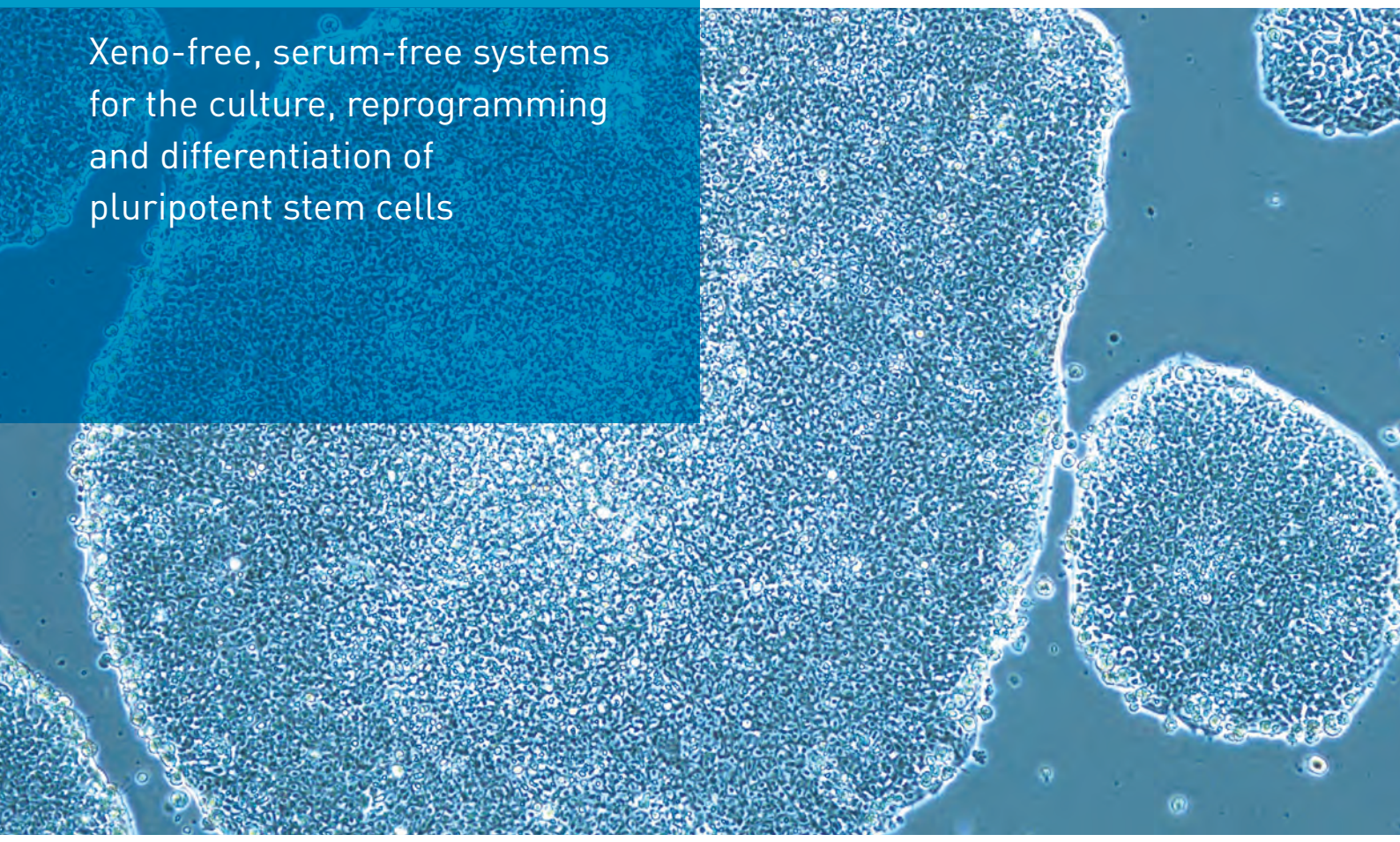
Maintenance of

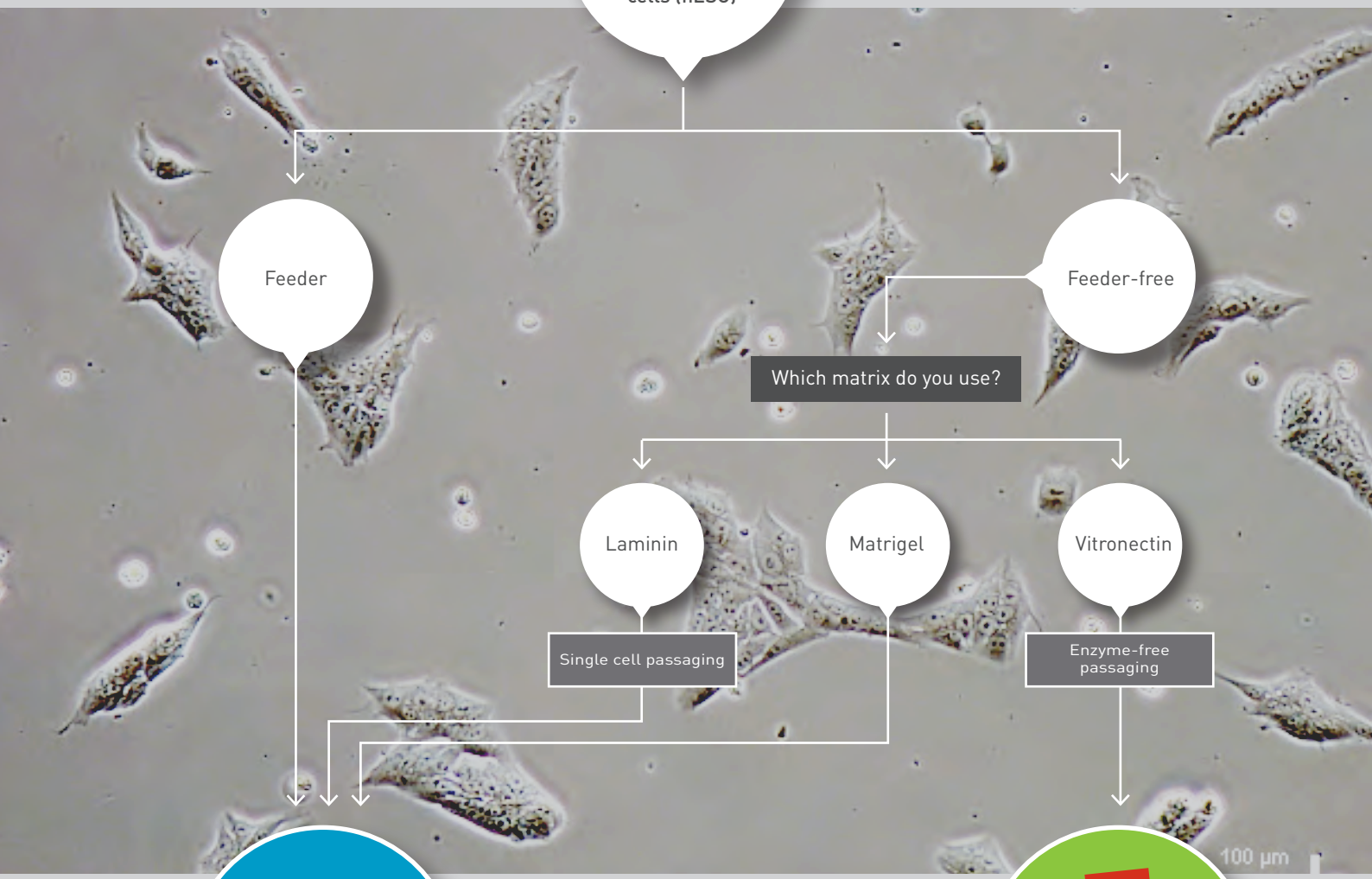
Pluripotent Stem Cells

Xeno-free, serum-free systems for the culture, reprogramming and differentiation of pluripotent stem cells



BI
Biological Industries
Culture of Excellence

Choose the hPSC culture medium that's right for you



NutriStem® hPSC XF culture medium

NEW

NutriStem® V9 XF culture medium

From Research to Cell Based Therapies

The transition from stem cell culture research models to clinical applications requires the design and implementation of qualified processes. Defined, high-quality culture systems and appropriate documentation are therefore an essential element in the development of regenerative stem cell therapies, where implantation in humans is the desired outcome.

Biological Industries (BI) provides an optimized cell culture environment for human pluripotent stem cell research, including the NutriStem® defined, serum free (SF), xeno-free (XF) media family and its auxiliary reagents, manufactured in a cGMP compliant facility. In addition, a Drug Master File (DMF) registered at the FDA is available.

Product Overview

Media

NutriStem® hPSC XF

Defined, xeno-free, serum-free medium for optimal growth and expansion of hPSC on feeder or feeder-free conditions, using laminin or Matrigel.

NutriStem® V9 XF

Defined, xeno-free, NutriStem® V9 XF serum-free medium for optimal growth and expansion of hPSC on feeder-free conditions using vitronectin.

Attachment

LaminStem™ 521

Defined, recombinant Laminin-521 for the attachment of human pluripotent stem cells in a feeder-free culture system.

Vitronectin ACF

Chemically defined, animal component-free (ACF) human recombinant lyophilized vitronectin protein for the attachment of human pluripotent stem cells in a feeder-free culture system.

Dissociation

Recombinant Trypsin EDTA Solution

ACF recombinant trypsin solution with EDTA for efficient single cell dissociation of adherent cell types from surfaces and tissues.

EDTA Solution 0.5M

Enzyme-free, chemically defined, ACF dissociation solution.

Cryopreservation

NutriFreez® D10 Cryopreservation Medium

ACF, protein-free and chemically defined freezing medium,
for hPSC cryopreservation both as single cells and aggregates.

hPSC Proliferation with

NutriStem® hPSC XF

Product Name	Cat. No.	Size	Storage
NutriStem® hPSC XF	05-100-1A 05-100-1B	500ml 100ml	-20°C
NutriStem® hPSC XF Medium (Modified, GF-free, bFGF-free)	06-5100-01-1A	500ml	-20°C

Defined, xeno-free, serum-free medium designed to support the growth and expansion of hESC and hiPSC.

Advantages

Excellent performance

- → Superior cell proliferation (low doubling time)
- → Maintenance of pluripotent stem cell characteristics and stable karyotype over long term passages (>50 passages)

User-friendly

- → One bottle formulation
- → Weekend-free feeding regime
- → Straightforward adaptation protocol

Flexible

- → Versatile coating and culture methods
- → Flexible packaging
- → Custom modifications

Defined, xeno-free, serum-free medium

- → Reproducible and consistent results throughout experiments
- → Batch-to-batch consistency

cGMP medium

- → Complete product dossier
- → Registered DMF
- → Produced under cGMP conditions

Low growth factor concentrations (bFGF, TGF Beta)

- → Improves cell quality, reprogramming and differentiation capabilities

Widely referenced in publications

- → Feel confident in your research

Excellent proliferation of undifferentiated hPSC

NutriStem® hPSC XF enables excellent proliferation of undifferentiated hESC and hiPSC.

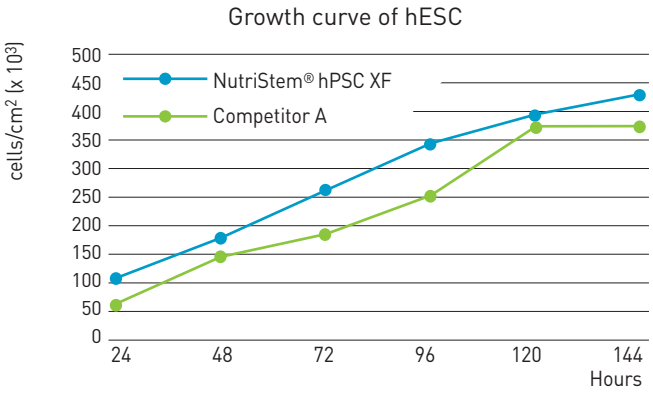


Figure 1: H1 cells (passage 6) were seeded in 96 well plates (Matrigel-coated) in the various media. Media were changed every 24 hours. The number of cells was determined using a CyQuant cell proliferation assay kit.

Embryoid Body (EB) Formation

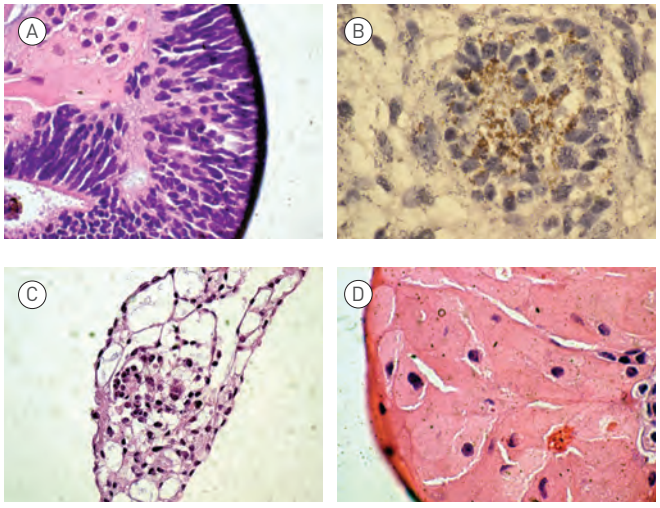
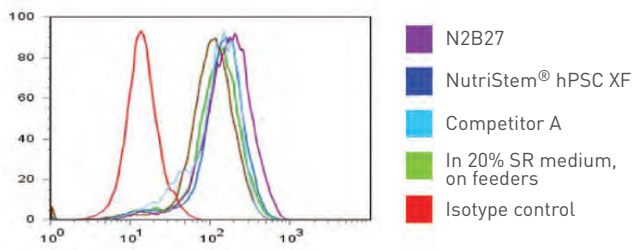


Figure 2: hESC from cell line H9.2 were cultured for 16 passages in NutriStem® hPSC XF using a Matrigel matrix and tested in vitro for pluripotency by EB formation. After suspension in serum supplemented medium the cells spontaneously formed embryoid bodies containing embryonic germ layers. Examining 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.

High expression of pluripotent stem cell markers

Oct-4



SSEA-4

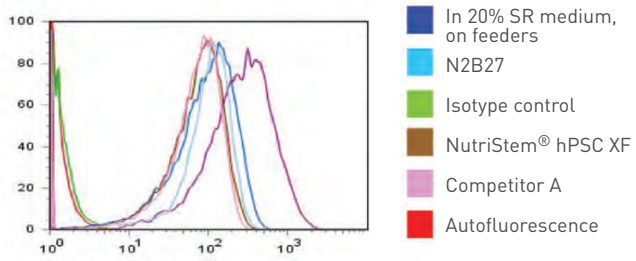


Figure 3: H1 cells cultured in different media for 6 passages were analyzed and compared using flow cytometry and gene expression. Cells cultured in NutriStem® hPSC XF were found to be >90% positive for SSEA-4 and Oct-4.

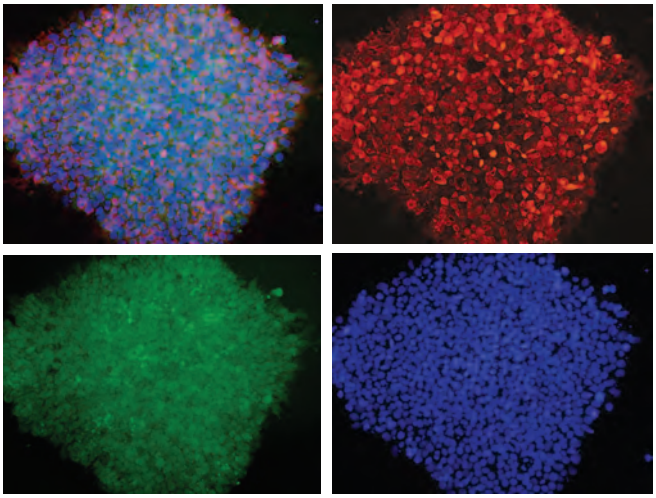


Figure 4: H1 cell morphology and immunofluorescence analysis of hESC markers: red SSEA-4, green OCT4 and blue DAPI. H1 cells stained positive for the expression of pluripotency markers.

NutriStem® hPSC XF gives you the freedom and versatility to derive and culture pluripotent stem cells in a variety of methods

NutriStem® hPSC XF medium supports both feeder-dependent and feeder-free culture systems. The medium is also suitable for culture as colonies or monolayer, and supports single cell applications.

Laminin-Based Culture System

LaminStem™ 521 with NutriStem® hPSC XF provide a superior culture environment for undifferentiated expansion and growth of hES and hiPS cells in a defined, xeno-free, and feeder-free culture system as a monolayer, while maintaining proper phenotype and genetic stability. Studies have shown that efficient clonal derivation of hES cell lines is possible with the combined use of NutriStem® hPSC XF medium and LaminStem™ 521 substrate, finding that the cells grew better in NutriStem® hPSC XF than any other defined medium tested, and that hES cells can be passaged and maintained using a single-cell expansion protocol (Rodin, S. et al. 2014).

Single cell passaging using LaminStem™ 521 and Recombinant Trypsin EDTA Solution

Culturing of hPSC using NutriStem® hPSC with LaminStem™ 521 enables easy and reliable single-cell passaging without artificial apoptosis inhibitors, such as ROCK inhibitor (Y-27632). This provides standardized procedures that are fast and easy to use. For the efficient dissociation and passaging Recombinant Trypsin EDTA Solution should be used.

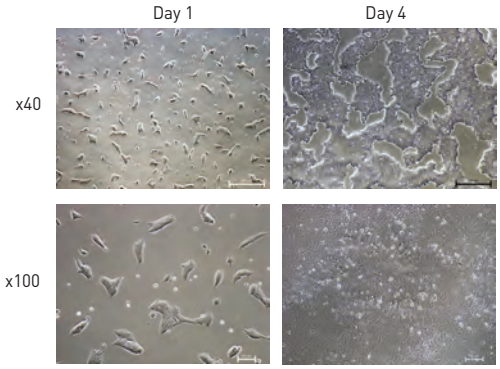


Figure 5: Typical recovery of H1 (61) hESC from single-cell passage using Recombinant Trypsin EDTA Solution and NutriStem® hPSC XF medium on 0.5µg /cm² LaminStem™ 521. Representative images for colony morphology one day and 4 days post-passage.

"hES cells grew better in the xeno-free chemically defined NutriStem® hESC XF"

(Rodin et al. 2014)

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Clinical Applications

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Matrigel™-Based Culture System

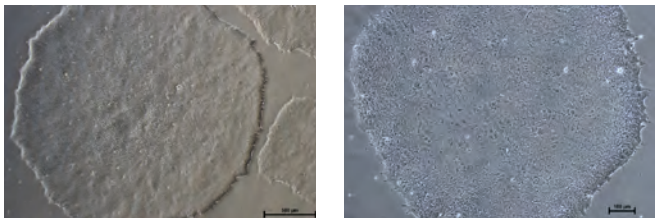

Figure 6: H1 hESC cultured in NutriStem® hPSC XF on Matrigel™ display compact colonies and distinct colony morphology typical of hPSC.

Enzyme-free passaging with EDTA

Small aggregate dissociation using EDTA is a gentle, enzyme-free method of passaging cells grown in feeder-free conditions.

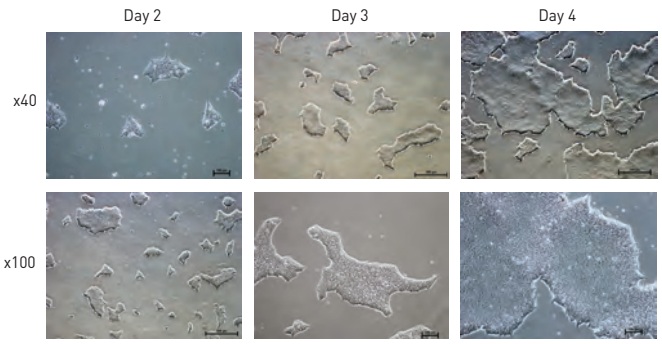

Figure 7: Typical recovery of hESC from enzyme-free passage (0.5mM EDTA) using NutriStem® hPSC XF medium on Matrigel™. Representative results for colony morphology of H1 hESC 2-4 days post-passage.

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Feeder-Dependent Culture (MEF/HFF)

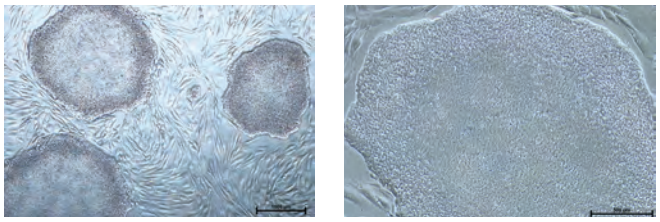

Figure 8: H1 hESC colonies on MEF feeder layer display compact colonies and distinct colony morphology typical of hPSC.

"NutriStem appears to support iPSC culture on feeders better than E8"

(T. Cerbini et al., 2015)

Key References

Gene Editing

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Clinical Applications

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- Y. Luo et al.,Stable Enhanced Green Fluorescent Protein Expression After Differentiation and Transplantation of Reporter Human Induced Pluripotent Stem Cells Generated by AAVS1 Transcription Activator-Like Effector Nucleases. STEM CELLS Translational Medicine: Volume 3, Issue 7, pp 821-35, 2014

Auxiliary Products

Product Name	Cat. No.	Size	Storage
EDTA Solution 0.5M	01-862-1B	100ml	RT

Diluted EDTA Solution 0.5mM is an enzyme-free, chemically defined, Animal Component Free (ACF) solution, suitable for the dissociation of human pluripotent stem cells. EDTA Solution 0.5mM mediates rapid cell dissociation by chelating calcium and magnesium ions that facilitate cell adhesion

Product Name	Cat. No.	Size	Storage
LaminStem™ 521	05-753-1F	1ml	-20°C

LaminStem™ 521 facilitates self-renewal hPSC in a defined, feeder-free and xeno-free cell culture system. LaminStem™ 521 is composed of purified laminin-521, a cell-type specific basement membrane protein proven to support excellent attachment proliferation of hES and hiPS cells.

Product Name	Cat. No.	Size	Storage
Recombinant Trypsin	03-079-1B	100ml	RT
EDTA Solution	03-079-1C	20ml	

Recombinant Trypsin EDTA Solution was developed for efficient single cell dissociation of adherent cell types from surfaces and tissues and were optimized for sensitive cells, such as hPSC.
Recombinant Trypsin EDTA Solution is ready-to-use and animal component free. The addition of EDTA accelerates the dissociation phase. The solution does not contain any chymotrypsin, carboxypeptidase A, or other protease contaminants.

hPSC Proliferation with

NutriStem® V9 XF

Product Name	Cat. No.	Size	Storage
NutriStem® V9 XF basal medium	05-105-1A	500ml	-10° to -20°C
NutriStem® V9 XF Supplement Mix	05-106-1F	1ml	-10° to -20°C

Defined, xeno-free, serum-free culture medium for hPSC optimized for vitronectin.

Advantages

Excellent performance

- → Superior proliferation rates in long-term culture on vitronectin coated culture ware
- → Maintenance of pluripotent stem cell characteristic over long term passages (>30 passages)
- → Supportive for difficult lines and routine culture

User-friendly

- → Weekend-free feeding regime
- → Pre-coating free protocol with BI's ACF Vitronectin
- → ROCK inhibitor free protocol for seeding, passaging and thawing

Defined, xeno-free, serum-free medium

- → Reproducible and consistent results throughout experiments
- → Batch-to-batch consistency

cGMP media

- → Produced under cGMP conditions

Cytokine-free basal medium

- → Improves cell quality and ability to reprogram and differentiate

Typical cell morphology during long-term culture

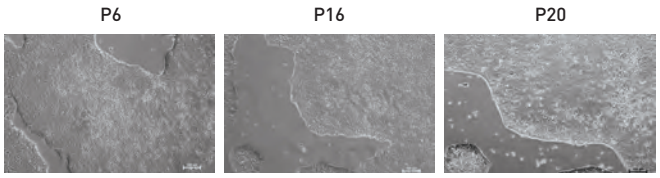

Figure 9: Phase contrast images (x100) of H1 hESC culture maintained in NutriStem® V9 XF using 0.5µg/cm² Vitronectin ACF-coated cultureware, show typical morphology.

Superior proliferation rate in long-term culture

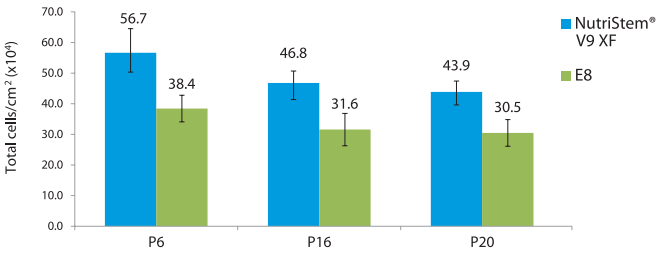

Figure 10: Nucleocounts performed on hESC suspension (Chemometec) during long-term expansion in NutriStem® V9 XF compared to E8 using 0.5µg/cm² Vitronectin ACF and passage as small aggregates every 3-5 days using 0.5mM EDTA solution.

Embryoid body (EB) Formation

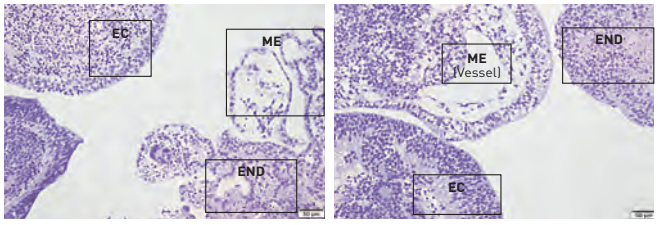

Figure 11: Embryoid bodies (EBs) generated from H1 hESC expanded for 6 passages in NutriStem® V9 XF medium on Vitronectin ACF as an evaluation of pluripotency. Cells were suspended in NutriStem® V9 XF basal, where they spontaneously formed EBs, for 18 days. Cell types identified by examination of EBs histological sections stained with H&E. See letters EC=neural rosettes, ME=primitive vessels, ED=primitive parenchyma (X100)

High expression of pluripotent stem cell markers

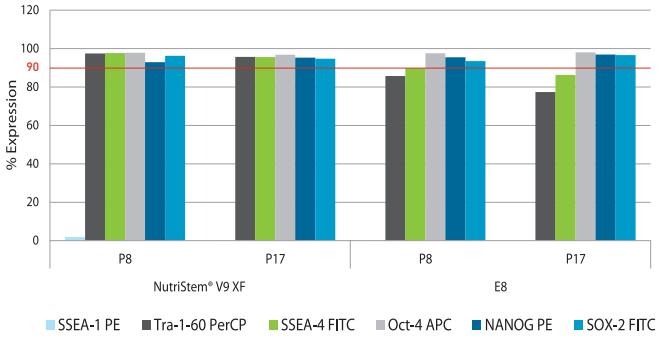

Figure 12: Immunophenotyping analysis of human pluripotent markers of H1 hESC cultured in NutriStem® V9 XF at P8 and P17 shows elevated marker expression over 90%. Data presented as % expression from gated viable cells.

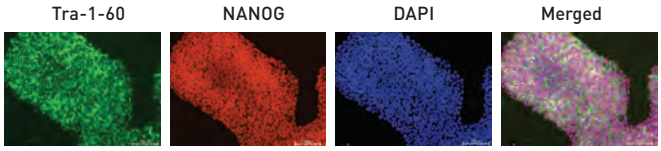

Figure 13: Immunofluorescence analysis of human pluripotent markers of H1 hPSC expanded in NutriStem® V9 XF medium under a weekend-free feeding regime at P8. Cells were stained for pluripotent surface markers: TRA 1-60 (Alexa Fluor) (green) and nuclear conjugated markers: NONOG-RRX (red), counterstained with DAPI (blue). Scale bar 200µm.

Save time with precoating-free procedure!

A user-friendly protocol has been developed to eliminate the precoating procedure. While seeding, vitronectin is added directly into NutriStem® V9 XF medium, making precoating unnecessary.

Novel precoating-free procedure		Traditional coating procedure	
Actual Vitronectin ACF coating	Vitronectin ACF volume added to 3ml medium	Vitronectin ACF coating	Vitronectin ACF volume
0.25-0.375µg/cm²	5-7.5µl	0.5µg/cm²	10µl
~30 minutes		90 minutes minimum	

Typical cell morphology

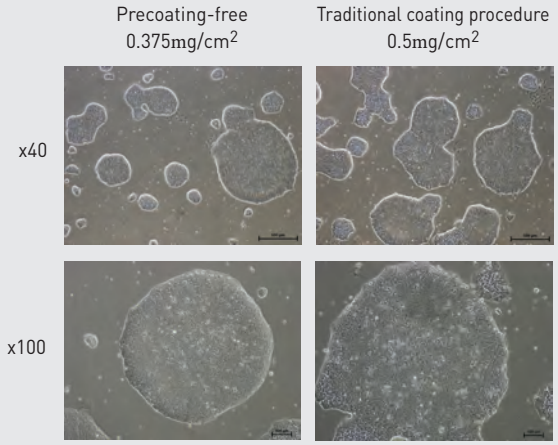

Figure 14: Colony morphology of hPSC cultured in NutriStem® V9 XF for 11 sequential passages using coated and precoating-free protocol (Vitronectin ACF added directly to NutriStem® V9 XF culture medium before cells seeding).

High proliferation rate of hPSC during long-term expansion

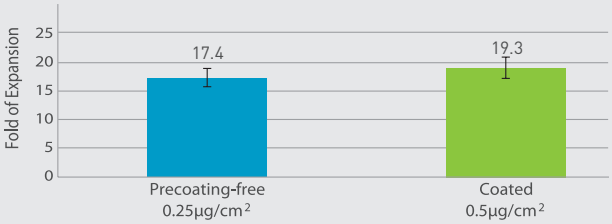

Figure 15: Nucleocounts performed on cells suspension (Chemometec) in NutriStem® V9 XF using traditional coating and precoating-free protocol and passaged as aggregates using 0.5mM EDTA solution. Equivalent proliferation rates were measured in both methods.

High expression of pluripotent markers

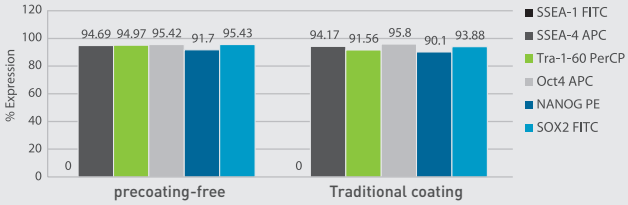

Figure 16: Flow cytometry analysis of H1 cultured in NutriStem® V9 XF for 11 passages using precoating-free protocol. Vitronectin ACF was added directly to NutriStem® V9 XF culture medium before cells seeding. High expression of pluripotent markers, over 90% is measured. Data presented as % expression from gated viable cells.

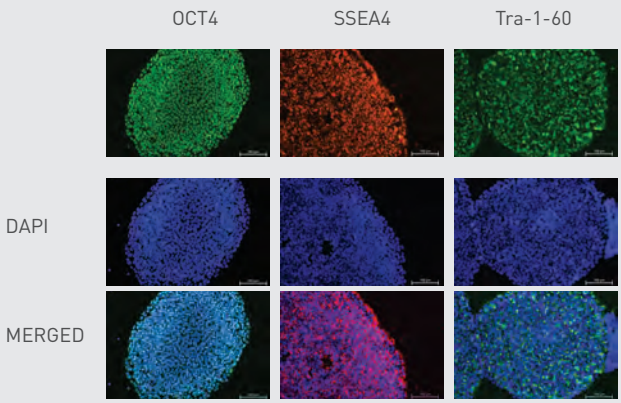

Figure 17: Immunofluorescence analysis of human pluripotent markers of H1 hPSC expanded in NutriStem® V9 XF medium using the precoating-free for 8 passages. Cells from P8 were fixed and stained for the classical pluripotent surface markers: SSEA4 (RRX) (red), TRA-1-60 (Alexa fluor) (green) and nuclear conjugated markers: OCT-4-Alexa fluor, counterstained with DAPI (blue). Scale bar 200 µm.

Auxiliary Products

Product Name	Cat. No.	Size	Storage
Vitronectin ACF	05-754-0002	200mg	-20° to -80°C

Vitronectin is a secreted glycoprotein that supports cell adhesion through binding to various integrins and proteoglycans. Vitronectin ACF (Animal Component Free) can function as a chemically-defined matrix component for the attachment of human embryonic and induced pluripotent stem cells in a feeder-free culture system. Vitronectin ACF is a 459 amino acid, single-chain, monomeric recombinant protein, which migrates at an apparent molecular weight of 75 kDa by SDS-PAGE under reducing conditions. The calculated molecular weight of Vitronectin ACF is 52.2 kDa.

Product Name	Cat. No.	Size	Storage
0.5M EDTA Solution	01-861-1B	100ml	RT

Diluted 0.5mM EDTA solution is an enzyme-free, chemically defined, ACF solution, Diluted 0.5mM EDTA solution suitable for the dissociation of human pluripotent stem cells. Diluted 0.5mM EDTA solution rapid cell dissociation by chelating calcium and Diluted 0.5mM EDTA solution magnesium ions that facilitate cell adhesion.

hPSC Cryopreservation

Product Name	Cat. No.	Unit Size	Storage
NutriFreez® D10 Cryopreservation Medium	05-713-1A 05-713-1B 05-713-1C 05-713-1D 05-713-1E	500ml 100ml 20ml 10ml 50ml	2-8°C

NutriFreez® D10 Cryopreservation Medium is an animal components-free, ready-to-use solution for the cryopreservation of animal cells.
NutriFreez® D10 Cryopreservation Medium was developed to maintain ACF conditions during cryopreservation when culturing cells in a XF culture system, and has been extensively validated with human ES cells (H1, H9 and HuES9). NutriFreez® D10 Cryopreservation Medium has shown to be very effective for the cryopreservation of hPSCs as single cells and cell aggregates. Cells preserved with NutriFreez® D10 Cryopreservation Medium show high viability, attachment, growth performance, and maintenance of pluripotency markers after thawing (Figure 1), with superior results compared to both serum-containing freezing media and other serum-free solutions.

Advantages

- Chemically defined, Animal component-free (ACF), Protein-free
- Works with various media
- Suitable for freezing hESC and hiPSC cultured in both feeder and feeder-free conditions
- High recovery efficiency: maintains excellent attachment ability as well as growth performance
- Maintains hESC and hiPSC pluripotency
- Complete formulation; Ready-to-use at 2-8°C
- For cryopreservation of hPSC clumps or single cells

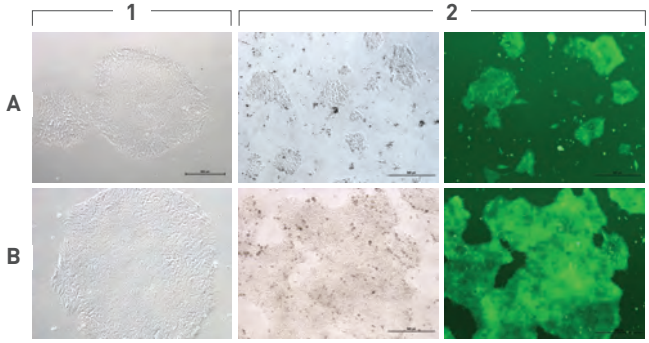

Figure 18: H1 hES cells (1) and BGO1V/hOG (2) GFP reporter cells frozen in NutriFreez® D10 Cryopreservation Medium. Cryopreserved hES cells were thawed into NutriStem® hPSC Medium on Matrigel-coated plates. Cells show high viability at day 1 (A) and at day 4 post-thaw (B).

"...cryopreservation with CryoStem* showed the best recovery rate for hPSCs after thawing"

(Nishishita N, et al., 2015)

* NutriFreez® replaces the brandname "CryoStem"

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hESC Derivation

NutriStem® hPSC XF medium enables successful derivation of new hESC lines, as well as long-term genetically stable growth of the clonal hESC lines in chemically defined, xeno-free environment.

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hiPSC Reprogramming

mRNA-based cellular reprogramming of human cells
NutriStem® hPSC XF medium supports mRNA-based cellular reprogramming of human cells. mRNA reprogramming is a fast, safe and efficient method for generating integration-free, virus-free, clinically relevant iPS cell lines from mature human cells. BI also offers the possibility for modified NutriStem® hPSC XF without growth factors.
Clonal mRNA reprogrammed iPSC lines can be expanded and maintained in NutriStem® hPSC XF.

Key References

- Protocol describes using laminin substrate and NutriStem™ hPSC XF Culture Medium to provide a complete xeno-free reprogramming environment:
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hPSC Differentiation

NutriStem® hPSC XF is widely referenced in publications, showing effective differentiation of hPSC into variety of cell types.

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Ordering information

Product Name	Cat. No.	Size	Storage
NutriStem® hPSC XF	05-100-1A 05-100-1B	500ml 100ml	-20°C
NutriStem® hPSC XF Medium (Modified, GF-free, bFGF-free)	06-5100-01-1A	500ml	-20°C
NutriStem® V9 XF Basal Medium NutriStem® V9 XF Supplement Mix	05-105-1A 05-106-1F	500ml 1ml	-20°C
LaminStem™ 521	05-753-1F	1ml	-20°C
Vitronectin ACF	05-754-0002	1ml	-20° to -80°C
Recombinant Trypsin Solution	03-078-1A 03-078-1B	500ml 100ml	RT
Recombinant Trypsin EDTA Solution	03-079-1A 03-079-1B	500ml 100ml	RT
EDTA Solution 0.5M	01-862-1B	100ml	RT
Accutase Solution	03-073-1B	100ml	-20°C
NutriFreez® D10 Cryopreservation Medium	05-713-1A 05-713-1B 05-713-1C 05-713-1D 05-713-1E	500ml 100ml 20ml 10ml 50ml	2-8°C

Biological Industries (BI) has been committed for over 30 years to provide optimal and innovative solutions for cell culture practice.
BI manufactures and supplies life science products to biopharmaceutical, academic and government research facilities, as well as to biopharma companies.

Our diverse portfolio of products and services includes all of the following:

- Liquid and powdered cell culture media
- Sterile sera (foetal bovine serum, newborn calf serum, donor horse, etc.)
- Novel serum-free and animal component-free media and supplements
- Products for stem cell research and cell-based therapies
- Products for cytogenetics
- Products for mycoplasma detection and treatment
- Disinfectants
- Products for molecular biology
- custom formulations and contract manufacturing services

All BI's products are manufactured via a quality management system ISO 9001:2015 and in regards of medical devices ISO 13485:2016. All aspects of the products life cycle fall under the QMS procedures. The set-up of clean zone and clean room facilities for manufacturing are following ISO 14644, whereas the production rooms are ISO 8, storage of sterile accessories ISO 7 and filling rooms ISO 5. Aseptic filling and validation is performed according to ISO 13408.

BI exports its products to more than 50 countries worldwide, via a network of exclusive distributors. Over the years we have established a reputation for fast delivery, and excellent technical support.
From the outset, the policy of BI has been based on the need to maintain an active Research and Development program in all facets of company activities. The company has its own in-house R&D department, and in addition, maintains active contact with science-based companies and research institutions in Israel and abroad, including know-how agreements with several such institutions. These ongoing efforts have led to the introduction of a series of serum-free medium products, as well as many other products for cell culture and molecular biology.

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