

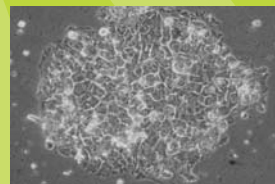
NutriStem™ hESC XF

FACILITATING THE SHIFT FROM STEM CELL RESEARCH TO CLINICAL APPLICATIONS

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

NutriStem™ hESC XF (with HSA)

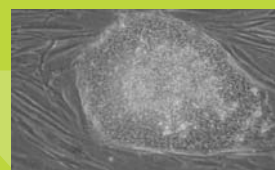
- Xeno-Free
- Defined
- Feeder-Independent
- Superior Performance on Matrigel™ as a Feeder-Free Matrix
- Ready-to-Use



Human ES cells cultured in NutriStem™ hESC XF on Matrigel™

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



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



Biological Industries



Technion R&D Foundation

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

Human ES Cell Research



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 (serum-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 (HSA), specifically optimized for hESC feeder-independent conditions. HSA is prepared from intravenous grade human albumin. Its superior performance has been demonstrated by Mouse Embryo Assay (MEA).

AF NutriStem™ hESC XF without HSA was optimized for use on feeder cells such as Mouse Embryo Fibroblasts (MEF) or Human Foreskin Fibroblasts (HFF).

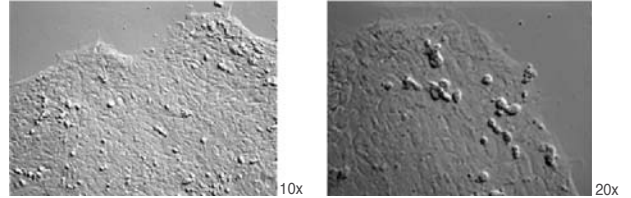
Both media were shown to support long-term (>50 passages) culture of undifferentiated hESCs while preserving normal karyotypes. Pluripotency was verified by testing the expression of multiple markers by Q-PCR, immunofluorescent staining, and FACS analysis, and by formation of embryoid bodies and teratomas.

Human ES Cell Culture

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. On the other hand, the presence of flat, cobblestone-like cells, loss of border integrity, or hole-riddled colonies, are all signs of differentiation.

Cell Morphology

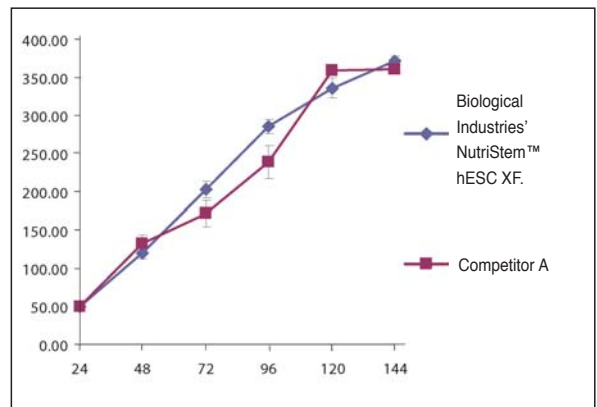
H1 cells cultured in NutriStem™ hESC XF medium at passage 5 showed compact colonies and distinct colony morphology typical of pluripotent hESCs.



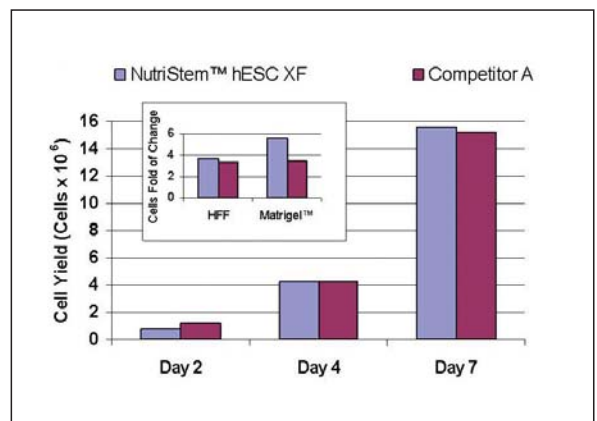
Proliferation

NutriStem™ hESC XF enables excellent proliferation of undifferentiated hESCs.

I. 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™ matrix. 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.



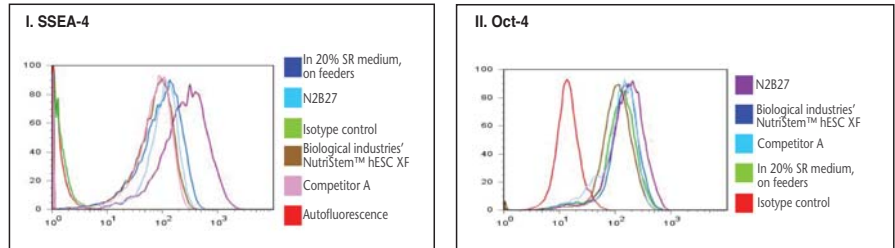
Human ES Cells Characteristics: Pluripotency and Differentiation Capabilities

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, FACS and immunofluorescence of cells cultured in NutriStem™ hESC XF verified pluripotency is maintained.

Gene expression analysis

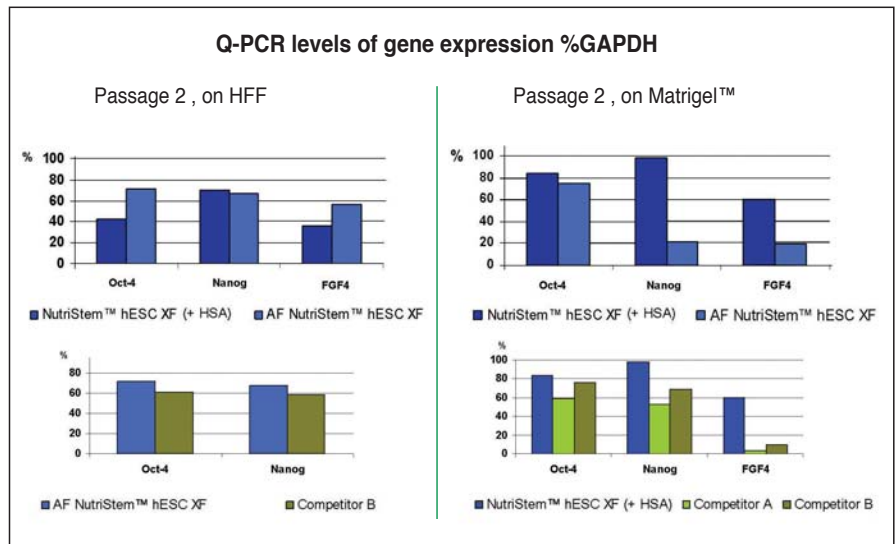
Flow cytometry gene expression analysis

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

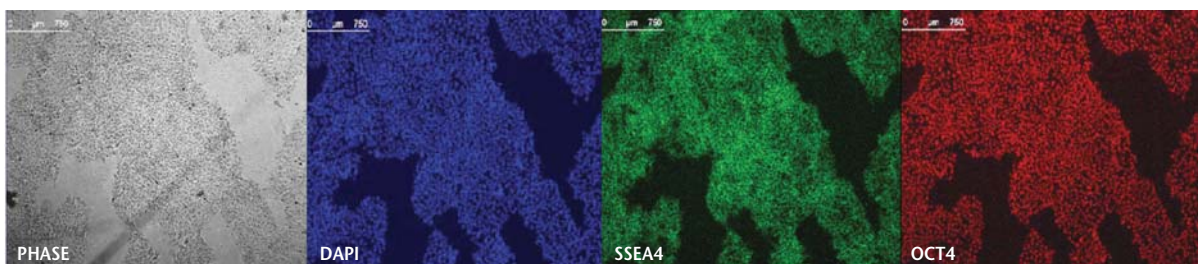
Superior H9.2 cell expansion is achieved using NutriStem™ hESC XF medium.



Immunostaining

H1 cell morphology and immunofluorescence analysis of hESC markers.

H1 cells stained positive for the expression of pluripotency markers.



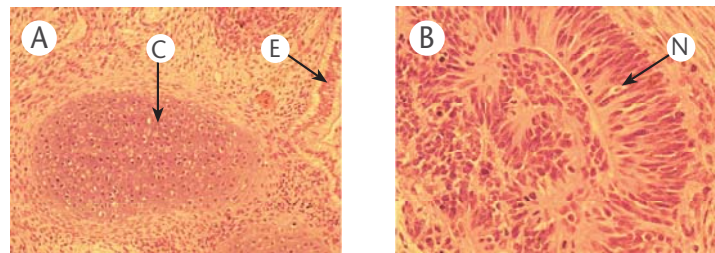
Human ES Cells Characteristics: Pluripotency and Differentiation Capabilities

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

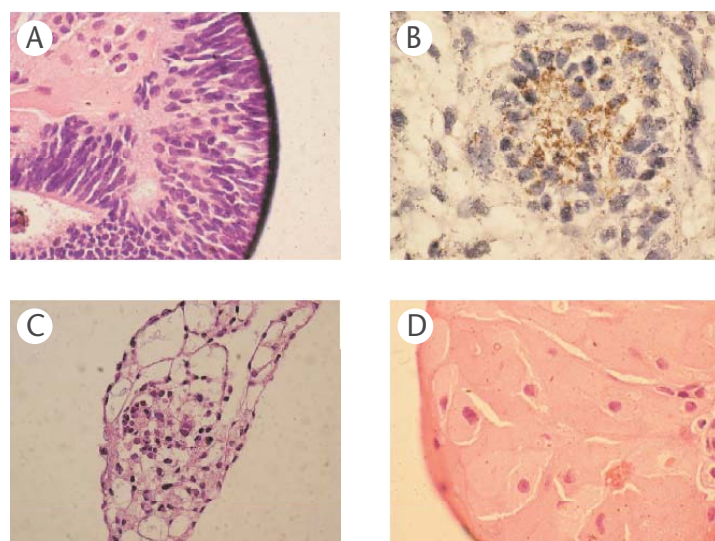
Teratoma formation

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).



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, Bar for A,C 300µM, and for B,D 150µM.



NutriStem™ hESC XF

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

Product Name	Catalogue No.	Unit Size
NutriStem™ hESC XF Xeno-Free Serum-Free Medium for hESCs. Optimized for Feeder-Free culture. May be used with feeder-dependent culture. Superior performance on Matrigel™. With HSA	05-100-1A 05-100-1B	500ml 100ml
AF NutriStem™ hESC XF Xeno-Free Serum-Free Medium for hESCs. Optimized for feeder-dependent culture. Superior on HFF feeder layer. Albumin Free (AF)	05-102-1A 05-102-1B	500ml 100ml

Traditional human Embryonic Stem Cells (hESC) culture methods require the use of mouse or human fibroblast feeder layers, or feeder-conditioned medium. These culture methods are labor-intensive, and difficult to scale up. Moreover, undefined conditions complicate the maintenance of hES cells in the undifferentiated state.

NutriStem™ hESC XF media enable the maintenance and expansion of hESCs with feeder cells or in feeder-independent cultures. NutriStem™ hESC XF media support cultures of undifferentiated hESCs in serum-free conditions on mouse feeder cells, Matrigel™, or human foreskin fibroblasts. Containing recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor β (rh TGF β), these media were successfully tested and proven to maintain hESC pluripotency.

For long-term growth of hESCs without feeder cells, the use of NutriStem™ hESC XF (with HSA) is recommended.

Features

Significant characteristics of serum-free NutriStem™ hESC XF media include:

- A ready-to-use formulation (no additions required), including Alanyl glutamine.
- Xeno-free: formulated with human-derived or human recombinant proteins
- Enables expansion of hESCs in feeder-free culture conditions (Matrigel™), on human-feeder layer (foreskin fibroblasts) or on Mouse feeder cells (MEFs)
- Enables superior expansion of hESCs (i.e. H9.2, I6, I3.2, H1)
- Supports long-term growth of hESCs (over 50 passages)
- Maintains hESC pluripotency
- Maintains hESC normal phenotype and genotype
- Low growth factor (bFGF, TGF β) levels
- Intended for use at a standard 5% CO₂ atmosphere
- Consistent media performance

NutriStem™ hESC XF

Storage

NutriStem™ hESC XF should be stored at -20°C. Once thawed, the medium may be stored at 2-8°C for 2 weeks.

Quality Control

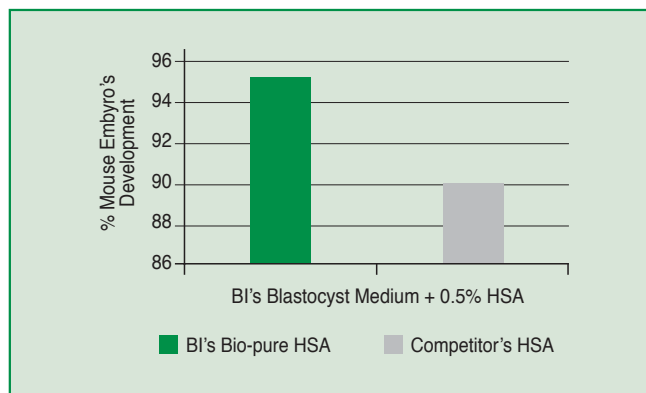
NutriStem™ hESC XF is routinely tested for optimal maintenance and expansion of undifferentiated hESCs.

Additional standard evaluations are pH, osmolality, endotoxins and sterility tests.

Bio-Pure Human Serum Albumin

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

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.

Product Name	Catalogue No.	Unit Size
Embryonic Stem Cells products		
Mouse Embryonic Stem Cells (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) Qualified for Human Embryonic Stem Cells	04-002-1A	500ml
	04-002-1B	100ml
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml
	04-222-1B	100ml
Mesenchymal Stem Cell Media		
Mesenchymal Stem Cell Growth Medium (Ready-to-use)	05-300-1A	500ml
Mesenchymal Stem Cell Adipogenic Differentiation Medium (Ready-to-use)	05-301-1B	100ml
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use)	05-302-1B	100ml
Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use)	05-303-1B	100ml
Related Solutions		
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1) Without L-Glutamine With Sodium Bicarbonate 1.2gm/l With Hepes 15mM With Sodium Pyruvate 55mg/l	01-170-1A	500ml
	01-170-1B	500ml
	01-340-1B	100ml
	01-818-1H	5ml
MEM Non-Essential Amino Acids Solution, 100X Conc. Human Recombinant Insulin Solution, 3.7 mg/ml Serum-Free Cell Freezing Medium	05-065-1A	500ml
	05-065-1C	20ml
L-Glutamine Solution, 29.2mg/ml in Saline, 200 mM	03-020-1A	500ml
	03-020-1B	100ml
	03-020-1C	20ml
L-Alanyl-L-Glutamine (Stable Glutamine), 200 mM	03-022-1B	100ml
	03-022-1C	20ml
Sodium Pyruvate Solution, 11.0mg/ml (100 mM)	03-042-1B	100ml
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml
	03-047-1B	100ml
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml
Papain Dissociation Solution	03-072-1B	100ml
Fibronectin Solution (Bovine), 1mg/ml	03-090-1-01	1ml
	03-090-1-05	5ml
Accutase Solution, primary human cell culture tested	03-073-1B	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
Insulin, Human Recombinant	41-975-100	100mg
Basic Fibroblast Growth Factor (FGF)	30-T-218A	10µg
	30-T-218B	50µg

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