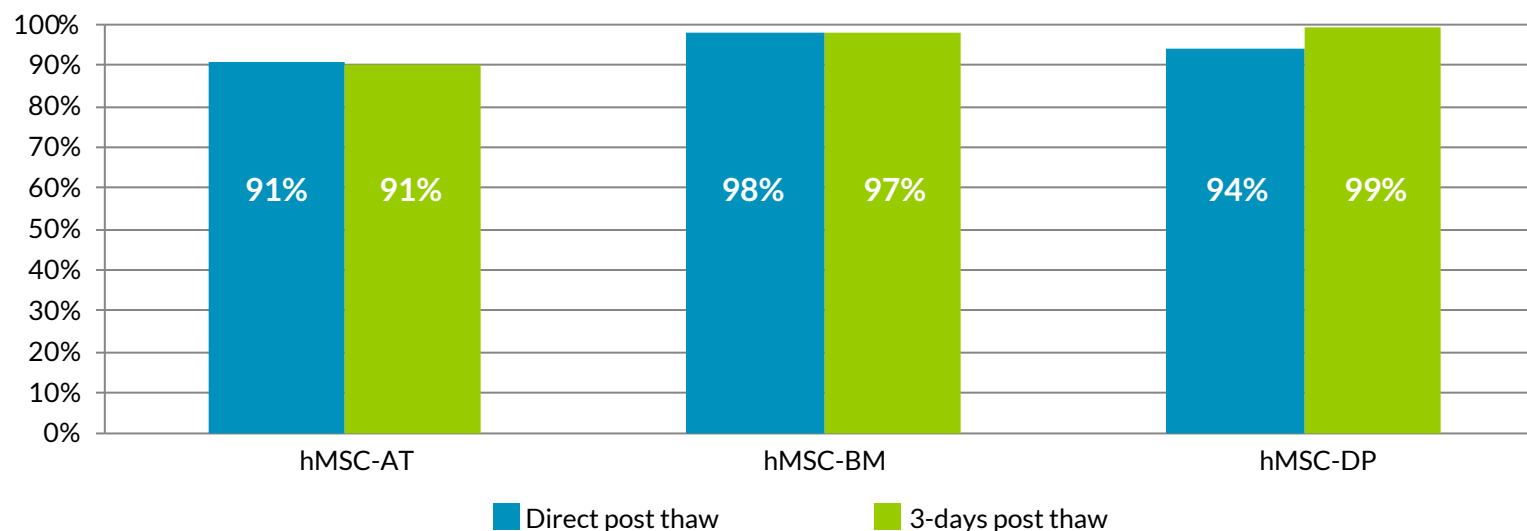
hMSC-AT show ≥ 94% viability after 5-years of cryopreservation



Viability comparison of various Human Mesenchymal Stem Cells post cryopreservation in NutriFreez™ D10 Medium.

High Viability

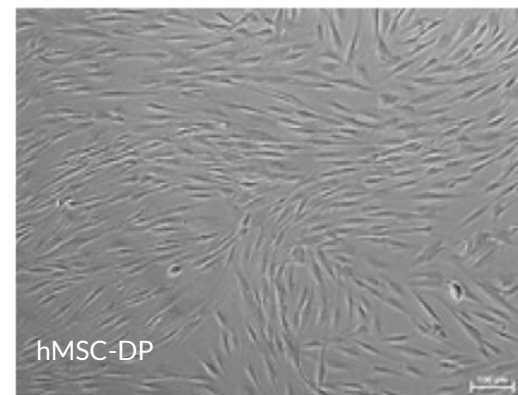
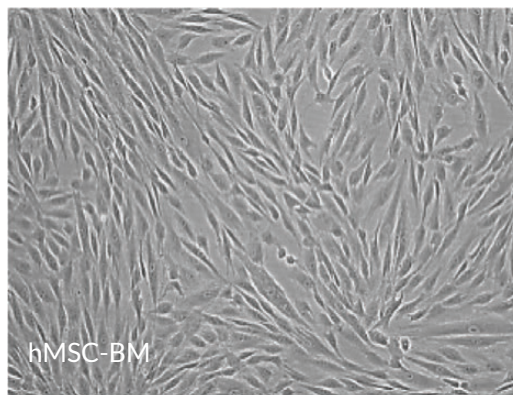
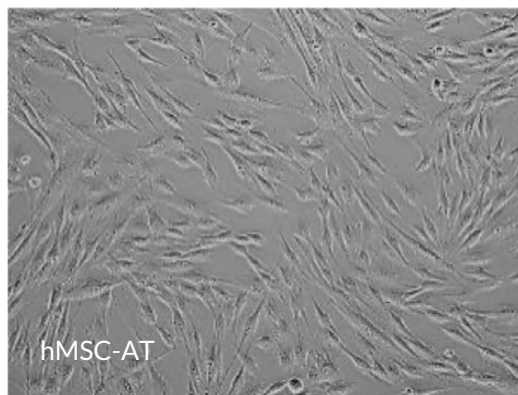
MSCs derived from adipose tissue (AT), bone marrow (BM), and dental pulp (DP) show $\geq 91\%$ viability after 3 days post thaw compared to direct post thaw



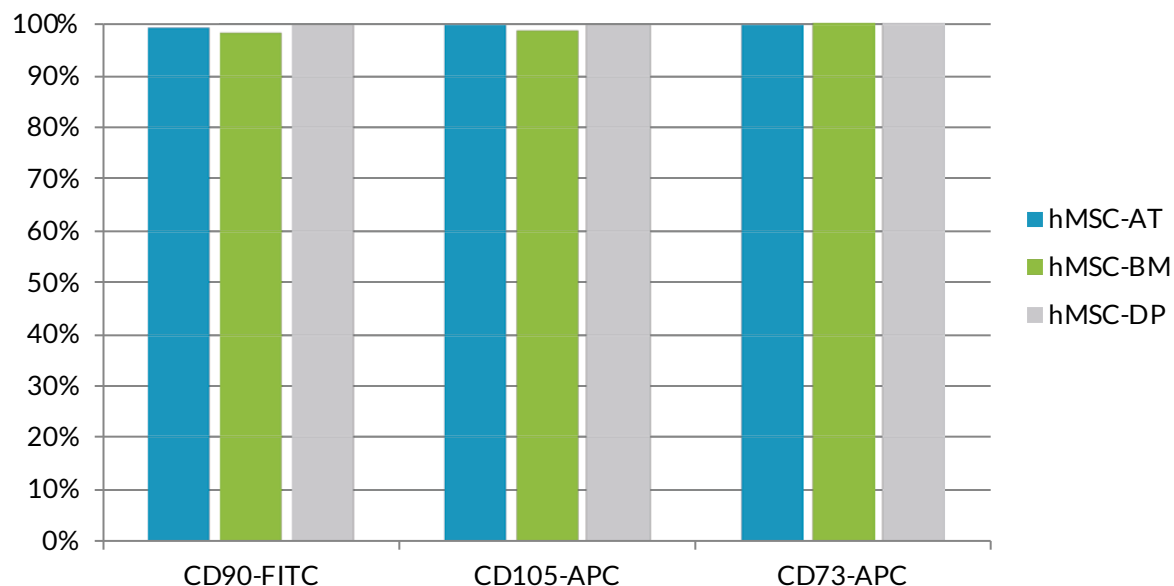
Morphology comparison of various Human Mesenchymal Stem Cells post cryopreservation in NutriFreez™ D10 Medium.

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



Various Human Mesenchymal Stem 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.

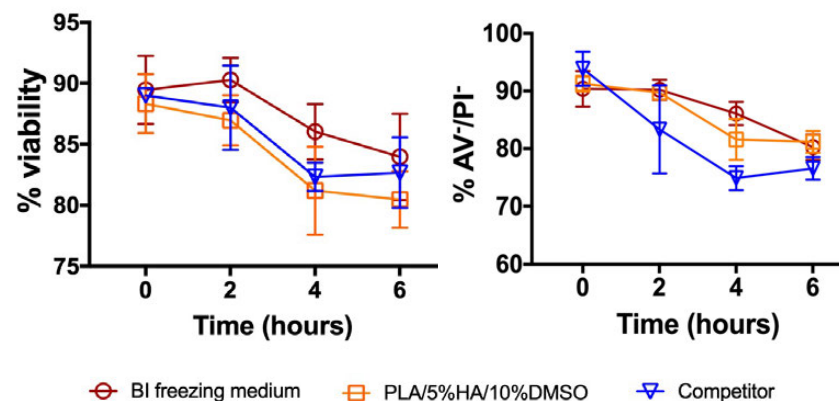


The Ottawa Hospital
RESEARCH INSTITUTE

L'Hôpital d'Ottawa
INSTITUT DE RECHERCHE

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.

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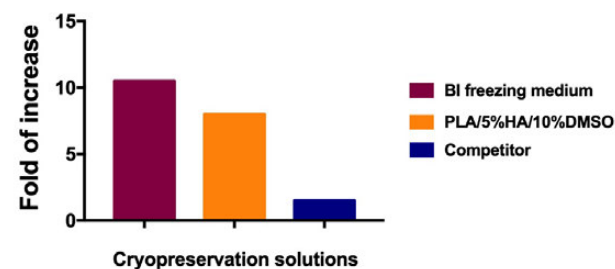
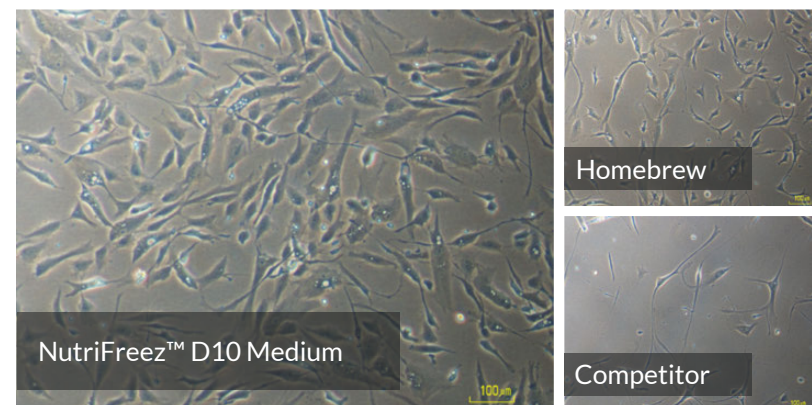
Thank you to Prof. Shirley H.J. Mei and research team Yuan Tan and Mahmoud Salkhordeh, Regenerative Medicine Program, Ottawa Hospital Research Institute.



**The Ottawa
Hospital** | **L'Hôpital
d'Ottawa**
RESEARCH
INSTITUTE | INSTITUT DE
RECHERCHE

Superior Recovery

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



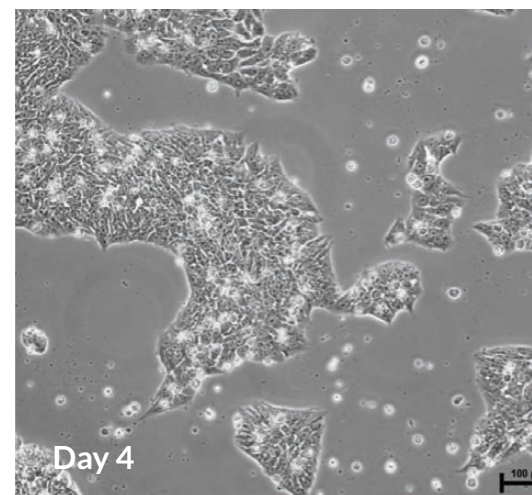
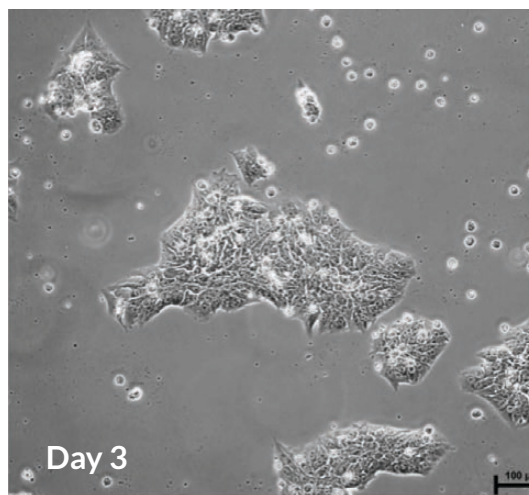
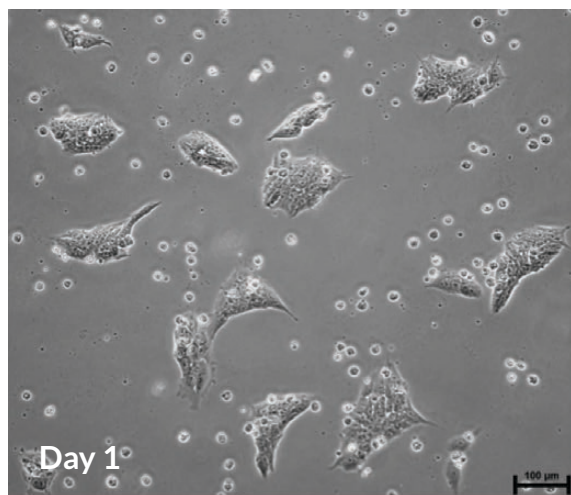
Reference: Salkhordeh, et. al. May 2018. Evaluation of different cryopreservation agents for mesenchymal stem cell as final study product. Cytotherapy.



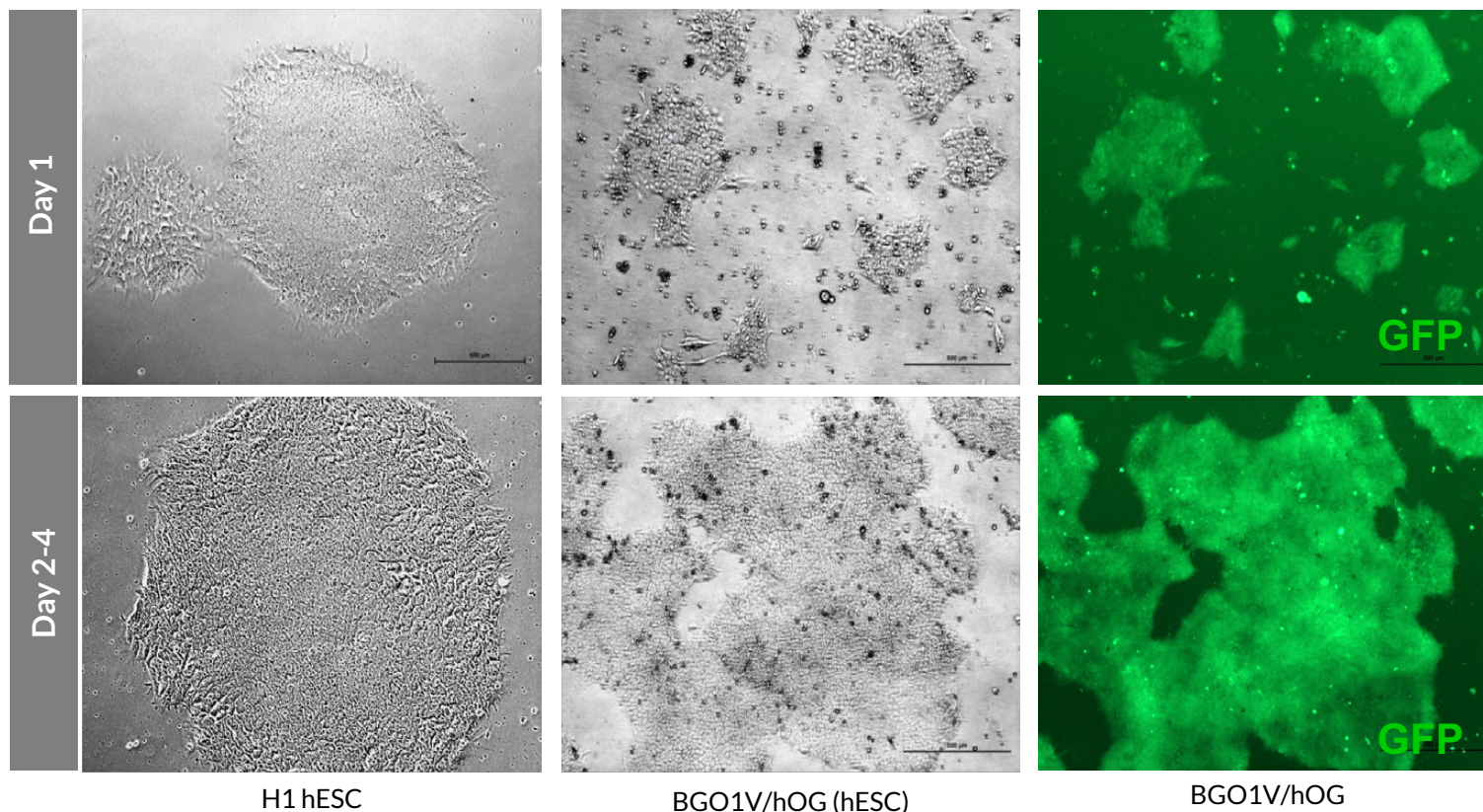
Human Pluripotent Stem Cells

DISCOVER PERFORMANCE

Human Embryonic Stem Cells exhibit superior recovery and morphology post cryopreservation in NutriFreez™ D10 Medium.



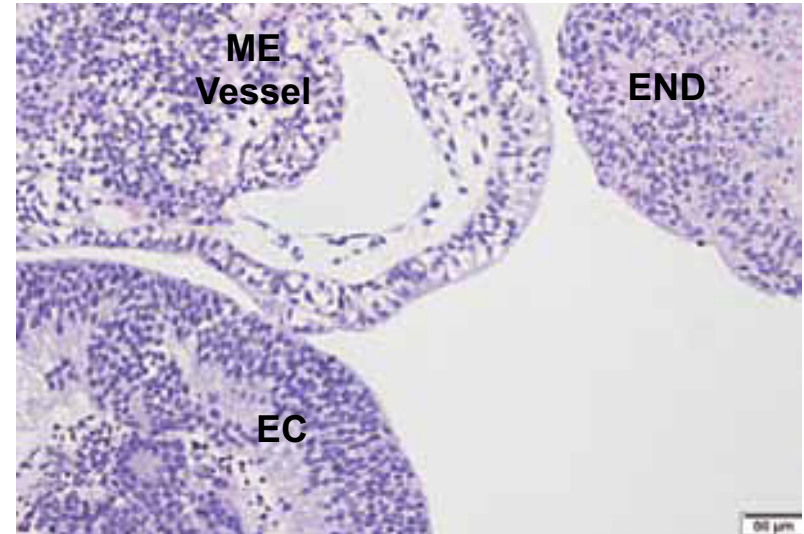
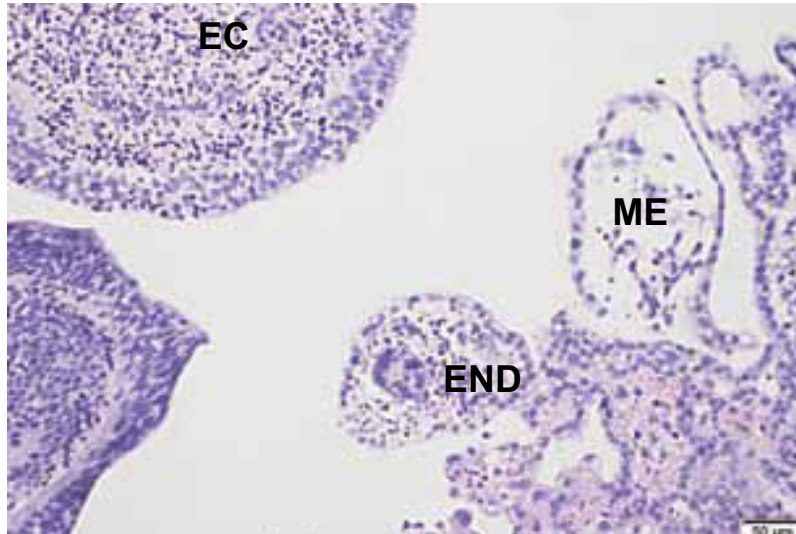
Human Embryonic Stem Cells exhibit superior recovery and morphology post cryopreservation of cell colonies in NutriFreez™ D10 Medium.



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

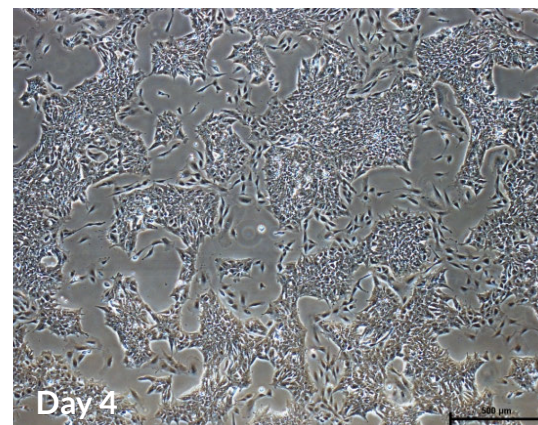
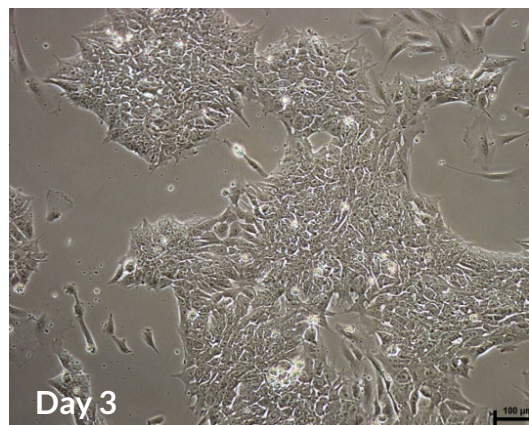
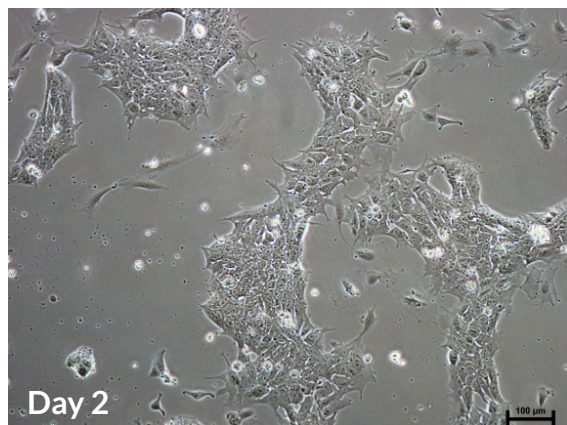


EC=neural rosettes, ME=primitive vessels, ED=primitive parenchyma (100X)

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



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:

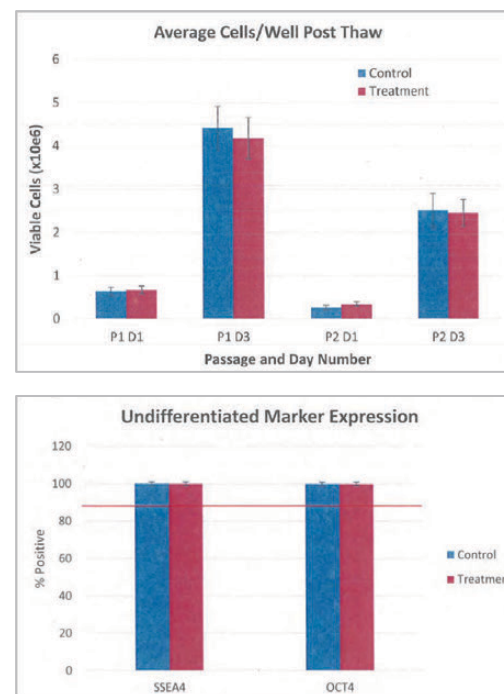
Study confirmed no negative effect on cell proliferation, differentiation, morphology, or karyotype was noted for human pluripotent cells cryopreserved using NutriFreez™ D10 Medium* (lot 1617350). NutriFreez™ D10 Medium was noted to have met all WiCell requirements for quality and when used as directed,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



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.

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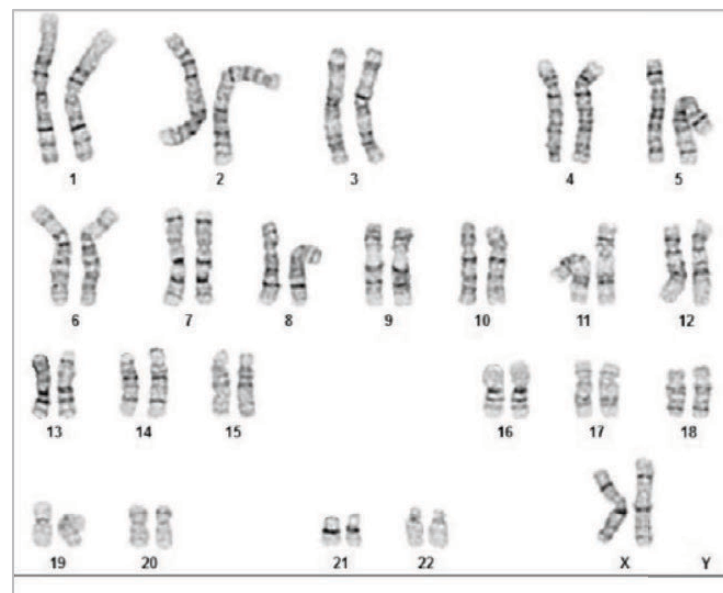
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** Please note that this test was conducted under the product brand name CryoStem™ Freezing Medium. The NutriFreez™ brand name replaces CryoStem™ and is the same formulation depicted here in this study.*



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.

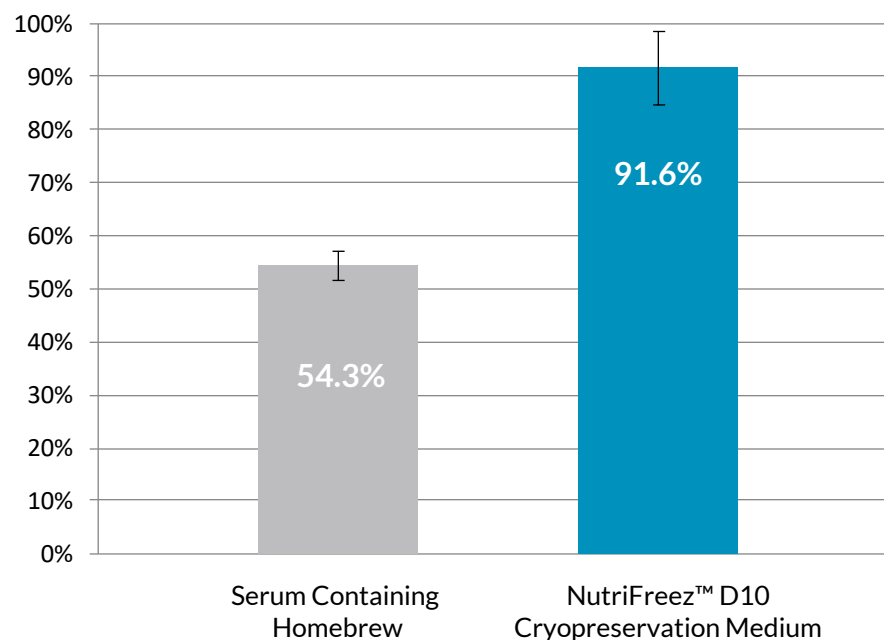
Primary Cells & Various Cell Lines

DISCOVER PERFORMANCE

Viability comparison of Human Peripheral Blood Mononuclear Cells following cryopreservation in NutriFreez™ D10 Medium.

High Viability

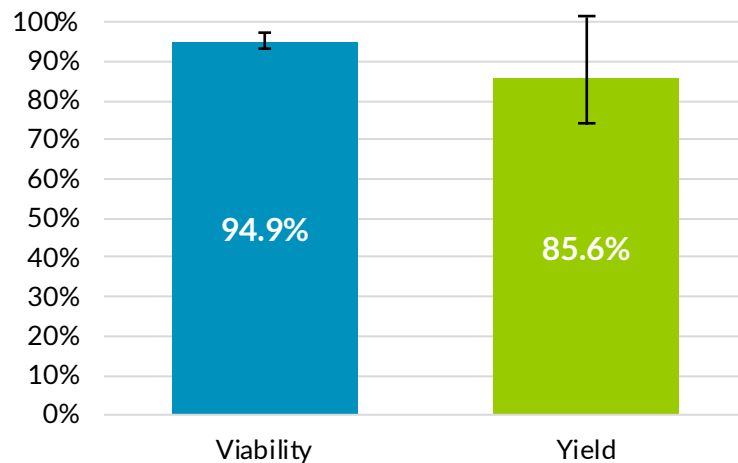
PBMCs show $\geq 91\%$ viability when compared to cells cryopreserved in homebrew freezing solutions



Viability and morphology of Human Umbilical Vein Endothelial Cells following cryopreservation in NutriFreez™ D10 Medium.

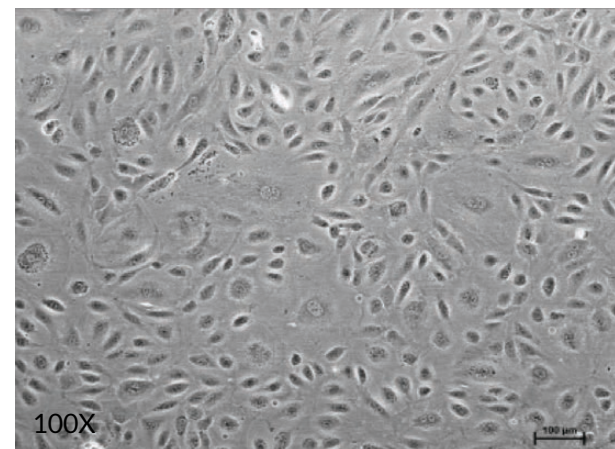
High Viability and Yield

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



Normal Morphology

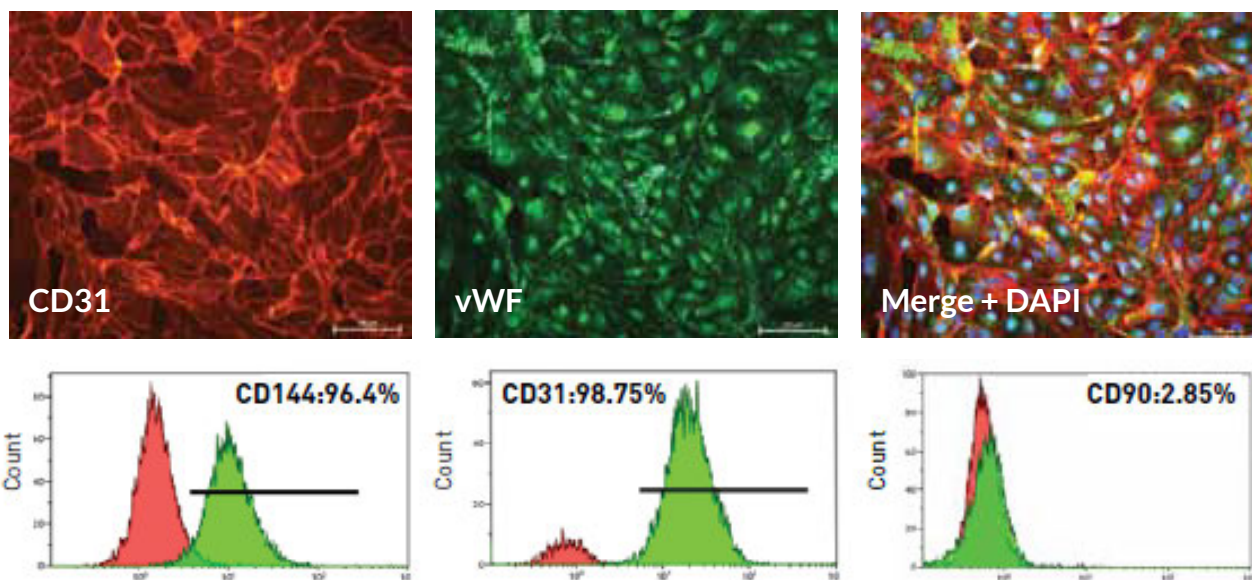
Normal morphology of HUVECs 4 days post thaw; cells cultured in EndoGo™ XF Medium



Human Umbilical Vein Endothelial Cells maintain surface markers via FACS analysis post cryopreservation in NutriFreez™ D10 Medium.

Typical Markers

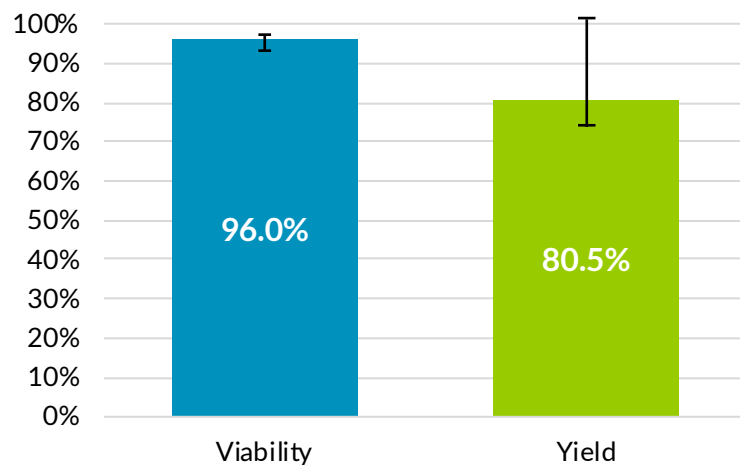
HUVECs were harvested and labeled with antibodies against endothelial cell surface markers CD31, CD144 and CD90



Viability and morphology of Human Dermal Microvascular Endothelial Cells following cryopreservation in NutriFreez™ D10 Medium.

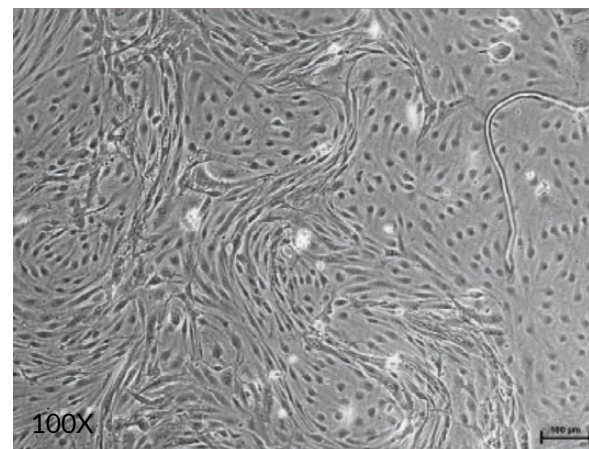
High Viability and Yield

HDMECs ≥ 96% viability and high cell yields post thaw

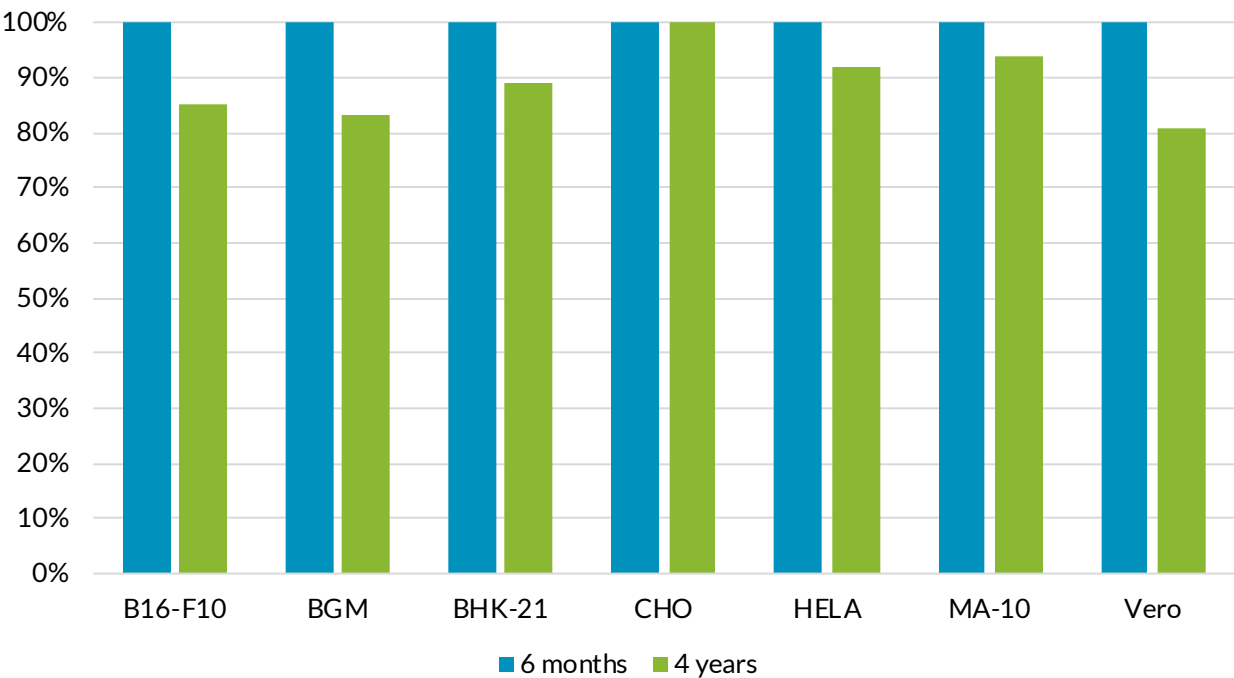


Normal Morphology

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

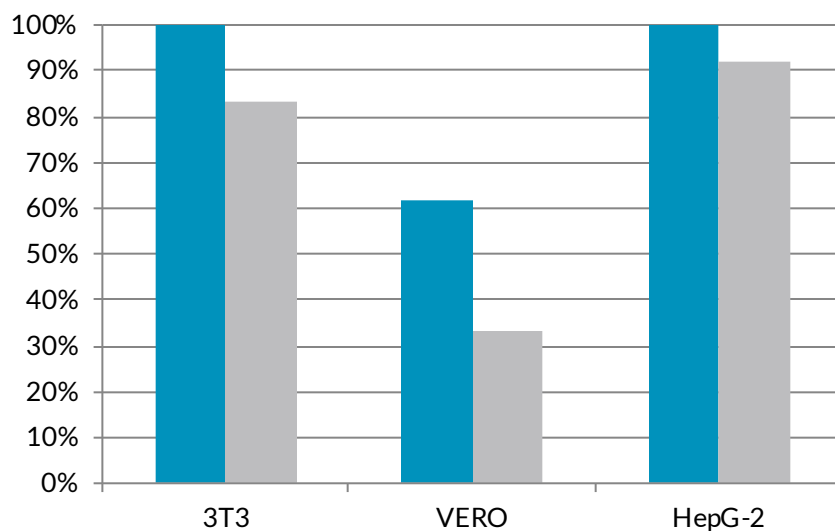


Viability comparison of various cell lines following long-term cryopreservation in NutriFreez™ D10 Medium.

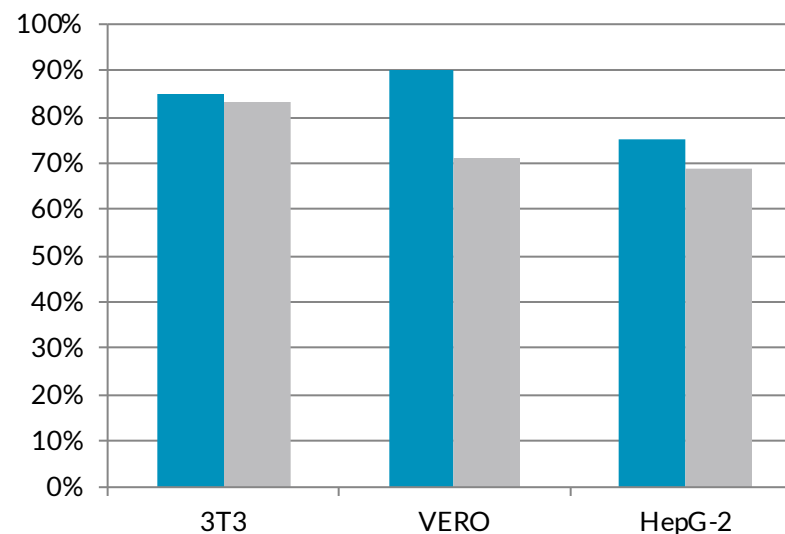


Attachment and viability comparison of various cell lines following cryopreservation in NutriFreez™ D10 Medium.

Superior Attachment



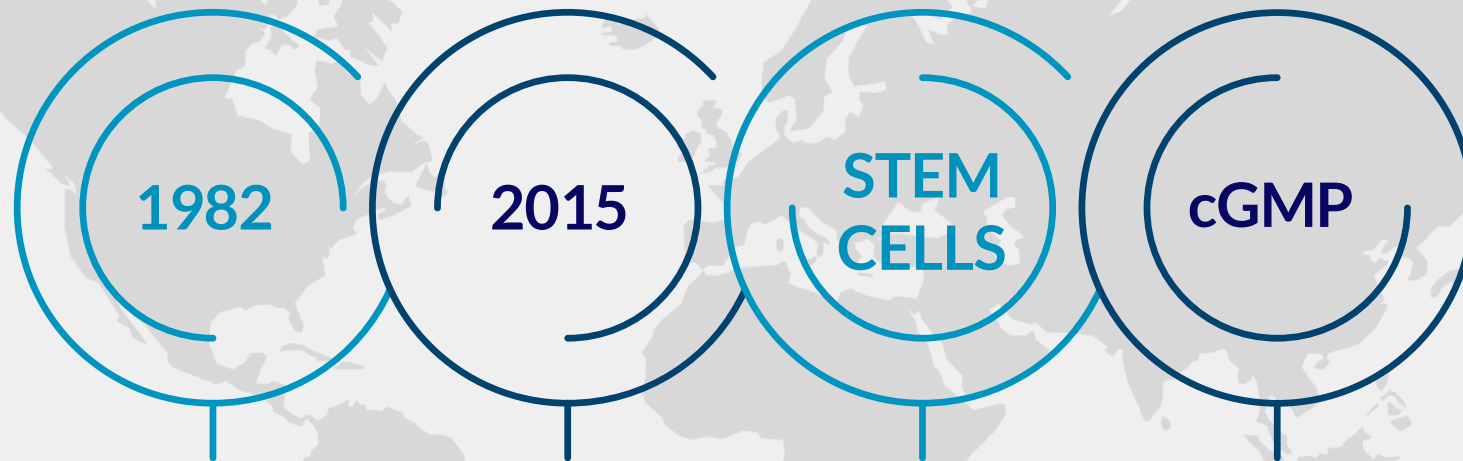
High Viability



■ NutriFreez™ D10 Medium ■ Homebrew Containing Serum

ABOUT BIOLOGICAL INDUSTRIES

A Culture of Excellence



35 YEARS

Backed by years of cell culture media manufacturing experts.

STRATEGIC

Global strategic reach expanded to include US, China, and Europe.

INNOVATION

First to develop xeno-free, serum-free cell culture systems.

QUALITY

Empowering translational and clinical research.

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