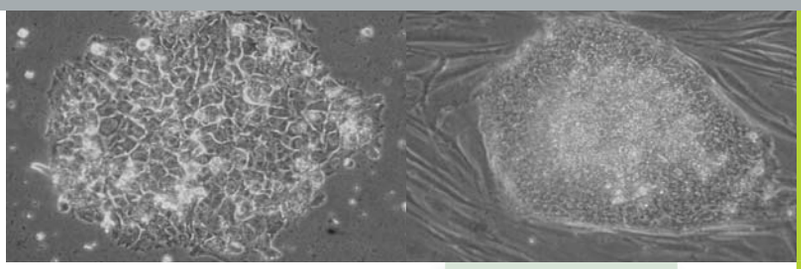
Xeno-Free Systems for **hESC & hiPSC**

Facilitating the shift from Stem Cell Research to Clinical Applications

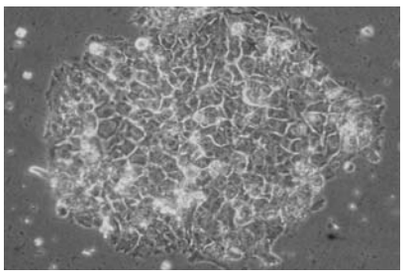


Biological Industries

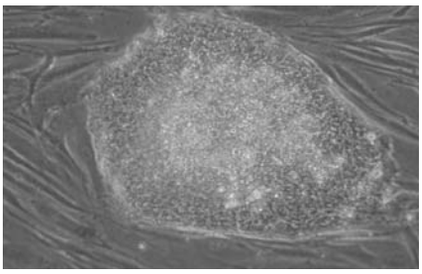
Co-developed in collaboration with the Technion R&D Foundation

NutriStem™

Defined, xeno-free (XF), serum-free media (SFM) specially formulated for growth and expansion of undifferentiated human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs)



Human ES cells cultured in NutriStem™ hESC XF on Matrigel



Human ES cells cultured in AF NutriStem™ hESC XF on HFF

NutriStem™ hESC XF

- Xeno-free
- Defined
- Feeder-independent
- Superior performance on Matrigel as a feeder-free Matrix
- Ready-to-use
- Complete (contains HSA)

AF NutriStem™ hESC XF (w/o HSA)

- Xeno-free
- Defined
- Optimized to support culturing on Human Foreskin Fibroblasts (HFF) or Mouse Embryonic Fibroblasts (MEF)
- Ready-to-use

Bio-Pure™ Human Serum Albumin (HSA)

Xeno-free supplement specially qualified for the growth of undifferentiated pluripotent human ES and iPS cells, in both feeder-dependent and feeder-free conditions

CryoStem™ New!

Animal component-free, protein-free and chemically defined freezing medium, for cryopreservation of human ES & iPS cells

Pluripotent Stem Cell Research

Biological Industries

Introduction

Human Embryonic Stem Cells (hESC) research is one of the most dynamic fields in modern biology. Human ES Cell based clinical applications are currently limited by xeno-contamination during the *in-vitro* derivation and propagation phases. Thus, bridging the gap between research models and clinical applications requires the design and implementation of xeno-free processes. Xeno Free (XF, or Animal Component-Free, ACF) media are therefore an essential element in the development of regenerative stem cell therapies, where implantation in humans is the desired outcome.

Biological Industries and the Technion- Israel Institute of Technology have established a strategic collaboration to develop optimized, feeder-independent cell culture environments for human embryonic stem cell research, including xeno-free defined media and qualified surfaces. Capitalizing on an outstanding pool of collective expertise, this collaboration will facilitate hESC research by developing innovative, reliable, high performance products.

The first products of this collaboration to be marketed are the proprietary serum-free NutriStem™ hESC XF formulations derived from xeno-free, defined components:

NutriStem™ hESC XF - is a ready-to-use, complete medium containing Human Serum Albumin, specifically optimized for human ES and iPS cell feeder-independent conditions.

AF NutriStem™ hESC XF - Albumin-Free (AF) medium is optimized for use on feeder cells such as Mouse Embryo Fibroblasts (MEF) or Human Foreskin Fibroblasts (HFF).

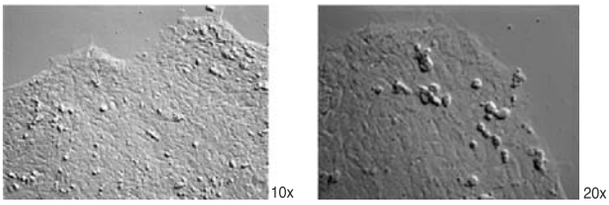
Both media were shown to support long-term (>50 passages) culture of undifferentiated hESCs (i.e. H9.2, I6, I3.2, H1) while preserving normal karyotypes. Pluripotency was verified by testing the expression of multiple markers by Q-PCR, immunofluorescent staining, and FACS analysis, and by formation of embryoid bodies and teratomas.

Human ES Cell Research

Formation of compact multicellular colonies of cells with a high nucleus-to-cytoplasm ratio, prominent nucleoli and distinct colony border is characteristic of undifferentiated hESCs. Thus, when viewed under a phase contrast microscope, healthy hESC colonies tend to exhibit “phase-bright” centers.

Cell Morphology

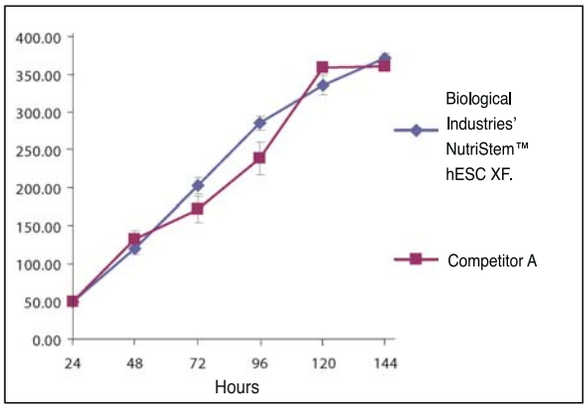
H1 cells cultured in NutriStem™ hESC XF medium at passage 5 display compact colonies and distinct colony morphology typical of pluripotent hESCs.



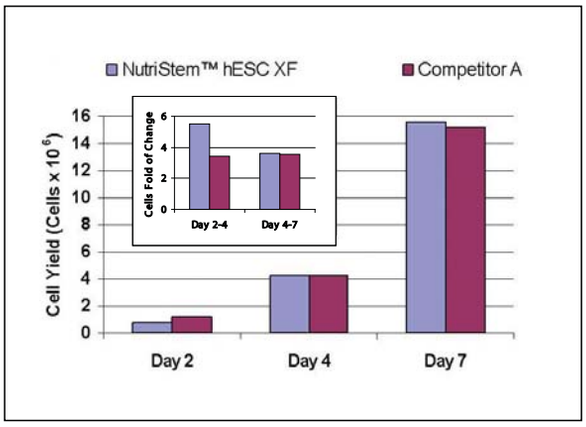
Proliferation

NutriStem™ hESC XF enables excellent proliferation of undifferentiated hESCs.

I. H1 cells (passage 6) were seeded in 96 well plates (Matrigel-coated) in the various media. Media were changed every 24 hours. The number of cells was determined using a CyQuant cell proliferation assay kit.



II. Evaluation of human embryonic stem cells (H9.2 cells) cultured in NutriStem™ hESC XF using Matrigel. Growth of hESCs cultured in NutriStem™ hESC XF was compared to growth using competitor A. Cell counts are reported for days 2, 4 and 7.



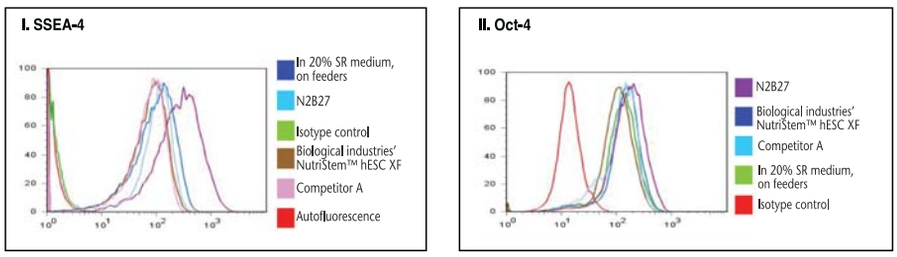
Human ES Cells Characteristics: Pluripotency and Differentiation Capabilities

Human ES cells are defined by their ability to proliferate indefinitely while remaining undifferentiated. Lack of differentiation is confirmed by monitoring the presence of specific cell surface markers (e.g. stage specific embryonic antigens SSEA-4), and the expression of certain transcription factors (e.g. Oct-4 and Nanog). Analysis by flow cytometry, Q-PCR and immunofluorescence of cells cultured in NutriStem™ hESC XF verified pluripotency is maintained.

Gene expression analysis

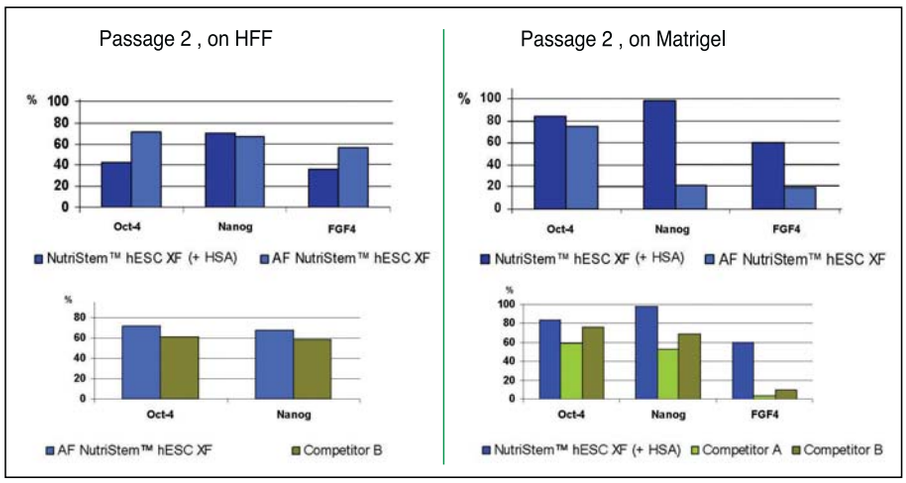
Flow cytometry gene expression analysis

H1 cells cultured in different media for 6 passages were analyzed and compared. Cells cultured in NutriStem™ hESC XF were found to be >90% positive for SSEA-4 and Oct-4.



Q-PCR

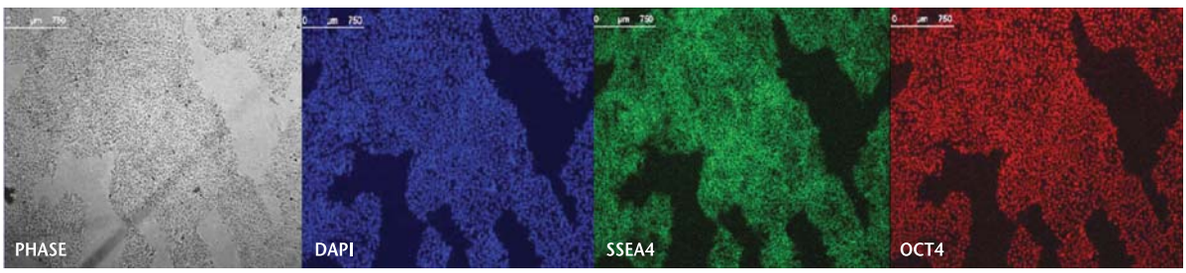
Superior H9.2 cell expansion is achieved using NutriStem™ hESC XF medium.



Immunostaining

H1 cell morphology and immunofluorescence analysis of hESC markers.

H1 cells stained positive for the expression of pluripotency markers.



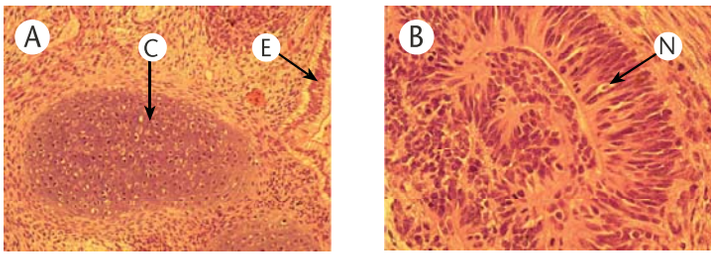
Human ES Cells Characteristics: Pluripotency and Differentiation Capabilities

Human ES cells differentiate into representatives of all three germ layers, i.e. endoderm, mesoderm and ectoderm, both in vitro and in vivo. This differentiation is defined by the formation of embryoid bodies in vitro and teratomas in vivo. Teratomas form when embryonic stem cells are injected into severe combined immunodeficient (SCID) mice and tissue types found include gut epithelium, cartilage, bone and neural epithelium among others.

Functional assessment of pluripotency

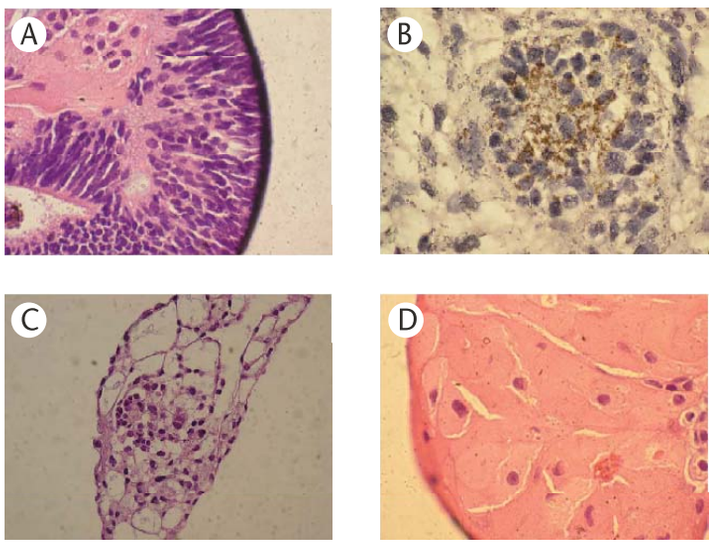
Teratoma formation

Human ESCs from cell line H9.2 were cultured for 11 passages in NutriStem™ hESC XF (with HSA) using foreskin fibroblasts as supportive layer and subsequently tested in vivo for pluripotency by teratoma formation. Cells were injected into the hind leg muscle of SCID-Beige mice. 12 weeks post injection the following tissues from all three germ layers were identified by histological sections; (A) Cartilage (mesoderm, marked arrow C), endoderm columnar epithelium (endoderm, marked arrow E), (B) Neural rosette (ectoderm, marked arrow N). Stained with H&E.



Embryoid body (EB) formation

Human ESCs from cell line H9.2 were cultured for 16 passages in NutriStem™ hESC XF (with HSA) using a Matrigel matrix and tested in vitro for pluripotency by EB formation. After suspension in serum supplemented medium the cells spontaneously formed embryoid bodies (EBs) containing embryonic germ layers. Examining the histological sections of 14-day-old EBs, the following cell types were identified; (A) Neural rosette (ectoderm), (B) Neural rosette stained with Tubulin, (C) Primitive blood vessels (mesoderm) and (D) Megakaryocytes (mesoderm). Stained with H&E.



NutriStem™

Defined, Xeno-Free media designed to support the growth of Human Embryonic Stem Cells (hESC)

Product Name	Catalogue No.	Unit Size	Storage
NutriStem™ hESC XF **Xeno-Free Medium for Human ES & iPS Cells** • Optimized for feeder-free culture • Superior performance on Matrigel • May be used with feeder-dependent culture • Complete (Contains HSA)	05-100-1A 05-100-1B	500ml 100ml	-20°C
AF NutriStem™ hESC XF **Xeno-Free Medium for hESCs** • Albumin-Free (AF) • Optimized for feeder-dependent culture • Superior performance on HFF feeder layer	05-102-1A 05-102-1B	500ml 100ml	-20°C

Human ES cells (i.e., H9.2, I6, I3.2, H1) cultured in NutriStem™ media displayed:

- The ability to self-renew, by expansion in feeder-free culture conditions (Matrigel), on human feeder layer (HFF) or on Mouse feeder cells (MEFs);
- Expression of hESC specific transcription factors and antigens;
- Pluripotency by the ability to differentiate into the three germ layers (endoderm, ectoderm and mesoderm) in-vitro by EB formation and in-vivo by teratoma formation in SCID mice; and
- Human ES cell normal phenotype and genotype.

NutriStem™ human ES & iPS cell culture media provides:

- **Consistent media performance** and predictable cellular behavior derived from a defined xeno-free formulation
- **Increased reproducibility** shown in long term growth of over 50 passages
- **Time saving** in eliminating the need to prepare feeder cells when choosing to culture on feeder-free layer
- **Cost efficiency** obtained in low human recombinant growth factor concentrations (rh bFGF, rh TGFβ).

NutriStem™ media are ready-to-use (no additions required), they contain alanyl glutamine and do not contain antibiotics.
For feeder-free culture (i.e., Matrigel), the use of NutriStem™ hESC XF (Cat.# 05-100-1) is recommended.
When culturing in AF NutriStem™ hESC XF (Cat.# 05-102-1) on feeder-free layer, an addition of 5% Bio-Pure™ Human Serum Albumin (HSA Solution, 10%) (Cat.# 05-720-1) is required.

Stability

18 months.

Quality Control

All NutriStem™ lots are tested for optimal maintenance and expansion of undifferentiated hESC. Additional standard evalutions are pH, osmolality, endotoxins and sterility tests.

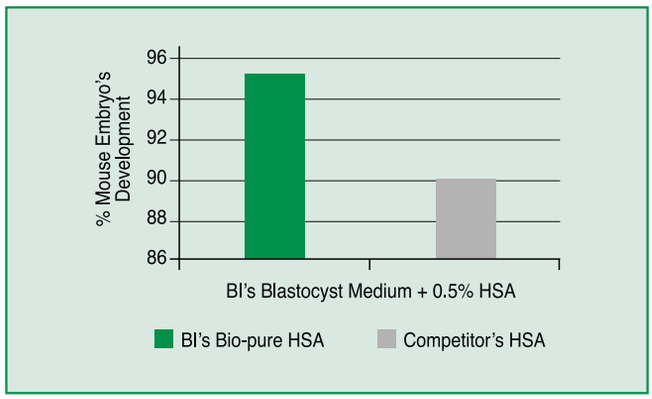
Bio-Pure™ Human Serum Albumin

Product Name	Catalogue No.	Unit Size	Storage
Bio-Pure™ Human Serum Albumin (HSA	05-720-1B	100ml	2-8°C
Solution, 10%), Optimized for hESC	05-720-1E	50ml	

Xeno-free supplement specially qualified for the growth of undifferentiated pluripotent human ES and iPS cells, in both feeder-dependent and feeder-free conditions.

HSA is a medium supplement that is a highly soluble osmolytic protein with a high molecular weight. HSA is effective in maintaining cell health, expansion, and growth of hESCs. It is particularly valuable in maintaining cell membrane stability. HSA is useful both for binding anionic, cationic and neutral molecules, as well as its ability to sequester and stabilize a wide array of ions and other small molecules.

HSA complies with strict manufacturing specifications and FDA requirements. All individual plasma donations, as well as the plasma pool, are tested for Hepatitis B Surface Antigen (HBsAg), Anti (Human Immunodeficiency Virus) HIV-I and II and anti-HCV and found to be negative.



96-hour one-cell mouse embryo development in blastocyst medium supplemented with Biological Industries' Bio-Pure 10% HSA solution (Cat. No.:05-720-1) versus competitor's 10% HSA solution.

CryoStem™

Product Name	Catalogue No.	Unit Size	Storage
CryoStem™	05-710-1D 05-710-1E	10ml 50ml	2-8°C

CryoStem™ freezing medium is an animal component-free formulation, designed for the cryopreservation of human ES and iPS cells.

Features

- Chemically defined
- Animal component-free (ACF)
- Protein-free
- For freezing human ES and iPS cells cultured in both feeder-free and feeder-dependent conditions
- Superior recovery efficiency: maintains excellent attachment ability as well as growth performance
- Maintains human ES and iPS cell pluripotency
- Complete formulation; Ready-to-use

Figure 1: *Alkaline phosphatase (AP) staining of H1 cells at passage 2 recovered from freezing condition in CryoStem™ freezing medium. Cells were maintained on feeder layer. Cell colony exhibits a distinct morphology typical of pluripotent hESCs.*

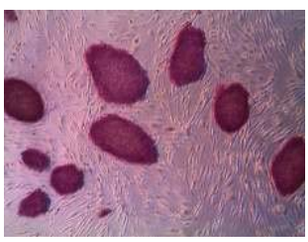


Figure 2: *AP staining of H1 cells at passage 2 recovered from freezing condition in CryoStem™ freezing medium. Cells were maintained in feeder-free conditions. Cell colony exhibits a distinct morphology typical of pluripotent hESCs.*

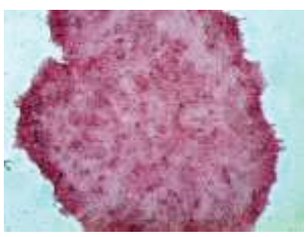
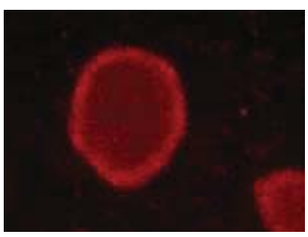


Figure 3: *H1 cell morphology and immunofluorescence analysis of hESC marker SSEA-4 at passage 2, recovered from freezing condition in CryoStem™ freezing medium.*



Product Name	Catalogue No.	Unit Size
Mouse Mouse Embryonic Stem Cell Products		
Mouse Embryonic Stem Cell (ESC) Basal Medium, with L-Alanyl L-Glutamine	01-171-1A	500ml
	01-171-1B	100ml
Gelatin Solution (0.1%)	01-944-1A	500ml
	01-944-1B	100ml
Foetal Bovine Serum Qualified for hESC		
Certified Foetal Bovine Serum (FBS)	04-002-1A	500ml
Qualified for Human Embryonic Stem Cells	04-002-1B	100ml
Certified Foetal Bovine Serum (FBS)	04-222-1A	500ml
Qualified for Human Embryonic Stem Cells		
Heat Inactivated	04-222-1B	100ml
Related Supplements & Reagents		
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml
Papain Dissociation Solution	03-072-1B	100ml
Transferrin, Human, Substantially Iron-Free (APO)	41-951-100	100mg
	41-951-500	500mg
Transferrin, Human, Iron-Saturated (HOLO)	41-952-100	100mg
	41-952-500	500mg
Basic Fibroblast Growth Factor (FGF)	30-T-218A	10μg
	30-T-218B	50μg
Crystalline Trypsin Solution (0.02%)	03-047-1A	500ml
Without Phenol Red	03-047-1B	100ml
Human Recombinant Insulin Solution, 3.7 mg/ml	01-818-1H	5ml

CE

ISO 13485: 2003

ISO 9001: 2008

cGMP
Manufacturing Facility

Worldwide Distributors

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E-mail: info@xpbiomed.com

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Website: www.ascomed.cz

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GERMANY
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INDIA AND SOUTH ASIA
Life Technologies (India) Pvt Ltd
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IRELAND
Alpha Technolgies
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ITALY
International PBI S.p.A
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JAPAN
Cosmo Bio Co., Ltd
Website: http://www.cosmobio.co.jp/

KOREA
GeneAll Biotechnology Co.Ltd.
Website: www.geneall.com

LATVIA
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LITHUANIA
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UNITED KINGDOM
Cadama Medical Ltd.
Website: www.cadama.co.uk

UNITED STATES
Stemgent
Website: www.stemgent.com

Stemgent inc., a leading provider of proprietary reagents and tools, developed and proven for reproducibility by some of the world's leading stem cell scientists, is the exclusive distributor for Biological Industries' NutriStem™ hESC XF (Cat. no. 05-100-1) and CryoStem™ (Cat. No. 05-710-1) in the USA.
American customers and dealers will now have a single distributor to obtain NutriStem™ and CryoStem™.
Stemgent is marketing and distributing NutriStem™ in the name of Stemedia™ NutriStem™ XF/ FF Culture Medium (Cat. No. 01-0005) and CryoStem™ in the name of CryoStem™ Freezing Medium (Cat. No. 01-0013).

Acknowledgment goes to Stemgent Inc., for the independent validation and the data provided for the success of this project

STEMGENT

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