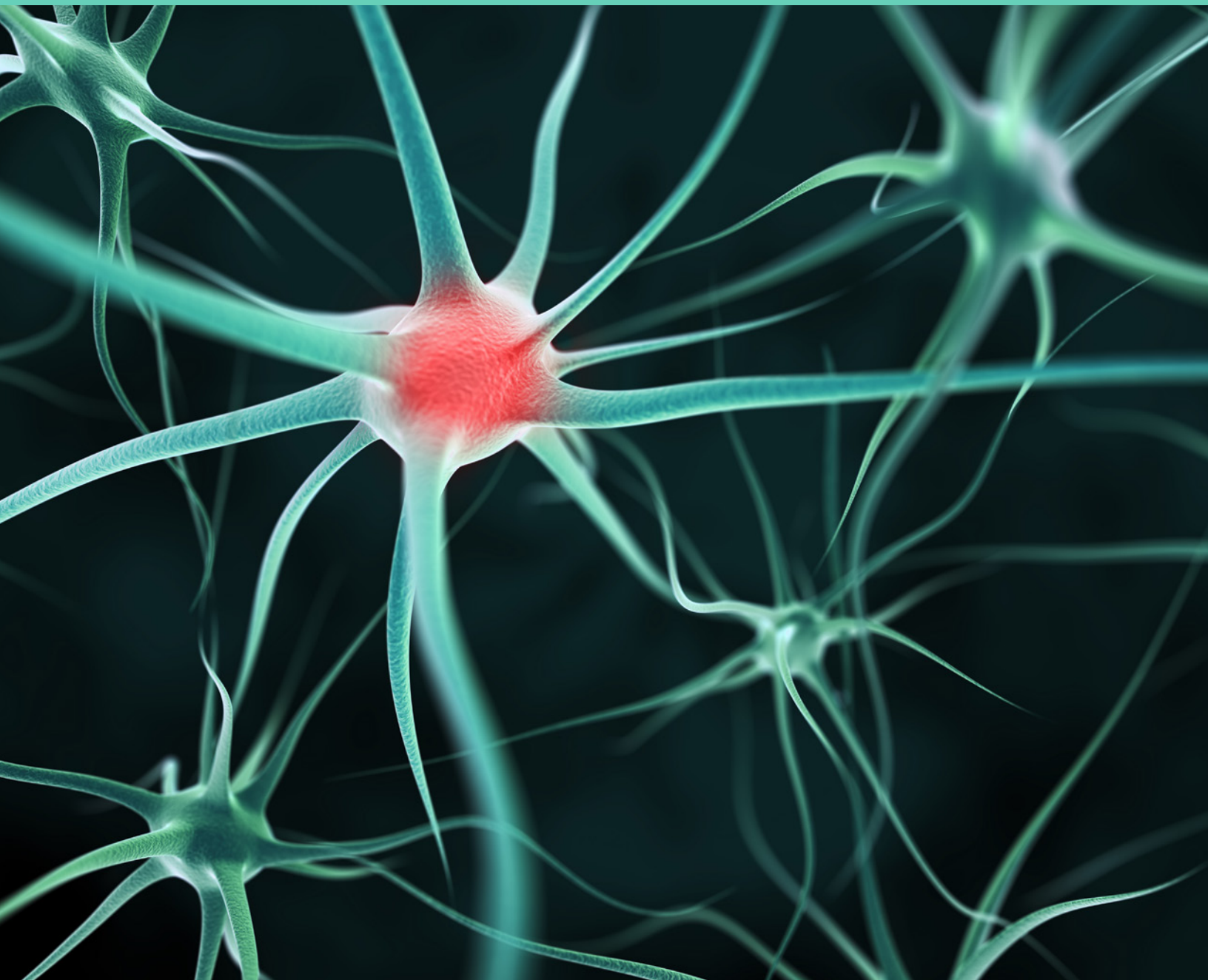
amsbio

Derivation, Culture & Characterization

STEM CELL GUIDE



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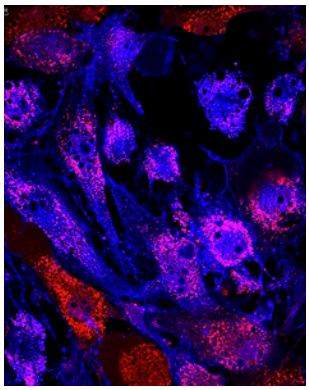


Kick Start Your Stem Cell Research With AMSBIO!

From quality tested ES and adult stem cell lines to iPS cell generation, we're here to help you progress with your research!

Try our cutting edge, off-the-shelf stem cells, reprogramming reagents and our small molecule stem cell modulators.

Stem cells are defined by their ability to undergo self-renewal and their biological potency to differentiate into distinct mature cell types. Stem cells are distinct from primary cells that have limited or no ability to undergo self-renewal and lack the ability to differentiate into other cell types. It is generally accepted that progenitor cells differ from stem cells because of their limited ability to divide and their predetermined differentiation into mature cell types. The current categorization of stem cells is largely based upon the number of progeny the stem cell is able to differentiate into and the tissue from which the stem cell is derived.

Adult stem cells, commonly also referred to as somatic stem cells, reside in most tissues within mammalian species and are required to maintain homeostasis as well as to regenerate injured or damaged tissue. Adult stem cells have a limited potential as to the number of mature cell types that they are able to differentiate into. Those adult stem cells that are able to differentiate into multiple (3 or more) mature cell types are referred to as 'multipotent'; whereas, at the other side of the spectrum adult stem cells that can differentiate into one cell type are called 'unipotent'. Classic examples of adult multipotent stem cells include: Hematopoietic stem cells (HSCs), and Mesenchymal stem cells (MSCs), derived from bone marrow or cord blood; and, adipose derived multipotent stem cells (ADSCs).

Perhaps the biggest scientific discovery of the last decade was the finding that any primary cell can be reprogrammed into pluripotent stem cells, termed induced pluripotent stem cells (iPSCs). This exciting discovery was made by Shinya Yamanaka in 2006 through the finding that transduction of a skin cell with four transcription factors could revert it to a pluripotent state. This discovery led to the Nobel prize in medicine being awarded to Professor Yamanaka in 2012 and is transforming stem cell research and regenerative medicine as we know it. AMSBIO supplies all of the tools and technologies needed to advance your stem cell research.

STEM CELL NOMENCLATURE BASED ON DIFFERENTIATION POTENTIAL

	Potency	Description	Example Stem Cell
	Totipotent (Omnipotent)	Ability to differentiate into any cell type and can construct a complete viable organism	Cells from the morula
Pluripotent Stem Cells	Pluripotent	Descendants of totipotent cells with the ability to differentiate into any cell type in the body	Embryonic Stem Cells.
	Pluripotent	Adult cells reprogrammed into an embryonic-stem cell-like state using factors to induce pluripotency.	Induced Pluripotent Stem Cells
Adult Stem Cells	Multipotent	Differentiate into closely related cell types	Neural Stem Cells, Mesenchymal Stem Cells
	Oligopotent	Limited differentiation into only a few cell types	Lymphoid or Myeloid Stem Cells
	Unipotent	Differentiate into only one cell type	Muscle Stem Cells

READY-TO-USE FEEDER CELLS

Mouse Embryonic Fibroblasts (MEFs)

We offer Mouse Embryonic Fibroblasts (MEFs) to support the growth of undifferentiated mouse or human embryonic stem cells and induced pluripotent stem cells. We supply CF-1, DR4, Neo-resistant and SNL (STO) feeder cells, offered either as untreated cells (P2) for further expansion and treated cells that can be directly used as a feeder layer.

BENEFITS

- ✓ Meticulously derived and comprehensively tested on mouse and human ES stem cells to ensure robust and consistent performance with every lot
- ✓ Comprehensive contamination tests include human pathogen and mycoplasma
- ✓ Save time and trouble dealing with an animal facility, performing time-consuming dissections and cell expansion

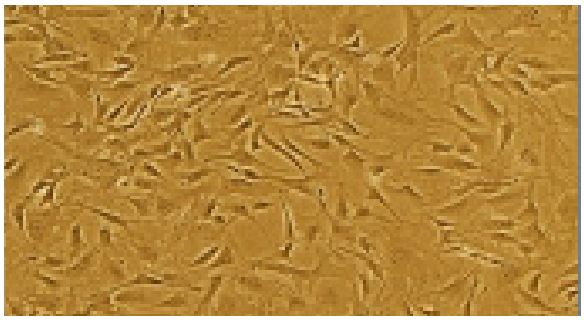


Figure 1: *Mouse Embryonic Fibroblasts*

QUALITY CONTROL

1. PCR-based assay for 22 mouse pathogens with sensitivity equal to or greater than traditional MAP Testing.
2. Direct inoculation and culture of blood agar plates, thioglycolate broth, tryptocase soy broth, and sabaoraud dextrose agar to check for bacterial and fungal contamination.
3. PCR-based assay sensitive to the detection of species typically found as contaminants of cell culture.
4. Sparse population of mouse ES cells is plated and observed for overall plating efficiency, colony morphology and immunocytochemistry.
5. Qualification based on the morphology, growth, and immunocytochemisty of human ESC cultured for multiple passages.

CF-1-MEFs

Treatment	Pack Size	Cat. No.
Irradiated	1 vial of 4 x 10^6 cells	ASF-1213
Irradiated	1 vial of 2 x 10^6 cells	ASF-1215
Irradiated	1 vial of 1 x 10^6 cells	ASF-1217
Mitomycin-C	1 vial of 4 x 10^6 cells	ASF-1123
Mitomycin-C	1 vial of 2 x 10^6 cells	ASF-1225
Untreated: P2	1 vial of 1 x 10^6 cells	ASF-1201

SNL 76/7 MOUSE FIBROBLAST STO CELL LINE, P14

Treatment	Pack Size	Cat No.
Mitomycin-C	1 vial of 5 x 10^6 cells	ASF-1327

DR4 MEFS (NEOMYCIN, HYGROMYCIN, PUROMYCIN, AND 6-THIOGUANINE RESISTANT)

Treatment	Pack Size	Catalogue No.
Untreated	1 vial of 1 x 10^6 cells	ASF-1001
Irradiated	1 vial of 2 x 10^6 cells	ASF-1015
Irradiated	1 vial of 4 x 10^6 cells	ASF-1013
Mitomycin-C	1 vial of 2 x 10^6 cells	ASF-1025
Mitomycin-C	1 vial of 4 x 10^6 cells	ASF-1023

NEOMYCIN RESISTANT MEFs

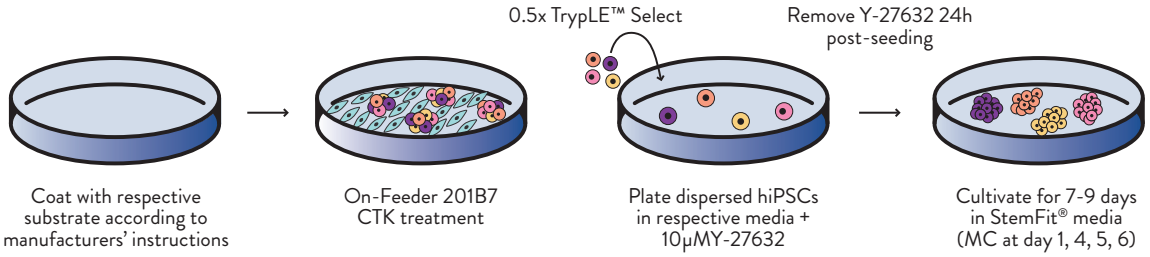
Treatment	Pack Size	Density	Cat. No.
Irradiated	1 vial of 4 x 106 cells	Standard	ASF-1113
Irradiated	1 vial of 2 x 106 cells	Low	ASF-1115
Mitomycin-C	1 vial of 4 x 106 cells	Standard	ASF-1123
Mitomycin-C	1 vial of 2 x 106 cells	Low	ASF-1125

StemFit® Feeder Free Medium ES/iPS Cell Culture

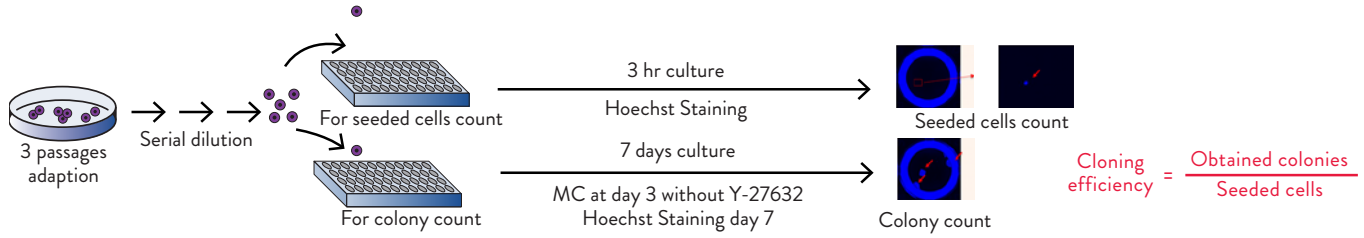
StemFit® is a xeno-free, defined medium proven to effectively maintain Induced Pluripotent Stem (iPS) and Embryonic Stem (ES) cells under feeder-free conditions during the reprogramming, expansion and differentiation phases of stem cell culture. StemFit® combines high colony forming efficiency with lower than standard media volume consumption to offer cost effective colony expansion when compared to leading competitors.

BENEFITS

- ✓ Highly stable and reproducible feeder-free culture system
- ✓ Easy transition from on-feeder to feeder-free culture
- ✓ High affinity to commercially available coating matrices
- ✓ Weekend-Free Cell Culture
- ✓ Superior colony forming efficiency from a single cell clone enables high quality and cost effective genome-edited clone generation



EASY ADAPTION



The Optimal Choice for Gene Editing Protocols

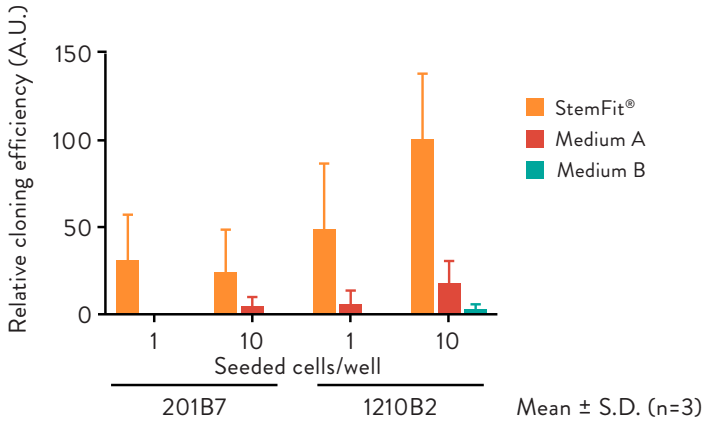


Figure 2: *Human iPSCs were adapted to StemFit®, or commercially available medium A or B on Matrigel® for more than 3 passages. Then, cells were serially diluted and seeded with each medium on Matrigel®-coated 96-well plates at 1 cell/well or 10 cells/well. The number of seeded cells was counted after 3 hours, and colonies were counted at day 7.*

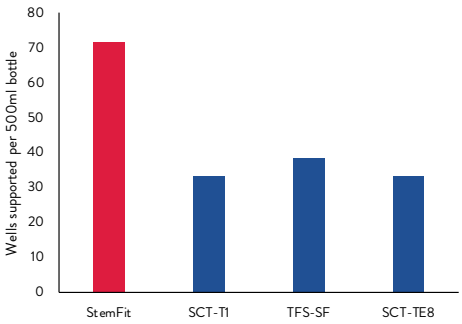


Figure 3: *With less frequent media changes and lower than standard medium volume consumption, one 500ml pack of StemFit® can support over 50% more wells than other leading competitor products. This makes it a highly cost effective solution for stem cell culture.*

Description	Pack Size	Cat No.
StemFit® Basic02- Stem Cell Culture Media	400 ml + 100 ml	SFB-500
StemFit® Basic03 - Clinical Grade Stem Cell Culture Media	400 ml + 100 ml	SFB-503

Laminin-511 E8 Fragments

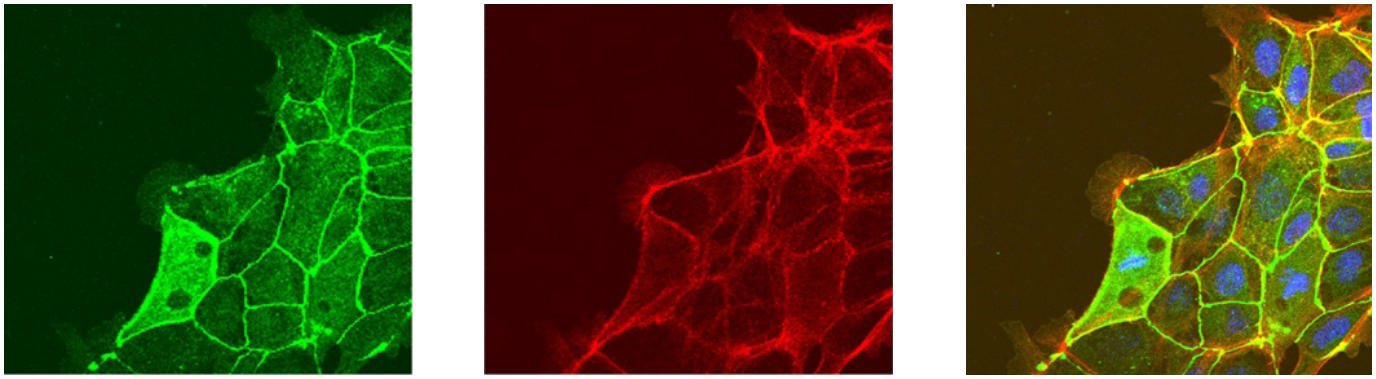


Figure 4: *Human iPS cells on laminin-511 E8 (coated laminin concentration 0.4 µg: ZO-1, Beta-Actin and Objective lens; X40)*

iMatrix-511 is an innovative cell culture matrix compatible with a wide variety of cell types, and exceptionally well suited for pluripotent stem cells. This product is comprised of recombinant Laminin-511 E8 protein fragments which permit ES/iPS cells to be maintained in xeno-free culture conditions, enable the passaging of single cells, and provide greater adhesion than full-length Laminin, Vitronectin or Matrigel®.

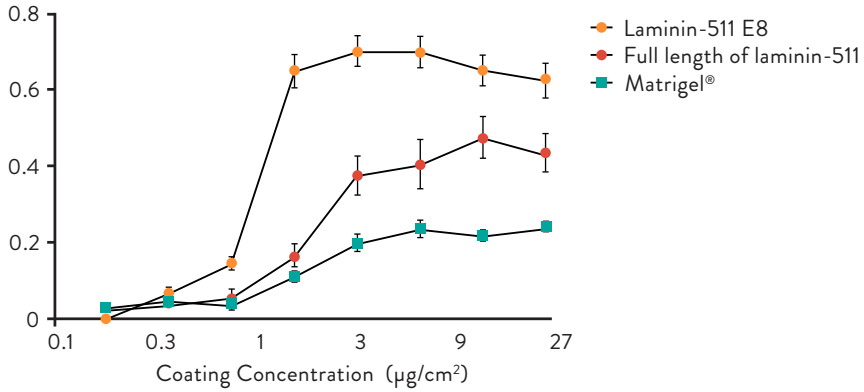
Laminin-511 is a major component of the basement membrane, which is expressed in early development of the embryo and can be used as a matrix for pluripotent (ES/iPS) stem cells, as it binds to integrin on cell surfaces. However, Laminin-511 is a large protein (800kDa) composed of three chains (alpha, beta and gamma), making it difficult to produce recombinantly. In order to overcome this challenge, Laminin-511 proteins were fragmented to find the smallest integrin-binding component and hES cells were found to adhere more strongly to the E8 fragment than to the full-length protein.

BENEFITS

- ✓ Ideal for Xeno-Free, Animal-Free, Feeder-Free Cell Culture
- ✓ Proven to provide superior adhesion of human ES and iPS cells
- ✓ Enables the passaging of single cells
- ✓ Eliminate need to coat plates
- ✓ Makes it easy to achieve extended cultures of hES/hiPS cells

Figure 5: *The Binding activity of Laminin 511 E8 Fragment against ES cell was better than full length 511 and traditional substrate.*

The horizontal axis of the graph shows the concentration of cell culture substrate, and the vertical axis shows the OD value (optical density at 570nm). This result shows that the Laminin-511 E8 fragment adheres to cells more strongly than its competitors.



Description	Pack Size	Catalogue No.
iMatrix-511	350 μL(175μL × 2 tubes)	AMS.892 011
iMatrix-511	1,050 μL(175μL × 6 tubes)	AMS.892 012
iMatrix-511silk	1,050 μL(175μL × 6 tubes)	AMS.892 021
iMatrix-411	1,050 μL(175μL × 6 tubes)	AMS.892 041
iMatrix-411	350 μL(175μL × 2 tubes)	AMS.892 042

STEM-CELLBANKER®

STEM-CELLBANKER® is a chemically defined cryopreservation media optimized for stem cells and iPS cells storage, as well as fragile primary cells. Furthermore, recent data supports its ability to cryopreserve organoids and tissues to allow the recovery of viable cells.

Available in both DMSO containing and DMSO-Free formulations, STEM-CELLBANKER® is an optimal freezing solution for basic research and clinical application of cell therapy products. It is ready-to-use and requires no special technical skills or devices, such as controlled rate freezers. It is animal component free and contains only chemically defined European and US Pharmacopoeia grade ingredients. Pluripotency, normal karyotype, high cell viability and proliferation following resuscitation from cryopreservation can be consistently achieved after long-term storage.

BENEFITS

- ✓ Chemically defined and animal product free
- ✓ FDA Drug Masterfile registered
- ✓ Manufactured in compliance with US, EU and PIC/S GMP guidelines
- ✓ Maintains pluripotency post thaw
- ✓ Enables long term cell storage at -80°C or -196°C
- ✓ No programmed freezer or liquid nitrogen required

STEM-CELLBANKER® vs Conventional Freezing

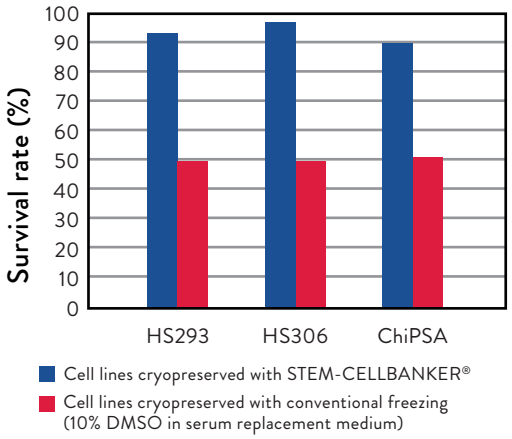


Figure 6: *Cell viability assessment: Trypan Blue & calcein-esterase based live-dead assay.*

Result: Significantly higher cell viability was observed while cell pluripotency, normal karyotype and proliferation ability were maintained in HS293, HS306 and ChiPSA cell lines.

-Study by Department of Clinical Science, Intervention and Technology, Karolinska Institute.

Description	Pack Size	Catalogue No.
STEM-CELLBANKER® - GMP	20 ml	11897
STEM-CELLBANKER® - GMP	100 ml	11890
STEM-CELLBANKER® - GMP - DMSO Free	20 ml	11897F
STEM-CELLBANKER® - GMP - DMSO Free	100 ml	11890F

HSC-BANKER®

HSC-BANKER® is an optimized GMP grade cryopreservation medium for hematopoietic stem cells. Studies show that the HSC-BANKER® is at least equivalent to conventional protocols using DMSO and DEXTRAN.

Description	Pack Size	Catalogue No.
HSC-BANKER® - GMP	15 ml	11900

CELLBANKER® is a series of easy-to-use cell freezing media offering superior protection against cell stress during freeze/thaw cycles, allowing successful cryopreservation of all mammalian cell types regardless of their sensitivity.

Fetal Bovine Serum, Pluripotent Stem Cell Qualified

It is essential to use pluripotent qualified FBS when culturing pluripotent stem cells. The large majority of FBS commercially available do not meet the performance needs for stem cells. AMSBIO supplies pluripotent cell qualified FBS extensively tested, selecting only lots that pass our stringent requirements for quality and the ability to maintain pluripotent stem cell cultures.

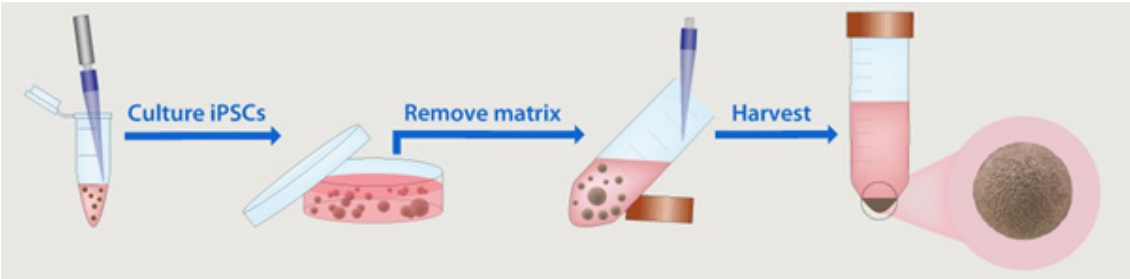
Description	Pack Size	Cat No.
Fetal Bovine Serum, US Sourced	100ml	SER-100
Fetal Bovine Serum, US Sourced	500 ml	SER-500
Fetal Bovine Serum, US Sourced-HEAT INACTIVATED	500 ml	SER-HI-500

MyEZGel™ 3D Stem Cell Culture Matrix

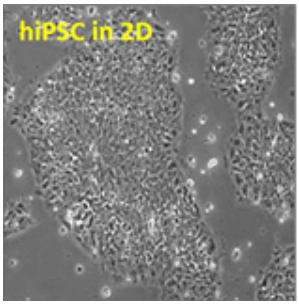
MyEZGel™ 3D-iPSC Matrix is a synthetic peptide hydrogel matrix inspired by muscle and spider silk proteins. It provides a solution to many of the basic problems associated with hydrogel-based matrices, such as complicated scaffold synthesis, temperature and pH sensitivity, low harvest/yield, and cellular toxicity from materials used. The MyEZGel™ 3D-iPSC Matrix is a powerful tool for in vitro 3D cultures of human induced pluripotent stem cells, with more accurate in vivo predictions for life science research and development. The MyEZGel™ nanofibrils are easily formulated into any cell culture medium of choice, in neutral pH and at room temperature of 37°C. The cells no longer suffer acidic or chill conditions and cultured cells can be easily harvested from the matrix.

BENEFITS

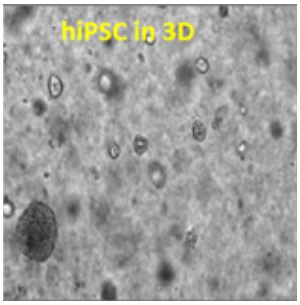
- ✓ Bridges the gap between conventional 2D cell cultures and complex native in vivo environments
- ✓ Nanofibrils are easily formulated into any cell culture medium
- ✓ Forms spheroids
- ✓ Easy cell encapsulation and harvest
- ✓ Fast hydrogel formation (~30 min)
- ✓ Does not require icing or acidic conditions, neutral pH is sufficient
- ✓ Requires room temperature/37°C culture conditions
- ✓ Low seeding density and high yield
- ✓ Maintains iPSCs in pluripotent state



COMPARISON OF HIPSCS CULTURED IN MYEZGEL™ 3D-IPSC MATRIX VS. 2D-HIPSCS CULTURED IN REGULAR MEDIA



hiPSC p10, day 3



hiPSC p10 -3Dp5, day 3

Figure 7: *Cellular morphology of 3D iPSCs. MyEZGel™ 3D cultured iPSCs forms spheroids in the hydrogel matrix.*

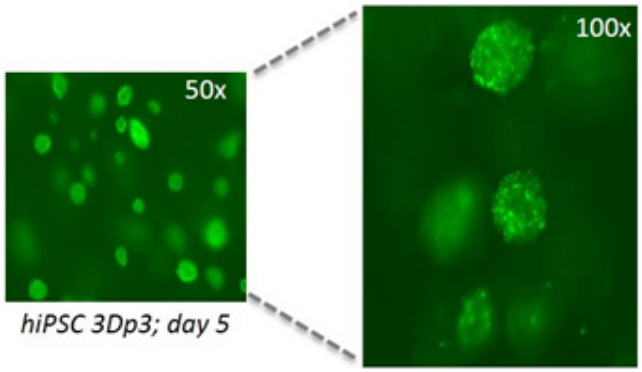


Figure 8: *In situ Immunohistochemical characterization of 3D cultured iPSCs. HiPSCs were cultured in MyEZGel™ 3D Matrix, fixed and immunostained for pluripotency marker, OCT4 on day 5 (p3) directly in the matrix, without the need for harvesting iPSCs for characterization.*

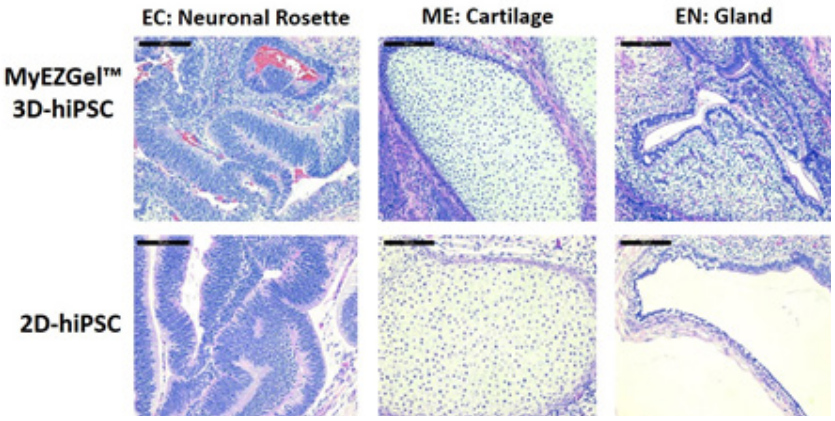


Figure 9: *Pluripotency of iPSCs (at passage #34) grown in MyEZGel™ 3D Matrix was analyzed by teratoma formation in 8 week old Fox chase SCID mice (beige; male). One million (1 x 10^6) cells were injected into two sites, kidney capsule and testis of the mice. Tumor formation was observed in both locations in all mice injected with 3D-Matrix grown iPSCs and tumors were collected 7 weeks post-injection. Histological analysis of the tumors confirmed the presence of the three germ cell layers, endoderm, mesoderm, and ectoderm, indicated by variously differentiated tissue lineages, thereby confirming the pluripotency and differentiation potential of the 3D cultured iPSCs.*

Description	Pack Size	Cat. No
MyEZGel iPSC 3D media	2 ml	ASR-3053
MyEZGel iPSC 3D media	6 ml	ASR-3054

Mimetix® 3D Cell Culture

Mimetix® scaffolds mimics the extracellular matrix by providing an ideal architectural environment to support the growth of cells in 3D. They are created by electrospinning the medical-grade polymer poly(L-lactide) (PLLA) into microfibres, which are highly consistent with regard to fibre diameter and pore size, resulting in excellent reproducibility of cell-based assays.

BENEFITS

- ✓ True 3D micro-environment
- ✓ Minimal protocol adaption required to switch from 2D to 3D
- ✓ Compatible with industry-standard automated handling and imaging equipment
- ✓ High well-to-well and batch-to-batch consistency
- ✓ Scaffolds are free from animal-derived products and synthesised using medical-grade polymers.

STEM CELLS DIFFERENTIATE INTO MATURE NEURONS IN THE MIMETIX SCAFFOLD

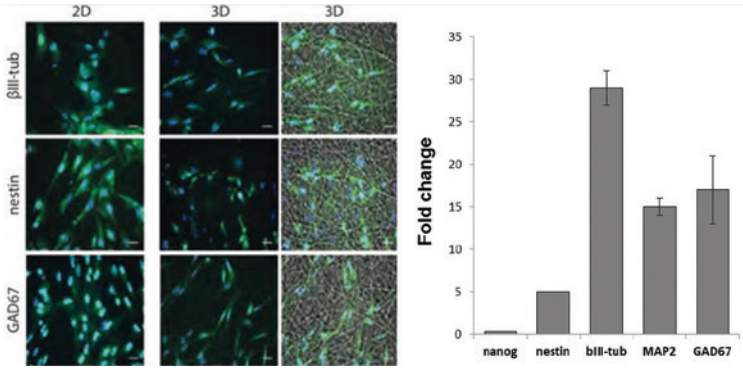


Figure 10: *HeSC cultured in the Mimetix scaffold differentiate into mature neurons. Cells were stained after 21 days, qPCR reveals an increase for post-mitotic markers GAD67 and MAP2. Scale bars represent 30μm.**

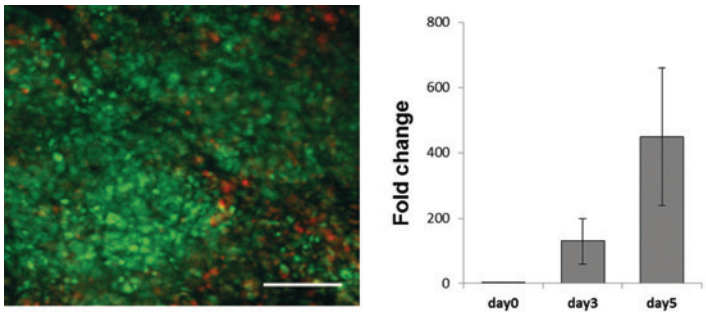


Figure 11: *hiPS cells differentiate into mature neurons in the Mimetix scaffolds. Cells were stained after 6 days with Pax6 (green) and Oct3/4 (red). RNA expression of Pax6 was analysed with qPCR.**
** (a. and b.) Experimental work performed at KTH, Sweden. The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-13) under grant agreement No. 601700.*

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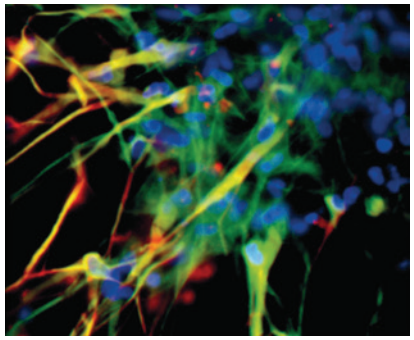


Figure 12: *Fetal cortical stem cells differentiate into mature neurons in the Mimetix scaffold. Cells were stained after 4 weeks with βIII-tub (green), DCX (red) and DAPI (blue), yellow staining is due to the co-expression of βIII-tub and DCX.**

** Image courtesy of Lara Stevanato, ReNeuron, UK.*

Description	Pack Size	Cat. No
Mimetix® 384-well plate	1 pack	AMS.TECL-001-1X
	8 packs	AMS.TECL-001-8X
	64 packs	AMS.TECL-001-64X
Mimetix® 96-well plate	1 pack	AMS.TECL-002-1X
	8 packs	AMS.TECL-002-8X
	64 packs	AMS.TECL-002-64X
Mimetix® 12-well plate	1 pack	AMS.TECL-003-1X
	8 packs	AMS.TECL-003-8X
	64 packs	AMS.TECL-003-64X
Mimetix® 6-well plate	1 pack	AMS.TECL-004-1X
	8 packs	AMS.TECL-004-8X
	64 packs	AMS.TECL-004-64X
Mimetix® 96-well plate aligned scaffold (2µm fibre diameter)	1 pack	AMS.TECL-005-1X
	8 packs	AMS.TECL-005-8X
	64 packs	AMS.TECL-005-64X
Mimetix® 12-well plate aligned scaffold (2µm fibre diameter)	1 pack	AMS.TECL-006-1X
	8 packs	AMS.TECL-006-8X
	64 packs	AMS.TECL-006-64X
Multiwell starter pack 1 : 1x12-well and 1x96-well plates containing Mimetix scaffold (PLLA, 4 micron fibre diameter, 50 micron thick)	1 pack	AMS.TECL-007-1X
Multiwell starter pack 2: 1x384-well and 1x96-well plates containing Mimetix scaffold (PLLA, 4 micron fibre diameter, 50 micron thick)	1 pack	AMS.TECL-008-1X
Multiwell insert starter pack 3: 1 x 12-well, 1 x 6-well with inserts (PLLA, Random, Fixed)	1 pack	AMS.TECL-009-1X

Alvetex®

AMSBIO offers Alvetex® a synthetic scaffold for routine 3D cell culture. Traditionally, cultured cells normally grow on treated-polystyrene 2D surfaces as in standard cell culture plastic-ware. alvetex® presents cells with the equivalent growth substrate but in a 3D format. These materials are readily adaptable to different types of existing tissue culture plastic-ware (e.g. multi-well plates, well inserts). The culture device is pre-fabricated, sterile, is ready to use off-the-shelf and can be handled in a similar manner as standard 2D plastic-ware

BENEFITS

- ✓ Consistent structure
- ✓ Based on existing cell culture material
- ✓ Stable and inert
- ✓ Adaptable to existing cell formats
- ✓ Compatible with current methods of analysis

PROPAGATION OF HUMAN PLURIPOTENT STEM CELLS IN 3D USING ALVETEX® STRATA

Human pluripotent stem cells have been shown to propagate continuously in 3D on the surface of Alvetex® Strata, a highly porous membrane in a well format. The stem cells were shown to adapt to their surroundings and acquire a more 3D shape. The cells appeared to become 'primed' for growth in 3D and showed improvements in their developmental potential when grown in a range of established 3D cell differentiation models. Alvetex® Strata was shown to offer a new approach for continuous cultivation of cells in 3D, leading to increased cell growth and function. Strata can therefore be used to routinely maintain proliferative cells with a 3D structural phenotype in preparation for their use in down-stream assays that involve 3D cell culture or cell transplantation.

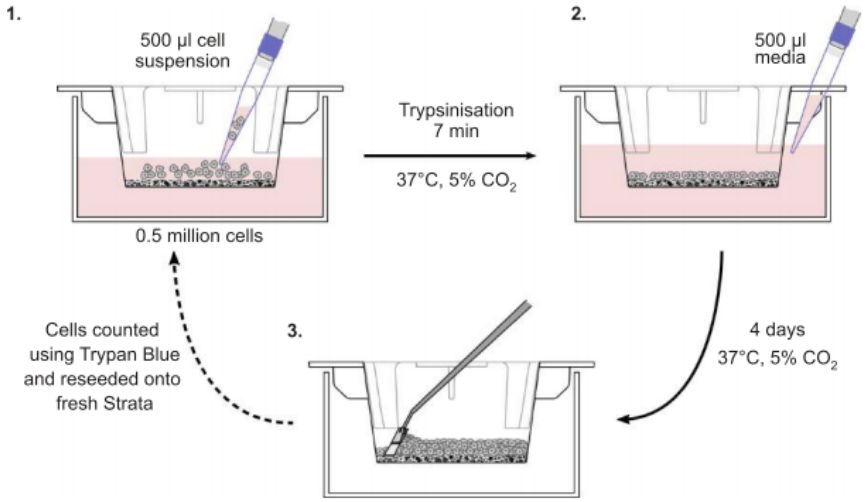


Figure 13: *Schematic illustrating the method of propagating pluripotent stem cells in 3D using Alvetex® Strata. Strata membranes are initially ethanol treated to render them hydrophilic and suspended in 6-well inserts.*

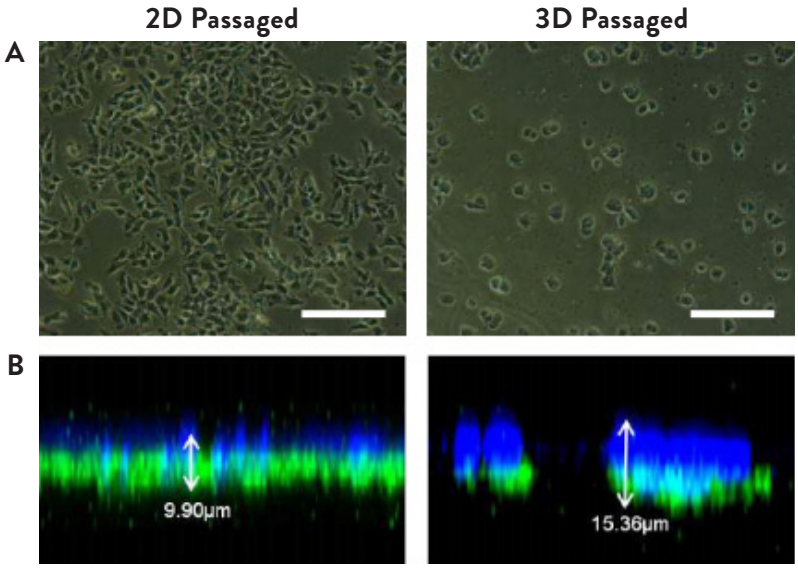


Figure 14: *Long-term propagation of pluripotent stem cells in 3D culture results in structural changes and adaptation to the growth substrate. Phase contrast (A) and confocal (B) imaging demonstrate the morphological differences between cells propagated in either 2D or 3D culture for 10 passages. A clear difference in cell morphology develops as passage number increases. Phase micrographs (A) show that 3D passaged cells appear rounder and are more likely to grow in colonies as cell numbers increase than the 2D cells which form a monolayer of more irregular shaped flattened cells. Confocal imaging (B) of cells stained with DAPI (blue), phalloidin (green) and α-tubulin (red) produced Z-stacks of the monolayer show a clear difference in height between cells propagated either in 2D or 3D. Scale bars: 100 µm.*

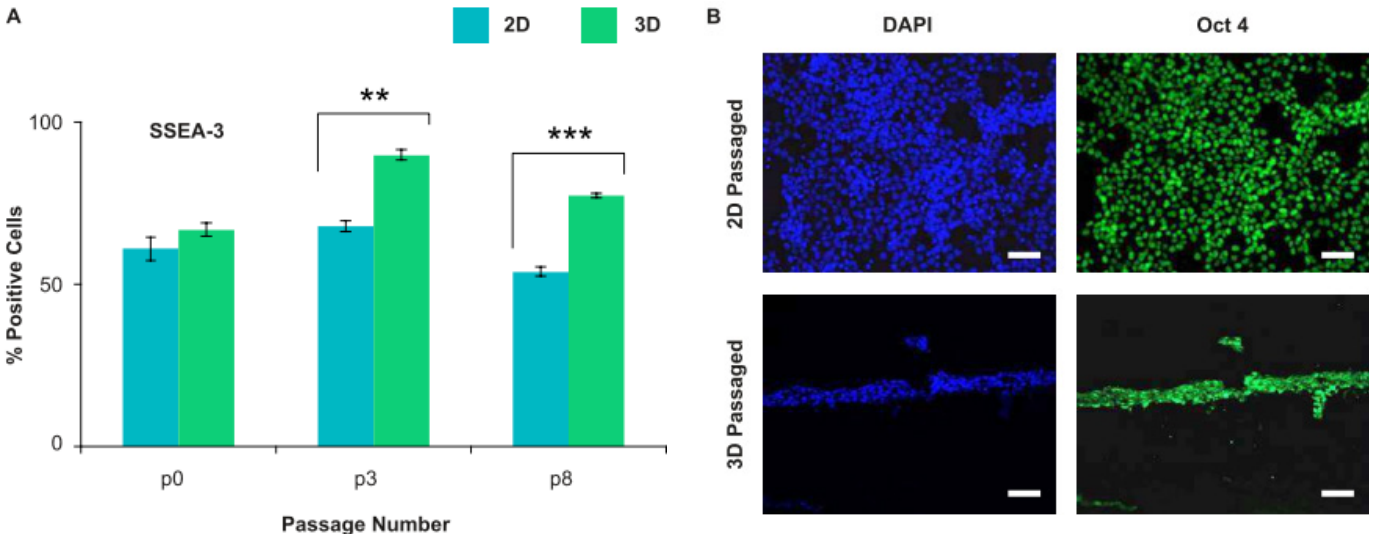


Figure 15: *Passaging pluripotent stem cells in 3D results in the enhanced expression of stem cell markers. A: TERA2.cl.SP12 cells propagated and maintained in 2D or 3D culture were analysed by flow cytometry for the expression of the stem cell marker SSEA-3 at passages P0, P3 and P8. Cells expressed higher levels of SSEA-3 when maintained in 3D culture compared to conventional 2D culture. Data represent mean, ± SEM, n=3, **p=0.01, ***p=0.001. B: Immunocytochemical staining showed that TERA2.cl.SP12 cells expressed high levels of the stem cell marker Oct 4 when passaged and maintained in either 2D or 3D culture. Scale bars: 100 µm.*

WELL PLATE

Description	Pack Size		
	1x	2x	10x
alvetex® 384-well plate for 3D Culture		AMS.AVP010-2	AMS.AVP010-10
alvetex® 96-well plate for 3D Culture	AMS.AVP009		AMS.AVP009-10
alvetex® 24-well plate for 3D Culture	AMS.AVP006		AMS.AVP006-10
alvetex® 12-well plate for 3D Culture	AMS.AVP002		AMS.AVP002-10

WELL INSERT

Description	Pack Size		
	6x	12x	48x
alvetex® 384-well plate for 3D Culture		AMS.AVP010-2	AMS.AVP010-10
alvetex® 96-well plate for 3D Culture	AMS.AVP009		AMS.AVP009-10
alvetex® 24-well plate for 3D Culture	AMS.AVP006		AMS.AVP006-10
alvetex® 12-well plate for 3D Culture	AMS.AVP002		AMS.AVP002-10

PERFUSION PLATE

Description	Pack Size	Catalogue No.
Perfusion plate	10x plates, luer locks	AMS.AVP011-10
Perfusion plate	2x plates, luer locks	AMS.AVP011-2
Perfusion plate with Alvetex 6-well inserts	2x plates, luer locks, 12x 6-well inserts	AMS.AVP-KIT-3
Perfusion plate with Alvetex 12-well inserts	2x plates, luer locks, 12x 12-well inserts	AMS.AVP-KIT-4
Perfusion plate with Alvetex 6-well inserts	5x plates, luer locks, 48x 6-well inserts	AMS.AVP-KIT-5
Perfusion plate with Alvetex 12-well inserts	5x plates, luer locks, 48x 12-well inserts	AMS.AVP-KIT-6

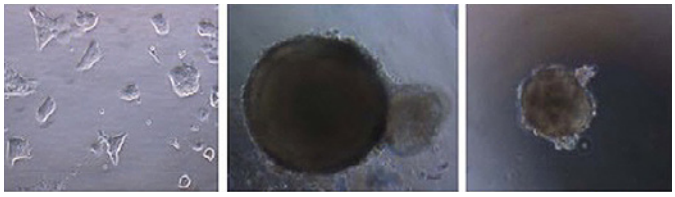
ANIMAL FREE GROWTH FACTORS

Defined, serum-free stem cell media as required by today's researchers is only made possible by the addition of the correct growth factors and cytokines to maintain the healthy growth of the cells. Furthermore, clinical translation of stem cell research requires higher quality reagents. Expansion and differentiation of stem cells is highly reliant on growth factor and cytokine supplements, with the activity and purity of these recombinants being critical to successful research and clinical applications. Despite recognized limitations, *E. coli* and mammalian cells remain the dominant expression systems for commercial recombinant protein production. Although significant improvements in manufacture have been made, both systems present a risk of unwanted contamination from pathogens and endotoxins.

AMSBIO now offers a new range of recombinant growth factors and cytokines produced in a plant-based expression system that utilizes barley seed to cut the risks associated with endotoxin, viral infection and other human pathogen infection. These low cost, animal-free and endotoxin-free growth factors and cytokines are ideal for stem cell research.

BENEFITS

- ✓ No contamination from serums, antibiotics or infectious agents
- ✓ No unwanted growth factor and cytokine carryover
- ✓ FDA G.R.A.S Status (Generally Recognized As Safe)
- ✓ Eliminates harmful cellular responses due to LPS contamination
- ✓ Low cost and high reproducibility



ES cells with LIF | Embryoid bodies | ES Cells without LIF

Figure 16: *Mouse ES cells either on feeders in complete medium/ without feeders in a conditioned medium. In both conditions mLIF was added using concentrations down to 0.1 ng/mL. All concentration conditions resulted colonies of pluripotent mouse ES cells. When the ES cells were deprived of mLIF cells spontaneously differentiated and formed embryoid bodies in hanging drops.*

Description	Pack Size*	Catalogue No.
Activin A	10µg	AMS-150-10
Activin B	10µg	AMS-260-10
IFN gamma	10µg	AMS-406-10
TNF alpha	10µg	AMS-524-10
hLIF	10µg	AMS-155-10
mLIF	10µg	AMS-263-10
G-CSF	10µg	AMS-273-10
M-CSF	10µg	AMS-375-10
GM-CSF	10µg	AMS-395-10
GDNF	10µg	AMS-162-10
hSCF	10µg	AMS-284-10

Description	Pack Size*	Cat No.
mSCF	10µg	AMS-931-10
FGF basic	10µg	AMS-480-10
VEGF164	10µg	AMS-792-10
VEGF165	10µg	AMS-295-10
BMP2	10µg	AMS-370-10
KGF	10µg	AMS-942-10
IL-2	10µg	AMS-517-10
IL-3	10µg	AMS-628-10
IL-4	10µg	AMS-729-10
IL-6	10µg	AMS-831-10
Flt-3 ligand	10µg	AMS-590-10
hEGF	10µg	AMS-060-10

All products available in 10µg, 50µg, 100µg and 1,000µg

All products are ANIMAL FREE RECOMBINANTS!

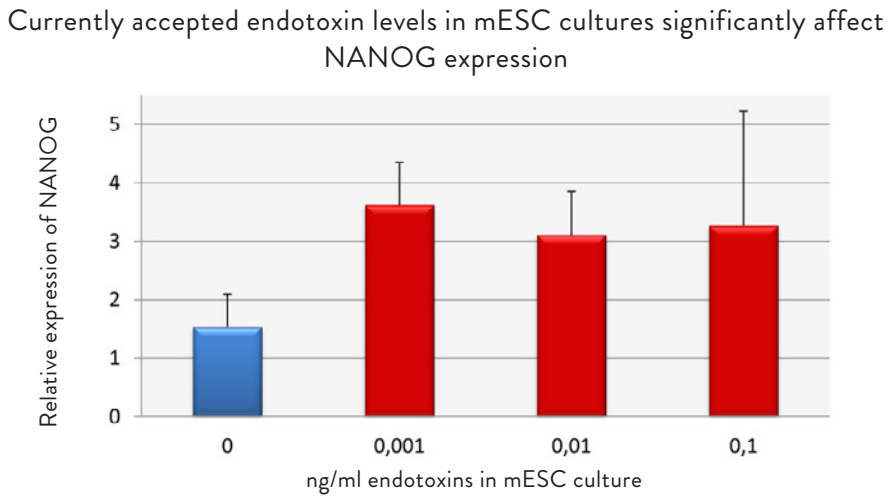


Figure 17: *Independent data has demonstrated that 0,001 ng/ml in a stem cell culture, induces NANOG expression (assuming a typical mLIF concentration of 10 ng/ml being used in the culture). According to these results, endotoxin levels in commercial growth factors, currently regarded as acceptable, may significantly affect stem cell status and compromise stem cell maintenance and differentiation.*

If you cannot find what you are looking for, please check online at: www.amsbio.com for our full product listing or contact us at: info@amsbio.com

EXOSOMES FROM STEM CELLS

AMSBIO human pre-adipocytes (adipose derived adult stem cells) and placental mesenchymal stem cells (MSC) are characterized by their self-renewing capacity and ability to differentiate into chondrocytes, adipocytes, and osteocytes. This makes them attractive starting materials for tissue engineering and regenerative medicine applications.

Since few transplanted cells persist in vivo, the beneficial effects of cell therapy may lie in the secreted factors being the active component of this treatment. A key part of paracrine secretion is exosomes. These membrane vesicles are stored intracellularly in endosomal compartments and are secreted when these structures fuse with the cell plasma membrane.

- ✓ Exosomes contain protein, DNA, and RNA, thus making them an attractive vector of paracrine signals delivered by stem cells.
- ✓ Exosomes may also be "loaded" with predetermined proteins and nucleic acid to achieve a desired effect (1).
- ✓ Exosomes can be stored as an "off-the-shelf" product having the potential for circumventing many of the limitations of viable cells for therapeutic applications in regenerative medicine.
- ✓ In vitro, exosomes from pre-adipocytes stimulate cell proliferation in a wound healing model.
- ✓ In vivo, adipose-graft derived exosomes have been shown to be a promising tool for skin repair and remodeling
- ✓ All cells have been screened negative for HIV-1, HIV-2, HTLV-1, HTLV-2, Hep-B, Hep-C.
- ✓ Quality Control: Particle mean diameter, Protein concentration, RNA concentration and Concentration of particles/ml.

Control ***Exosome Treated***

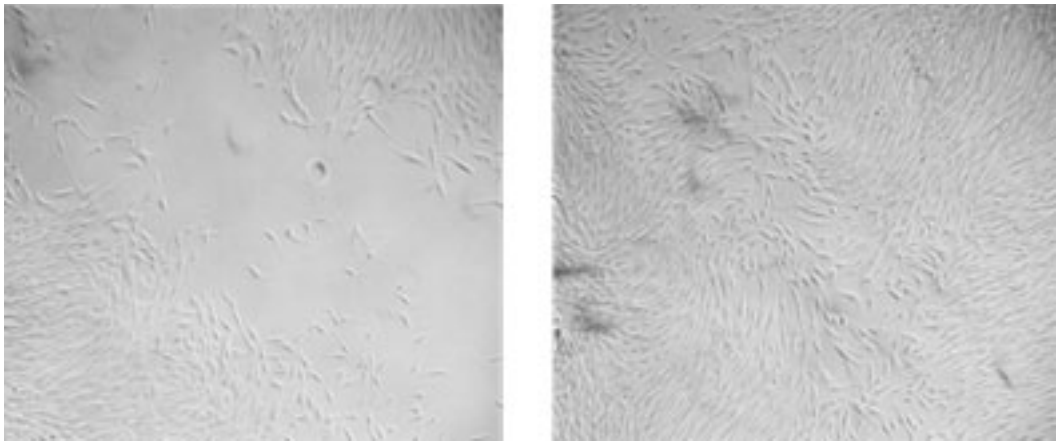


Figure 18: *After the cell monolayer was 'wounded' using a cell scraper, the cultures were washed and fed with DMEM + 10% exosome-free FBS (Control), or DMEM + 10% exosome-free FBS supplemented with 25µg of pre-adipocyte exosomes (Exosome Treated). After a 5 day incubation, fibroblast proliferation closed the gap between the two edges of the wounded monolayer only in the medium supplemented with exosomes. These data support the notion that factors including exosomes secreted by pre-adipocytes (adipose tissue-derived MSC) play a role in wound healing and tissue regeneration, by facilitating cellular proliferation*

Description	Pack Size	Cat. no
Human Preadipocyte (Mesenchymal Stem Cell) Exosome	100ug	EXP-F100
Human Placental Derived Mesenchymal Stem Cell Exosomes, Frozen	100ug	EXPLMSC-F100
Human Cord Blood Serum Exosomes, Frozen	100ug	EXCBS-F100

Adult Stem Cells

Adult stem cells are undifferentiated cells found throughout mature tissues or organs in the body. Also commonly referred to as somatic stem cells, the role of adult stem cells is to replenish cells that have died to maintain homeostasis and to regenerate damaged tissues. Adult stem cells are central to cellular therapies and regenerative medicine. The key advantages of adult stem cells as research tools are their ability to undergo cell division or self-renew and to differentiate into specialized cell types. Unlike embryonic or induced pluripotent stem cells (iPSC), adult stem cells are limited in their ability to differentiate into mature cell types. To take full advantage of adult stem cells as research tools it is vital to support the undifferentiated growth and expansion within an *in vitro* system. AMSBIO offers a wide range of adult stem cell optimized media that maintains the undifferentiated growth of multipotent stem cells. The range includes media for the culture of mesenchymal stem cells (MSCs) isolated from bone marrow and adipose tissue and neural stem cells (NSCs) isolated from brain tissue.

HEMATOPOIETIC STEM CELLS

Human CD34+ Hematopoietic stem cells are isolated from fresh umbilical cord blood Cord blood is obtained from US clinics and processed within 24 hours.

Description	Pack Size	Catalogue No.
Human CD34+ Stem Cells	0.2 M cells/vial	CBU-001-0219

MESENCHYMAL STEM CELLS

Mouse Bone Marrow Derived Mesenchymal Stem Cells

Rat and mouse mesenchymal stem cells are isolated from adult or fetal bone marrow and ideal for investigating pathways of pluripotent cell differentiation into bone, cartilage, fat and neuronal cells. Select from rat and mouse mesenchymal stem cells, all supplied with low passage cells and guaranteed growth.

Description	Pack Size	Catalogue No.
Cryopreserved, MSC from C57BL6	5 M cells/vial	Z7030061
Mouse MSC Growth Medium	500ml	Z7030063

Human Bone Marrow Derived Stromal Stem Cells

High quality fetal human marrow stromal Stem Cells provide a convenient *in vitro* model for investigating pathways of multipotent cell differentiation into bone, cartilage and adipose cells. Human marrow stromal stem cells are isolated from normal fetal human bone marrow. They are cryopreserved at second passage and can be cultured and propagated for 10 population doublings.

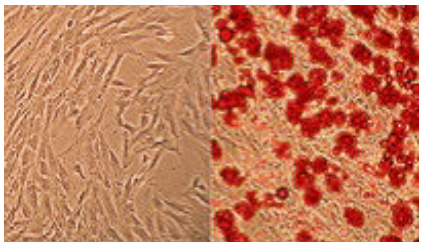


Figure 19: *Human Marrow Stromal cells*

Description	Pack Size	Cat No.
Human Marrow Stromal Cells (HMSC) Cryopreserved	0.5 M cells/vial	SH49205
Human Marrow Stromal Cells (HMSC) Complete System	1 Kit	SH49205K
Human Marrow Stromal Cell Medium	500ml	SMH419500
Human Marrow Stromal Stem Cell Adipocyte Differentiation Medium	250ml	SMH811D250
Human Marrow Stromal Stem Cell Osteoblast Differentiation Medium	250ml	SMH417D250

Adipose Derived Stem Cells

AMSBIO supplies human adult stem cells are isolated from subcutaneous adipose tissue from healthy individuals. Cells are capable of differentiating into adipocytes, osteoblasts and chondrocytes. The human adult stem cell preparations are positive for surface markers of mesenchymal stem cells, CD29, CD44 and CD105, while being negative for endothelial and macrophage markers, CD14, CD31, CD34, CD45 and CD133. Pre-adipocyte stem cell medium is used to plate adult stem cells prior to differentiation and contains growth factors necessary for expansion of subcutaneous pre-adipocyte derived adult stem cells.

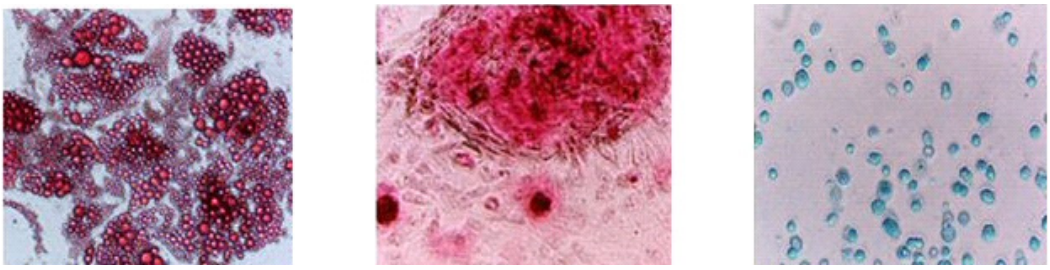


Figure 20: *Adult stem cells differentiated into adipocytes. Cells shown are two weeks old and have been stained with the neutral lipid dye Oil Red O. (Middle) Adult stem cells differentiated into osteoblasts. Mineralized cells stained with Alizarin Red. (Right) Adult stem cells differentiated into chondrocytes. Cells stained with Alcian Blue.*

Description	Pack Size	Catalogue No.
Human Adult Stem Cells Adipose Derived	1 M cells/vial	ASC-F
Cryopreserved Adult Stem Cells Adipose Derived, Multiple Donors	1 M cells/vial	ASC-F-SL

MSC DIFFERENTIATION MEDIA

Adult stem cells derived from adipose tissue are multipotent and can be induced differentiate into osteoblasts, chondrocytes or adipocytes upon exposure to specific differentiation media formulations. Differentiation media supporting differentiation of human adipose derived stem cells are available for all three differentiation fates.

Description	Pack Size	Catalogue No.
Adult Adipose Derived Stem Cell Adipocyte Differentiation Medium	100ml	DM-2
Adult Adipose Derived Stem Cell Adipocyte Differentiation Medium	500ml	DM-2-500
Adult Adipose Derived Stem Cell Osteoblast Differentiation Medium	500ml	OB-1
Adult Adipose Derived Stem Cell Chondrocyte Differentiation Medium	100ml	CM-1-100
Adult Adipose Derived Stem Cell Chondrocyte Differentiation Medium	500ml	CM-1
Mesenchymal Stem Cell Adipogenic Differentiation Kit	1 Kit	5010-024-K
Mesenchymal Stem Cell Osteogenic Differentiation Kit	1 Kit	5011-024

Primary Cells

Primary cells are isolated directly from tissue with a minimal amount of processing and manipulation. Unlike cultured cells or established cell lines, primary cells are widely believed to be the most relevant biological *in vitro* tool because they retain as close as possible the physiological function of the cells within a tissue. In addition, cultured cells or established cell lines may acquire genetic abnormalities. However due to the fact that primary cells must be obtained from different donors and are limited, this may introduce a degree of variability in experimental outcomes when using primary cells. To maximize lot-to-lot consistency, all primary cells supplied by AMSBIO undergo rigorous testing that guarantees physiological function and performance when used in combination with AMSBIO's optimized mediums, reagents and protocols. Furthermore, all primary cells are supplied with a Certificate of Analysis (CoA) and undergo testing for sterility and test negative for HIV-1, Hepatitis B and C and mycoplasma.

PRIMARY RAT NEURONAL CELLS

NeuroPure™ - Live Ready-To-Use Neurons

NeuroPure™ Primary Rat Neurons are live neuronal tissues isolated from micro-surgically dissected regions of Sprague/Dawley rat. These neurons are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 14 days under refrigeration.

NeuroPure™ Primary Rat Neuronal Cells are live neuronal tissues isolated from micro-surgically dissected regions of Sprague/Dawley rat. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 14 days under refrigeration. NeuroPure™ cells are ideal for a wide variety of applications including: transfection, pharmacology studies, immunocytochemistry, and neuron development studies.

BENEFITS

- ✓ Freshly isolated healthy neurons - not frozen
- ✓ Ready to use - get your primary culture up and running within 1 hour
- ✓ Pure neuronal cells - 99.9% glial cell free
- ✓ Guaranteed quality and consistency

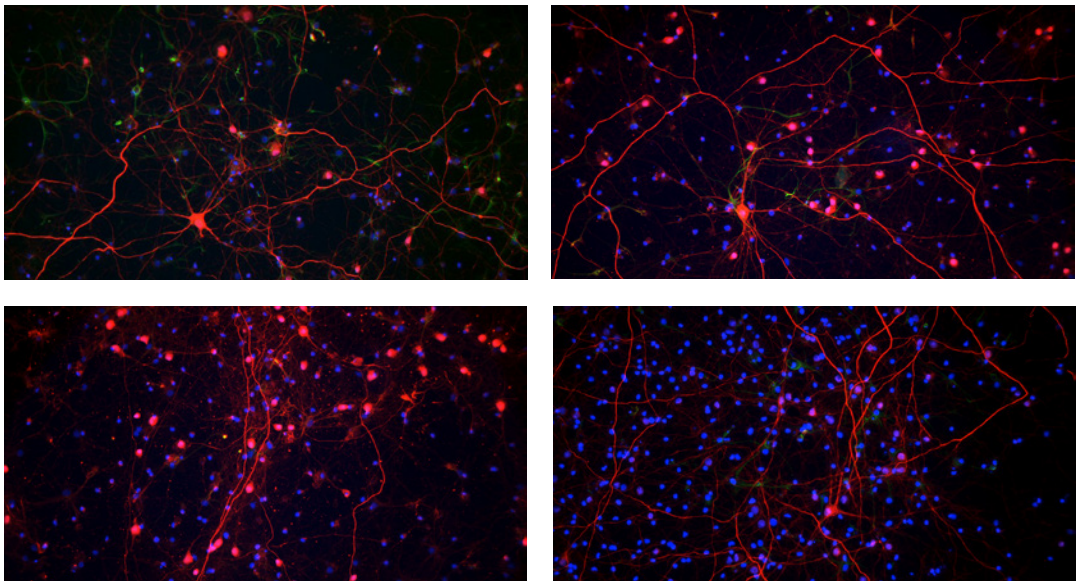


***Figure 21:**: (A & B) Rat Hippocampal cells (C & D) NeuroPure™ Rat Cortical Cells. Triple stained NeuroPure™ neurons after 21 days in culture. Neurofilaments-red were immunostained with rabbit anti-neurofilament antibody and goat anti-rabbit AlexaFluor 568. Glial cells-green were immunostained with a mouse anti-GFAP antibody AlexaFluor 488. DNA-blue was stained with Hoechst 33258 (bis-benzamide).*

Description	Pack Size	Catalogue No.
E18 Hippocampal Neurons	1 M cells/vial	N100200
E18 Cortical Neurons	1 M cells/vial	N200200
P8 Cerebellar Neurons	4 Pairs	N300200
E18 Hypothalamus Neurons	1 Pair	N400200
E18 Striatal Neurons	1 Pair	N500200
E18 Spinal Cord Neurons	1 Cord	N600200
E18 Midbrain Cells	1 Midbrain	N700200

NORMAL CELL TYPES

AMSBIO stocks an extensive range of primary cells isolated from human tissue and various mammalian species. Primary cells are supplied cryopreserved or plated in a ready-to-use format that offers a high level of quality control and cell viability. The majority of the primary cells are isolated from normal tissue; however, AMSBIO uniquely provides diseased primary cell types to the research community. A small selection of diseased primary cell types are stocked but any diseased cell type can be custom sourced. Please contact us with your custom request.

NEURAL STEM CELL CULTURE REAGENTS

Synthetic Retinoid ec23®

Controlling cell differentiation in a predictable way is a major challenge in stem cell research. Although natural retinoids can be used to trigger neuronal stem cell differentiation, they are inherently unstable, leading to partially differentiated cultures and highly variable result. ec23® synthetic retinoid derivative represents a new chemically and light stable alternative to ATRA that does not degrade. ec23® enables the robust and reproducible differentiation of stem cells and progenitor cells. With higher stability in tissue culture medium and more potency as differentiation factor than retinoic acid, it produces down regulation of markers associated with the pluripotent stem cell phenotype and increased expression of differentiation markers.

BENEFITS

- ✓ Mimics the activity of natural retinoid
- ✓ Immune to the disruptive influence of temperature and light
- ✓ Induces neurogenesis with enhanced potency
- ✓ Maximizes the consistency, reliability of neural stem cell differentiation
- ✓ Minimizes culture heterogeneity

Results show that ec23® is a more potent inducer of neurogenesis than ATRA. In direct comparison to ATRA, we found that synthetic retinoid ec23® induces larger numbers of neural cells with less variability and with the added advantage of compound stability.

Description	Pack Size	Catalogue No.
Synthetic Retinoid ec23®	2x 5mg (powder)	AMS.SRP002-2

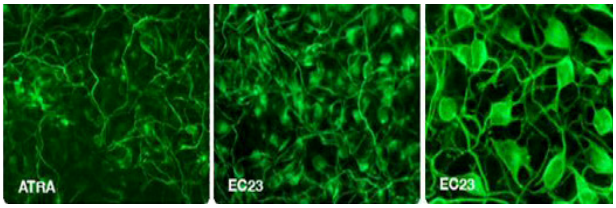


Figure 22: *Human TERA2.cl.SP12 pluripotent stem cells exposed to either 10uM ec23® or ATRA form populations of terminally differentiated neurons. Immunocytochemical staining for neurofilament-200 was performed on differentiated cultures and the number of positive cells quantified.*

Isolation & Purification

CELLOTION®

CELLOTION® wash solution provides superior performance prior to cryopreservation and post thawing. CELLOTION® is safe, chemically defined and contains no serum, protein or animal derived components. It offers excellent performance with increased recovery rates of cells over conventional washing buffers following centrifugation.

BENEFITS

- ✓ Significantly increase cell yields after washing - maintaining high cell viability
- ✓ Chemically defined solution, free of animal derived components
- ✓ Easy to use protocol

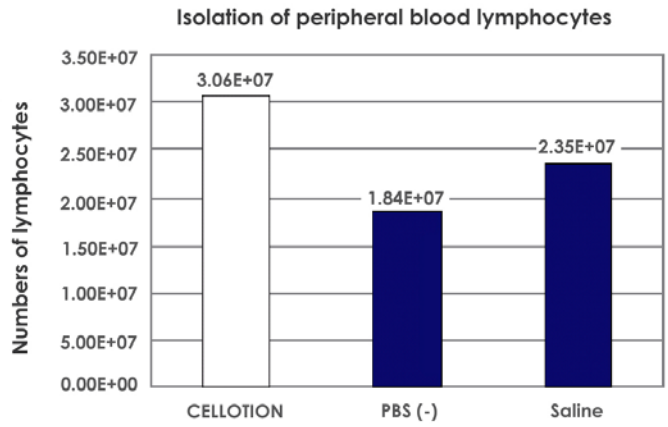


Figure 23: *Comparative analysis of CELLOTION® cell wash and recovery solution vs in-house cell wash methods.*

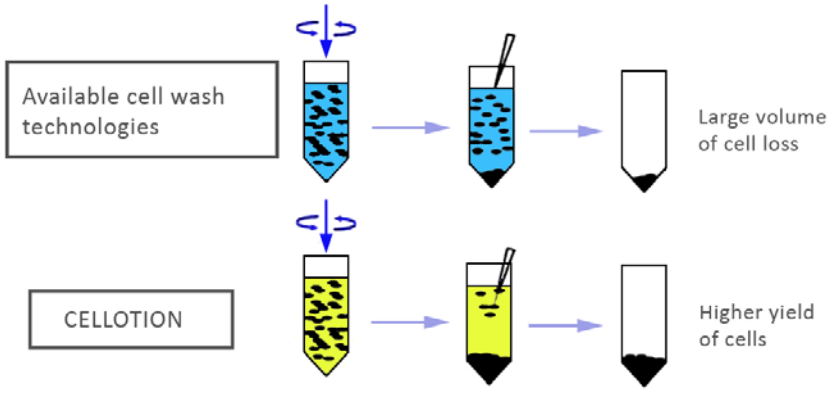


Figure 24: *CELLOTION® protects against unnecessary cell loss during general wash procedures.*

Description	Pack Size	Cat No.
CELLOTION®	100ml	11898

FACSMAX™ - EFFECTIVE SINGLE CELL DISSOCIATION

FACSMax™ proprietary formulation of proteolytic, collagenolytic and DNAse enzymes is highly effective in creating single cell suspensions from clumped cell cultures for accurate cell counting, flow cytometry, viral transfection assays, cell sorting, and bioreactor scale-up.

BENEFITS

- ✓ Dissociates clumped cells in minutes
- ✓ Results in homogeneous single cell suspension
- ✓ Gentle cell disaggregation for maximum cell viability
- ✓ Yields accurate, reproducible cell counts
- ✓ Saves time - No need for extra PBS washing steps
- ✓ Ready to use solution

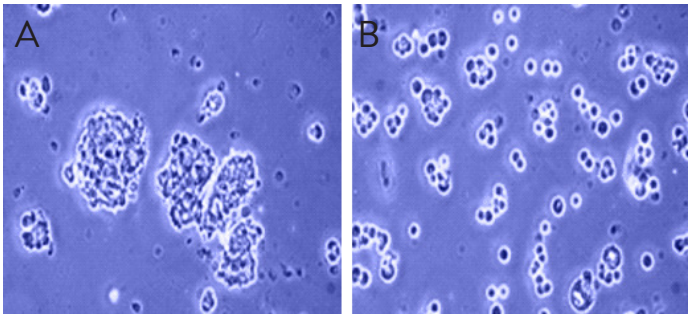


Figure 25: *MCF-7 cells were harvested at 90% confluency from a T150 culture flask using a cell scraper, centrifuged and resuspended in 10 mls of complete medium. A. MCF-7 cells are adherent and grow in clumps. They are very difficult to dissociate. B. After treatment with FACSMax™ a single cell suspension makes for easy and accurate cell counting.*

Description	Pack Size	Cat No.
FACSMax™ Cell Dissociation Solution	100ml	T200100
FACSMax™ Cell Dissociation Solution	10x 100ml	T200110

DETACHIN™ - THE SUPERIOR ALTERNATIVE TO TRYPSIN

Detachin™ Cell Detachment Solution is a superior alternative to Trypsin/EDTA for gently detaching adherent cells from *in vitro* growth vessels. Detachin™ provides quick, gentle, and effective detachment of a wide variety of adherent cells, including primary cells, from all known tissue culture plastic ware.

BENEFITS

- ✓ Maximum cell viability over Trypsin
- ✓ Effective on a wide range of cells
- ✓ No mammalian or bacterial by products
- ✓ No need to wash detached cells
- ✓ Convenient format that reduces contamination and footprint
- ✓ Preserve unused Detachin™ for longer term storage

Based on a propriatory formulation of protease, and collagenase activities in an isotonic, phosphate buffer solution with EDTA.

Detachin™ provides consistent, safe, and efficient results. It has been tested successfully on a wide variety of different cells and cell types like bone marrow stem, fibroblasts, hepatocytes, mouse germ, keratinocytes, A-375, BHK, CHO, COS , D54, HEK 293, HeLa, and many others

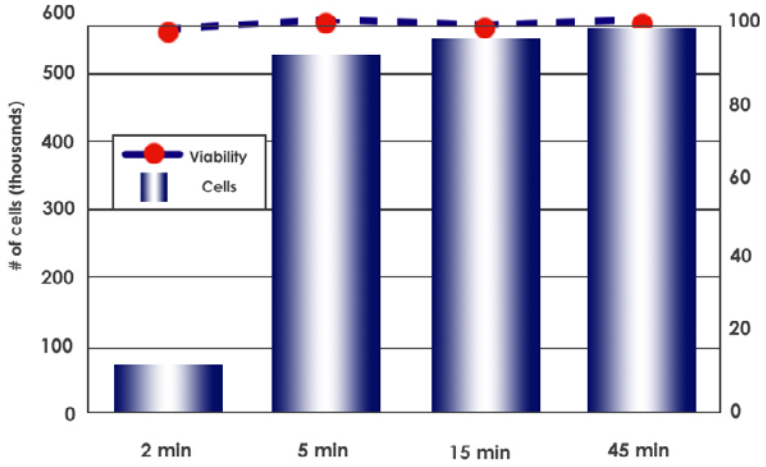


Figure 26: *Cell detachment and viability with increasing incubation time*

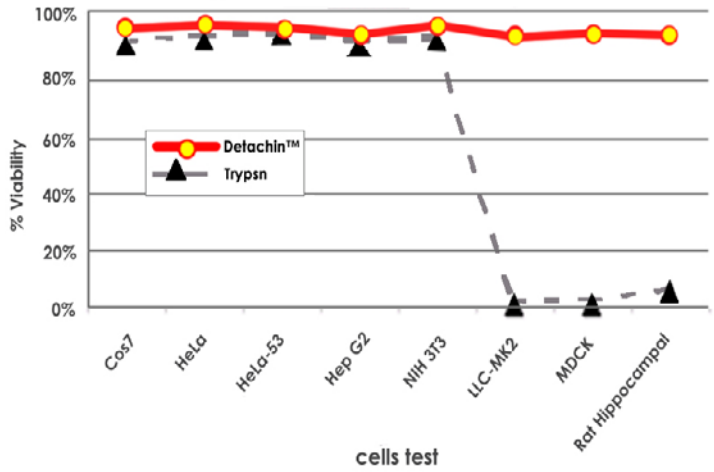


Figure 27: *Viability of cells after full detachment*

Description	Pack Size	Catalogue No.
Detachin™ Cell Detachment Solution	100ml	T100100
Detachin™ Cell Detachment Solution	6x 50ml	T100106
Detachin™ Cell Detachment Solution	10x 100ml	T100110

TISSUE DISSOCIATION

Collagenases from *Clostridium histolyticum*, are proteolytic enzymes that are able to cleave peptide bonds in the triple helical collagen molecule of human or animal tissue *in situ*. The collagenases are divided into class I and class II collagenase isoforms on the basis of their activities towards synthetic peptides. Both collagenase isoforms act on triple helical type I, II, III and IV collagens, but in slightly different modes of action.

Our Collagenase NB qualities are particularly suitable for various tissue isolation applications. The application of pharmaceutical manufacturing standards guarantees stringent quality control, high quality, reliable lot-to-lot consistency and excellent performance of the product.

Collagenase NB 6 GMP Grade is particularly suited for the clinical preparation of stem cells in regenerative medicine applications. This enzyme contains collagenase classes I and II as well as proteolytic activities such as neutral protease and clostripain and is suitable for stem cell passaging (e.g. ES cells). The pharmaceutical manufacturing standards used to produce Collagenase NB 6 GMP Grade guarantees stringent quality control, high quality, reliable lot-to-lot consistency and excellent performance.

BENEFITS

- ✓ High cell yields and viability
- ✓ Reliable lot-to-lot consistency
- ✓ TSE safe manufacturing
- ✓ Low endotoxin
- ✓ Animal-Free GMP Grade Collagenase

AMSBIO now offers the world's first highly purified collagenase free of any animal-based components. Using raw materials of animal origin often causes concerns regarding the introduction of potential animal-derived pathogens. Collagenase AF-1 GMP Grade is derived from Clostridium histolyticum grown in a medium containing carefully selected plant-based ingredients.

For research applications, non-GMP grade Collagenase NB4 Standard Grade and Collagenase NB 5 Sterile Grade are economical alternatives with comparable enzymatic properties to Collagenase NB 6 GMP Grade. Collagenase NB4 is also an ideal for the passage of stem cells (see Passaging Stem Cells - Page 38).

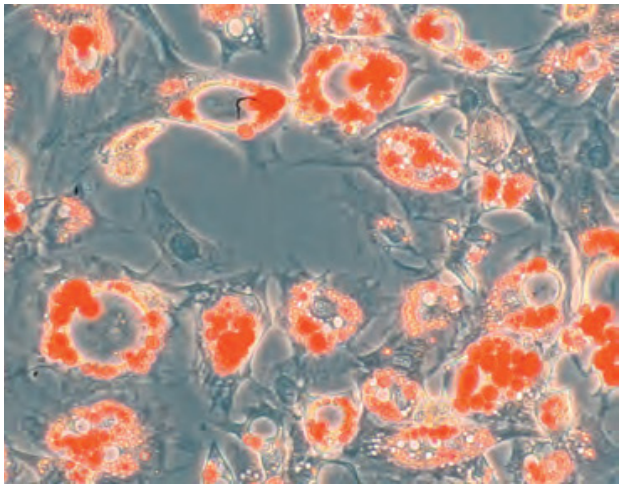


Figure 28: *Human adipocytes, by courtesy of H. Sell, Deutsches Diabetes-Zentrum, Germany*

Description	Pack Size	Catalogue No.
Collagenase NB4 Standard Grade	1g	17454.01
Collagenase NB4 Standard Grade	500mg	17454.02
Collagenase NB5 Sterile Grade	1g	17459.03
Collagenase NB6 GMP Grade	1g	17458.03
Collagenase Animal Free GMP Grade	≥ 2000 PZ U/Vial	17457.01
Neutral Protease Animal Free GMP Grade	≥ 100 DMC U/Vial	30306.01

MAGNETIC BEAD CELL ISOLATION

AMSBIO offers a range of superior magnetic products for use in protein purification, proteomics, and genomics applications. High quality coating of magnetic silica beads with Streptavidin, Protein A, Protein G or other ligand specific molecules allows isolation of specific target molecules or cells out of a large volume or complex matrix.

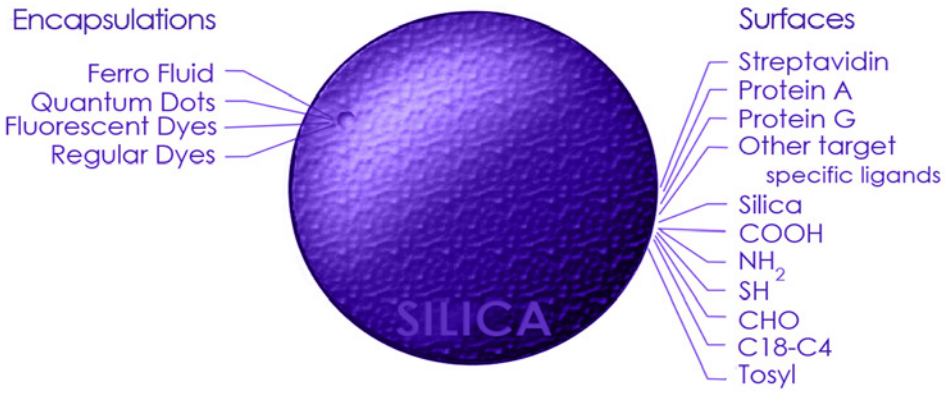


Figure 29: *Encapsulation and surface possibilities for MagSi*

BENEFITS

- ✓ High binding capacity
- ✓ Minimal non-specific absorption
- ✓ nm & µm sized magnetic beads
- ✓ High customization potential

Description	Pack Size	Cat No.
MagSi-STA Streptavidin	10mg/ml, 600nm	MD16001
MagSi-STA Streptavidin	10mg/ml, 1µm	MD01001
MagSi-protein A	10mg/ml, 600nm	MD10011
MagSi-protein A	10mg/ml, 1µm	MD01011
MagSi-protein G	10mg/ml, 600nm	MD10012
MagSi-protein G	10mg/ml, 1µm	MD01012

Bead	Application	Surface	Encapsulation Possibilities
MagSi Tools	Customer development Tailored products	Silica COOH NH_2 SH CHO	Ferro fluid (30-50%) Quantum dots Fluorescent dye Regular Dye
MagSi-STA	Isolate biotinylated target molecules Immuno assays Bind biotinylated Ab Cell/bacteria isolation Protein purification	Streptavidin covalently bound to silica	Ferro fluid (30-50%) Quantum dots Fluorescent dye Regular Dye
MagSi-pA MagSi-pG	Bind IgG antibodies Antibody purification Immuno precipitation from small sample volumes	Protein A or G covalently bound to silica	Ferro fluid (30-50%) Quantum dots Fluorescent dye Regular Dye

Stem Cell Characterization

STEM CELL ANTIBODIES

Antibodies are powerful and essential tools in stem cell research. Due to the inherent labile nature of stem cells *in vitro* culture, stem cells must always be monitored for their differentiation status. Antibodies provide definitive markers of antigens expressed by undifferentiated stem cells and are used to characterize the differentiated progeny.

AMSBIO is your one stop center for a vast selection of polyclonal and monoclonal antibodies. We provide over >35,000 antibodies and custom polyclonal and monoclonal development services.

AMSBIO also offer over 12000 VERIFY™ tagged antigens, gene-specific over-expression lysates as positive controls. A small selection of AMSBIO's stem cell antibody portfolio is shown below for pluripotent, mesenchymal, adipose, neural and cancer stem cells.

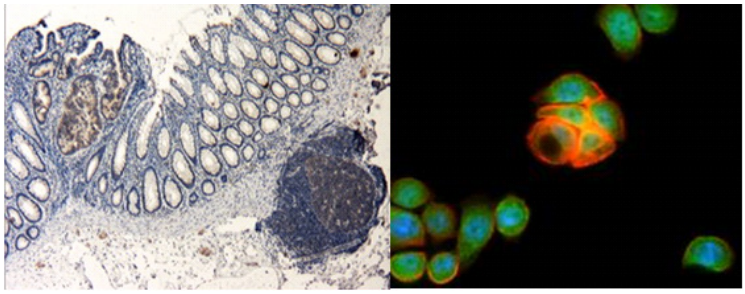


Figure 30: *(Left) Immunohistochemical staining of paraffin-embedded Human colon tissue using anti-LGR5 mouse monoclonal antibody. (Heat-induced epitope retrieval by Tris-EDTA, pH8.0, Cat No. TA503316). (Right) Immunofluorescent staining of HT29 cells using anti-LGR5 mouse monoclonal antibody (green) (Cat No. TA503316). Actin filaments were labeled with TRITC-phalloidin (red), and nuclear with DAPI (blue).*

Description	Pack Size	Catalogue No.
Rabbit Monoclonal Antibody against Oct-4 (clone EPR2054)	100 µl	TA307418
Purified Rabbit Polyclonal Antibody against NANOG (Center)	100 µg	TA302154
Rabbit anti-Human CD105 Polyclonal Antibody	100 µg	500-4234
Rabbit Monoclonal Antibody against ALCAM/CD166 (clone EPR2759(2)	100 µl	TA307534
Goat Anti-AREB6 / ZEB1 Antibody	100 µg	TA305688
Rabbit Polyclonal Slug Antibody	100 µg	TA306360
Rabbit Monoclonal Antibody against p53 (C-Term) (E47)	100 µl	TA303724
Purified LGR5 Mouse Monoclonal Antibody, Clone 2A2	100 µl	TA503316
Rabbit Monoclonal Antibody against LGR5 (Clone EPR3065Y)	100 µl	TA301323

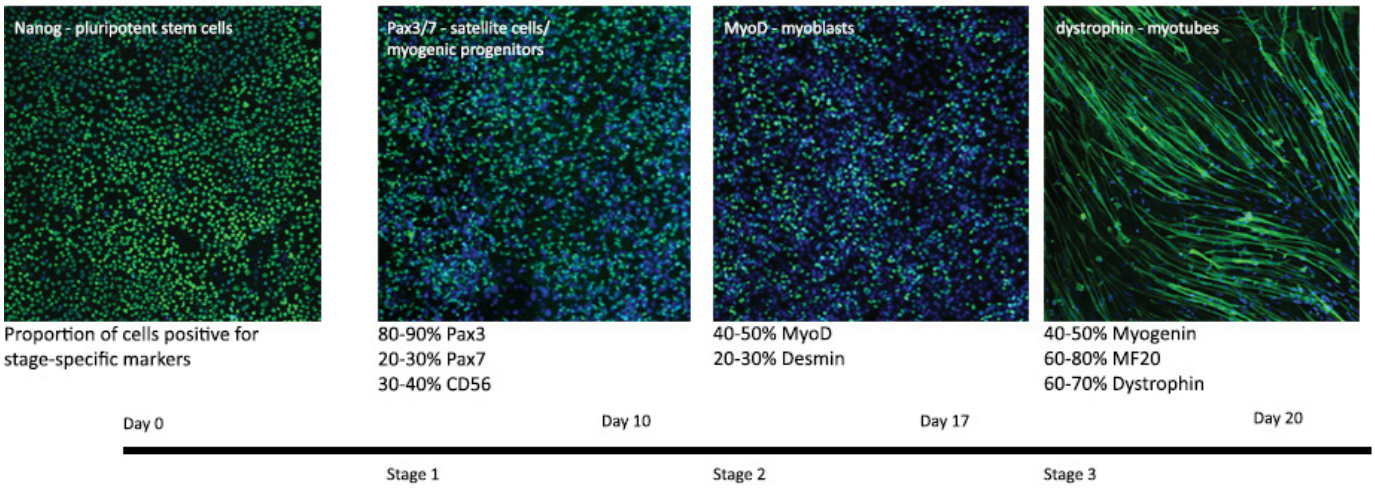
Please visit our website for a complete listing our AMSBIO's extensive range of antibodies or contact us today for customized requests.

Skeletal Muscle Differentiation Kit

AMSBIO has the world's first commercially available media system to differentiate human pluripotent stem cells into functional myotubules.

BENEFITS

- ✓ Achieves typically 70% MF20-positive myotubes
- ✓ Simple 3-step process involves only media changes and cell passaging
- ✓ No cell sorting steps required
- ✓ No transfection of myogenic transcription factors
- ✓ Tested on a wide range of human embryonic & induced pluripotent stem cell lines



Description	Pack Size	Catalogue No.
Skeletal muscle differentiation kit (with control myoblasts)	1 kit	SKM-KIT
Skeletal muscle differentiation kit (without control myoblasts)	1 kit	SKM-KITM

ALL KIT COMPONENTS ARE AVAILABLE TO ORDER SEPARATELY.

Description	Pack Size	Catalogue No.
Skeletal Muscle Induction Medium	250 ml	SKM01
Myoblast Cell Culture Media	250 ml	SKM02
Myotube Cell Culture Medium	250 ml	SKM03
Myotube Fusion Medium	250 ml	SKM03plus

Reprogramming & Genetic Modification

OFF-THE-SHELF LENTIVIRUS AND ADENOVIRUS SYSTEMS FOR IPSC GENERATION

AMSBIO's lentivirus system provides ready-to-use high-titre lentiviral particles for all six human and mouse iPSC factors (OCT4, SOX2, NANOG, LIN28, c-Myc, and Klf4). For efficient transduction, simply add the lentiviral particle solution to your tissue culture medium without the need for additional reagents or equipment. These self-inactivating particles are safe to-use and offer unparalleled high transduction efficiencies in dividing or even non-dividing cells. The reprogramming factors in our lentiviral particles are expressed under EF1α (see Figure 31) or the optional inducible suCMV promoter (see Figures 32 and 33) and with different selection markers. We also offer adenoviral particles for non-integrating reprogramming.

BENEFITS

- ✓ Ready-to-use particles - directly added to cells
- ✓ No need for transfection reagents
- ✓ Constitutive or tetracycline inducible expression
- ✓ High true titre: 1 x 10e7 IFU/ml
- ✓ Monitor expression in real-time via co-expressed markers
- ✓ Highly reproducible gene delivery
- ✓ Safe replication-incompetent virus

APPLICATIONS

- ✓ Transduction of adherent and suspension cells
- ✓ Gene delivery into hard-to-transfect, primary or drug-arrested cells
- ✓ Suitable for *in vitro* and *in vivo* studies
- ✓ Cost effective generation of stable cell lines

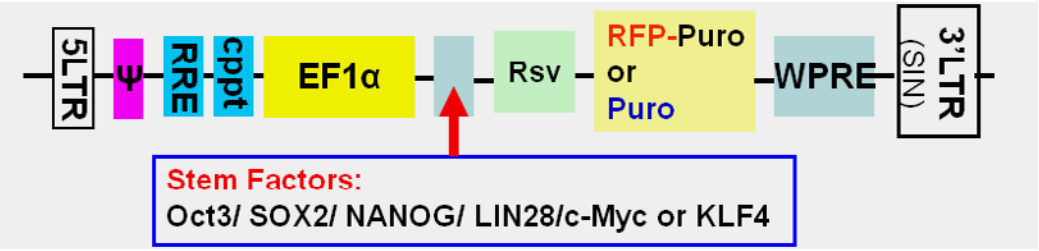


Figure 31: *Schematic representation of lentivector (EF1α) for iPSC*

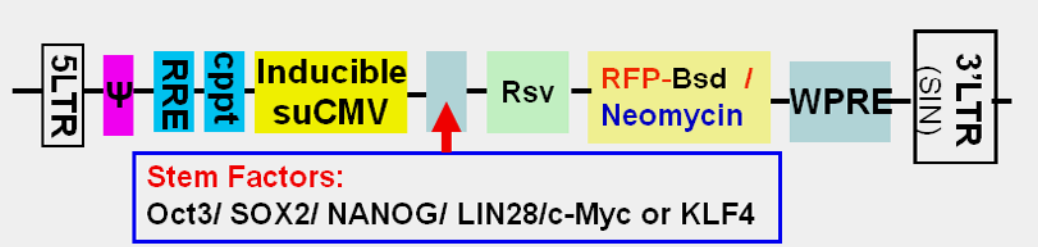


Figure 32: *Schematic representation of inducible lentivector for iPSC*

HUMAN STEM FACTOR LENTIVIRAL PARTICLES

Expressed Human Stem Factor	Pack Size	Promoter/Markers			
		Optional inducible suCMV/RFP-Blasticidin	Optional inducible suCMV/Neomycin	EF1α/Puromycin	EF1α/RFP-Puromycin
OCT4	200 µl x (1 x107 IFU/ml)	LVP003	LVP311	LVP317	LVP588
SOX2	200 µl x (1 x107 IFU/ml)	LVP003	LVP312	LVP318	LVP589
NANOG	200 µl x (1 x107 IFU/ml)	LVP005	LVP313	LVP319	LVP590
LIN28	200 µl x (1 x107 IFU/ml)	LVP006	LVP314	LVP320	LVP591
c-Myc	200 µl x (1 x107 IFU/ml)	LVP007	LVP315	LVP321	LVP592
Klf4	200 µl x (1 x107 IFU/ml)	LVP008	LVP316	LVP322	LVP593

MOUSE STEM FACTOR LENTIVIRAL PARTICLES

Expressed Mouse Stem Factor	Pack Size	Promoter/Markers	
		Optional inducible suCMV/RFP-Blasticidin	Optional inducible suCMV/Neomycin
OCT4	200 µl x (1x108 IFU/ml)	LVP003m	LVP311m
SOX2	200 µl x (1x108 IFU/ml)	LVP004m	LVP312m
NANOG	200 µl x (1x108 IFU/ml)	LVP005m	LVP313m
LIN28	200 µl x (1x108 IFU/ml)	LVP006m	LVP314m
c-Myc	200 µl x (1x108 IFU/ml)	LVP007m	LVP315m
Klf4	200 µl x (1x108 IFU/ml)	LVP008m	LVP316m

MOUSE ADENOVIRAL PARTICLES

Description	Pack Size	Catalogue No.
OCT4	50 µl x (1x1011 IFU/ml)	AVP016-PBS

Custom Made Lentivirus

Lentivirus Custom Services from AMSBIO generate high titer lentivirus expressing your gene of interest or shRNA expression, and provide stable cell lines. Lentivirus cloning kits and packaging systems are also available.

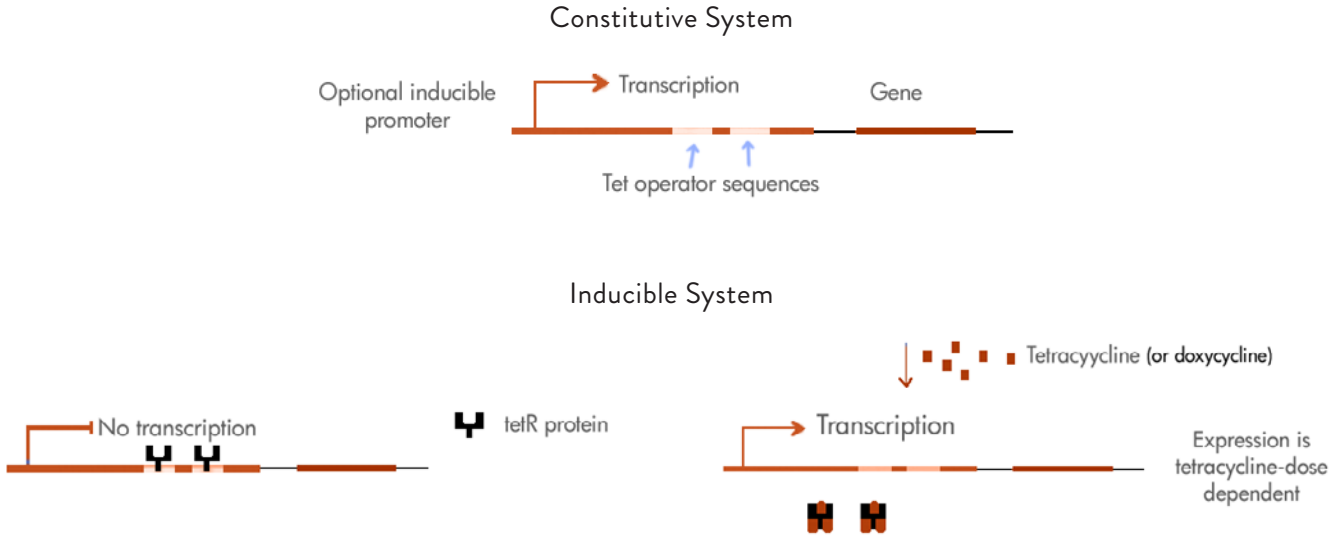


Figure 33: *Lentiviral optional inducible system: Without further intervention, our optional inducible lentiviral particles drive regular constitutive expression. To take advantage of the promoter's inducible properties, the tetracycline repressor protein (TetR) must be present in advance. The expression of TetR can be achieved by using our pre-made Tet-repressor lentiviral particles.*

CONSTITUTIVE OR INDUCIBLE: CONTROL WHEN YOUR TARGET IS EXPRESSED

Our optional inducible promoters have two copies of tetracycline (Tet) operator sequence integrated. This does not affect the efficiency of the promoters so without further intervention, the optional inducible lentiviral particles drive regular high constitutive expression of your gene or shRNA of interest. By transducing one of our ready-to-use Tet-Repressor lentiviral particles or by using our Tet-Repressor stable cell lines, the transcription of the transgene or shRNA will be repressed by the binding of TetR to the Tet operator sequences of the promoter.

Also available: shRNA lentivirus to knock-down Mbd3, a core member of the Mbd3/NuRD (nucleosome remodelling and deacetylation) repressor complex and achieve higher reprogramming efficiency. Mouse and human shRNA lentivirus available.

SYNTHETIC mRNA MEDIATED REPROGRAMMING

AMSBIO offers a non-integrating strategy for reprogramming stem cell fate based on the administration of synthetic modified mRNA that greatly increases efficiency whilst reducing the innate antiviral response of viral protocols. This ready-to-use synthetically optimized mRNA cocktail of Klf4, c-Myc, Oct4, Sox-2 and Lin28, with GFP mRNA (collectively referred to as KMOSL+G) is proven to reprogram a wide variety of human cell types.

The method for deriving iPS cells originally described by Yamanaka remains the most established technique. However, several barriers have hampered the wide-spread applicability of iPS cells using this technique. Traditional methods used retrovirus transduction of the reprogramming factors, which results in viral integration into the genome and has low reprogramming efficiencies. Viral integration and low efficiency presents downstream problems to the therapeutic use of iPS cells. Synthetic highly modified mRNA overcomes these obstacles by inducing pluripotency without incurring genetic change. Messenger RNA (mRNA) is the key intermediary in gene expression; translating the genetic code into the amino acids that make up proteins. The direct link of mRNA to protein makes this technique ideally suited for nuclear reprogramming The high efficiency of synthetic mRNA based reprogramming was reported in *Cell Stem Cell (Warren, 2010).* In addition, synthetic mRNA is a powerful tool to direct stem cell fate and transdifferentiation.

BENEFITS

- ✓ Pre-made, highly purified and ready-to-use
- ✓ Simple, safe, non-integrating technique
- ✓ Modified to overcome innate antiviral responses
- ✓ Reprogram multiple human cell types to pluripotency
- ✓ Higher Efficiencies than traditional protocols
- ✓ Direct cell fate and transdifferentiation

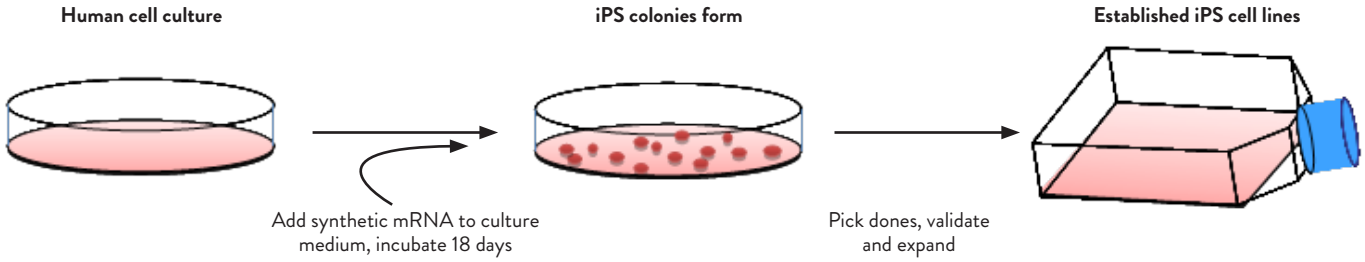


Figure 34: *Synthetic mRNA Mediated Reprogramming*

Synthetic mRNA is the most advanced and safest method for deriving your iPS cells. AMSBIO offers ready-to-order synthetic modified mRNA proven to reprogram a wide variety of human cell types. Alternatively made-to-order custom mRNA is available to meet your unique requirements.

INHIBITORY RNA FOR GENE KNOCKDOWN

The use of short hairpin RNA (shRNA) constructs and small interfering RNA (siRNA) enable discoveries at an accelerated pace and at a relatively low cost. shRNA plasmids and siRNA constructs are pre-designed with genome wide coverage of human, mouse and rat. Four shRNA constructs are provided per target, alternatively siRNA are supplied with three constructs per target. Whether using shRNA or siRNA, one of the constructs is guaranteed to produce 70% or more knock-down, provided a minimum transfection efficiency of 80% is achieved. shRNA plasmids are available in multiple formats, with or without tGFP or tRFP fluorescent markers driven by a CMV promoter providing easy identification of transfected cells and with Puromycin or Blasticidin as a selection marker for stable cell lines selection.

3 Unique Gene-Specific 27mer siRNA Duplexes

Trilencer-27 siRNA kit contains Dicer-Substrate duplexes that provide two critical improvements over the use of traditional 21mer siRNA designs. 27mer siRNA takes advantage of the natural processing by Dicer producing 10-fold higher potency and specificity than shorter 21mer RNAi forms. 27mer dicer-substrate duplexes also evade the radar of the mammalian interferon response when expressed in mammalian cells and initiates strong and specific gene silencing. By its optimal design, Trilencer-27 siRNA has the advantages of improved efficacy and minimal interferon response.

BENEFITS

- ✓ Genome wide coverage against human, mouse and rat
- ✓ 27mer Dicer-substrate duplex--higher potency & minimal interferon response
- ✓ Guaranteed* gene knockdown (≥70%)
- ✓ 3 gene-specific siRNAs + 1 negative control

GENESILENCER® siRNA TRANSFECTION REAGENT

GeneSilencer® siRNA transfection Reagent is a novel cationic lipid formulation specifically designed for efficient delivery of siRNAs (small interfering RNAs) into a wide variety of cell types. siRNAs are short, gene-specific double-stranded RNAs that can cause gene silencing in mammalian cells by catalytically cleaving greater than 95% of the target mRNA.

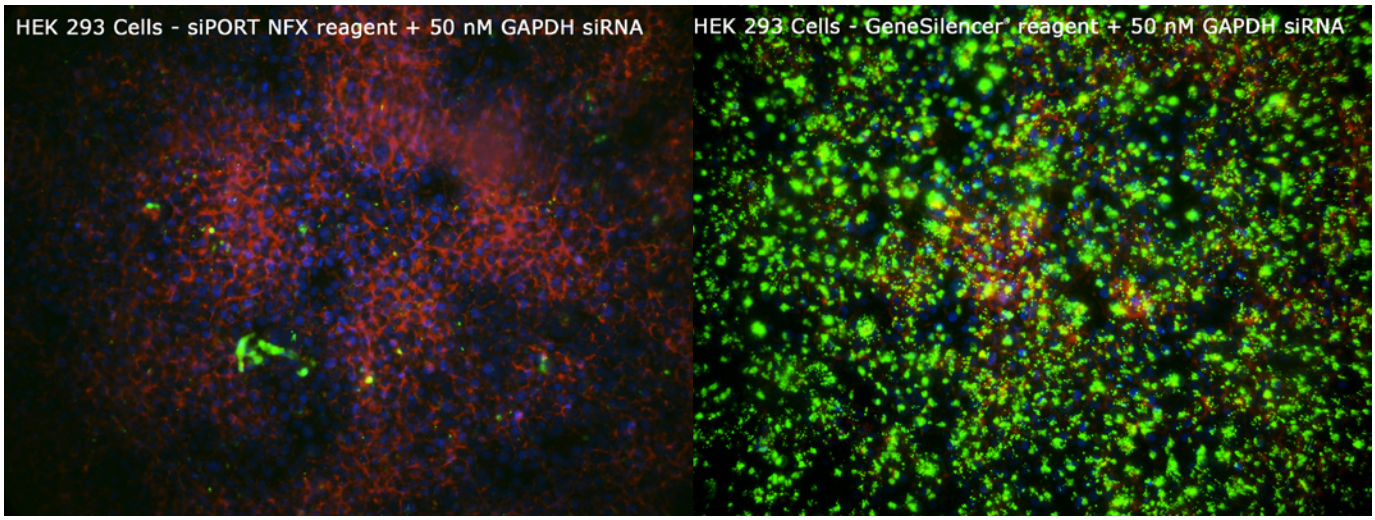


Figure 35: *HEK 293 cells were transfected with 50 nM of GeneSilencer® FAM Labeled GAPDH siRNA with either GeneSilencer® or siPORT NeoFX™ (Ambion) siRNA transfection reagents according to the manufacturer's recommended protocols. Cells were incubated for 48 hours then fixed, permeablized, and incubated with AlexaFluor® 555 phalloidin (Invitrogen), which stains cellular actin red. Cells were then mounted on slides and stained with DAPI (Invitrogen), which stains the cell nuclei blue. Transfected cells were visualized by fluorescence microscopy using identical exposure times for FITC, TRITC, and DAPI. The transfected GAPDH siRNA is localized in the cell cytosol and can be seen as green fluorescent specks or dots.*

BENEFITS

- ✓ High siRNA transfection efficiency
- ✓ Functional gene silencing post siRNA delivery
- ✓ Compatibility with diverse growth conditions (with and without serum)
- ✓ Low cytotoxicity
- ✓ Easy-to-use protocols for both adherent and suspension cells

Description	Pack Size	Catalogue No.
GeneSilencer® siRNA Transfection Reagent	50 rxn	T500020
GeneSilencer® siRNA Transfection Reagent	200 rxn	T500750
GeneSilencer® siRNA Transfection Reagent	1000 rxn	T505750

CELLSCRUB™ BUFFER

CellScrub™ Buffer is a unique washing buffer designed to remove all complexes of DNA and cationic lipids which associate with cell surfaces during transfection. Non-toxic, fast and effective removal of extracellular lipid/DNA complexes post-transfection to cells

Description	Pack Size	Catalogue No.
CellScrub™ Buffer	100 ml	B100001

Protein Delivery

BioPORTER®

BioPORTER® Protein Delivery Reagent is a unique lipid formulation that allows direct translocation of proteins into living cells.

BioPORTER® Protein Delivery Reagent provides researchers with a quick and easy method to study protein function without the need for cloning and DNA transfection. The BioPORTER® reagent lipid captures proteins and transports them inside the target cells. The delivered proteins retain their structure and function while leaving the transduced cells unharmed. The BioPORTER® Reagent is especially useful when studying protein function in cells that are difficult to transfect using traditional DNA transfection reagents.

BENEFITS

- ✓ Efficient protein delivery into a wide variety of cell types
- ✓ Efficient delivery of a variety of proteins and peptides
- ✓ Fast and Easy Protocol

For additional speed and convenience, the BioPORTER® Reagent is also available in pre-coated single-use BioPORTER® QuikEase™ tubes. The BioPORTER® QuikEase™ tubes save hours of time by eliminating the need to coat the BioPORTER® Reagent onto individual vials.

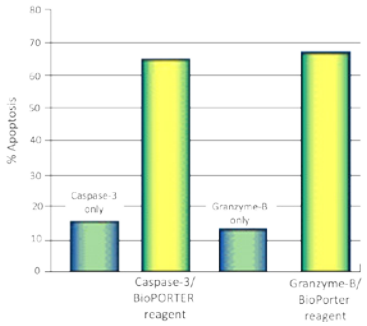
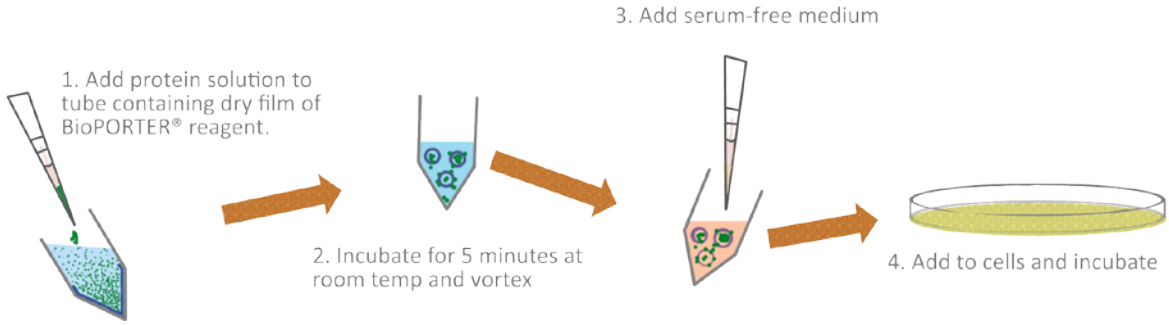


Figure 36: *Functional Apoptotic Protein Delivery in Ki-Ras 267 cells*



Description	Pack Size	Catalogue No.
BioPORTER® Protein Delivery Reagent	24 Reactions	BP502401
BioPORTER® Protein Delivery Reagent - QuikEase™ Kit	24 Single-Use Tubes	BP502424
BioPORTER® Protein Delivery Reagent	96 Reactions	BP509604
BioPORTER® Protein Delivery Reagent - QuikEase™ Kit	96 Single-Use Tubes	BP509696

Protocols

PASSAGING STEM CELLS

The following Application Note is to give technical support for passaging human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) using enzymatic digest.

Passaging Stem Cells Using Collagenase IV

Collagenase IV is commonly used to passage human pluripotent stem cells (hESC & hiPSC). It is gentler than trypsin, but requires longer incubation times. Periodic tests to confirm karyotypic stability during long-term culturing and expansion is highly recommended.

Collagenase IV is commonly used to passage human pluripotent stem cells (hESC & hiPSC). It is gentler than trypsin, but requires longer incubation times. Periodic tests to confirm karyotypic stability during long-term culturing and expansion is highly recommended.

1. Plate the feeder cells roughly 24 hours prior to passaging hESC/iPSCs.

2. Aspirate the medium from the culture flask and wash with enough 1X PBS to cover the entire cell growth area.

3. Cover the entire growth area of each flask with Collagenase IV solution (approx. 1 mg/mL
AMSBIO Cat No. 17454.01) and return flask to 37°C incubator.

4. Observe hESC/iPSC colonies under the microscope after 30 minutes. Some of the hESC/iPSC colonies will start to curl up on the edges. *
5. Continue incubation until a majority of the colonies are curled up or floating. This can take between 0.5–1 h or possibly longer.

6. Add complete growth medium (ES-DMEM/F12, GSM-1002, supplemented with serum replacer and basic FGF, GSR-2001).

Wash the cell growth area with a pipette to dislodge colonies from the surface.

7. Collect cells and centrifuge at 270 x g for 5 min. (Alternatively, allow the colonies to settle by gravity. This decreases the transfer of fibroblasts to the new culture.)

8. Remove most of the supernatant. Pipet the cell suspension in order to break the colonies into smaller pieces. Be careful not to pipet too much. The colonies should not be passaged to single cells.

9. Resuspend the suspension in complete growth medium.

10. Plate hESC/iPSCs into new cell culture vessel.

11. We recommend 1:3 – 1:5 split ratio depending on the growth rate of the individual cell line.

*For plates or dishes, a cell scraper can be used to detach the colonies at this point. If the colonies have started to round up at the edges, remove the Collagenase and add media. Scrape the surface and transfer the suspension to a centrifuge tube. Skip to step 7.

Passaging Human Stem Cells Using Trypsin

Trypsin is an enzyme used to dissociate human stem cells to smaller aggregates or single cell suspension. Extra care should be taken when using trypsin as there is some research suggesting that it may facilitate chromosomal mutation. Therefore it is crucial not to over-trypsinize the hESC/iPSC colonies and allow them to become single cells unless required by a specific assay. Periodic tests to confirm the karyotypic stability of the cell line during expansion is highly recommended.

1. Prepare flasks/dishes to receive the cells by thawing fibroblasts 24 hours before passaging. We recommend a 1:3 – 1:5 split ratio depending on the growth rate of the individual cell line.

2. Aspirate the medium from each flask/dish and wash once with 1X PBS.

3. Add enough 0.05% Trypsin/EDTA** to cover the cell growth area and quickly return flask to 37°C incubator.

4. Observe hESC/iPSC colonies under the microscope. As soon as the cells have started to round up (approx. 1 min.), tap the culture vessel gently and add equal volume serum containing growth medium to inactivate trypsin. Do not trypsinize to single cells unless required.

5. Wash the cell growth area gently with a pipette from the top down until the sticky fibroblast layer has detached.

MEF CELL CULTURE INSTRUCTIONS

All media and reagents used in the culture of this product should be warmed to 37°C before use. Perform all activities under aseptic culture conditions.

MEF Plating Density

There is a wide range of MEF plating densities used by researchers for mouse and human pluripotent stem cell culturing. The density depends on the ESC or iPSC lines you are culturing. We recommend that you use the feeder density previously used by the source lab from which you received the cell line; or the density determined through personal experience to be appropriate for your specific stem cell line. Our MEFs have been successfully used to support undifferentiated pluripotent stem cells at densities ranging from 2×10^4-5.3×10^4 cells/cm^2.

Cell Culture Plate Preparation

Our MEFs do not require gelatin coating of the plates prior to use. Using gelatin does not benefit or inhibit the ability of the MEFs to support pluripotent stem cells. We recommend not using gelatin with our MEFs because it adds in an extra step and could be a source off variation in culturing the cells. The use of gelatin is up to the individual researcher.

Cell Culturing

1. Place the frozen vial into a 37°C water bath as soon as possible and retrieve the vial before the contents are completely thawed (1–2 minutes).

2. Immediately transfer the contents of the vial to a 15-mL tube and dilute 1:10 with complete growth medium.

3. Spin the tube at 270 x g for 5 minutes in order to pellet the cells.

4. Resuspend the pellet in complete growth medium and plate the cells to appropriate size tissue culture flask at the required density.
5. Feeder cells should be plated 24 hours prior to plating the pluripotent cells.

6. Once the pluripotent cells are plated they should be passage within 4-7 days.

7. The MEFs should not be used for longer than 7–10 days.

AMSBIO RECOMMEND THE FOLLOWING PLATING DENSITIES OF MEFS TO SUPPORT HUMAN OR MOUSE EMBRYONIC STEM CELL CULTURE:

Vessel type	Surface area	MEF number for mESCs	MEF number for hESCs
12 well plate	3.8 cm^2/well	0.2 x 10^6/well	0.28 x 10^6/well
6 well plate	9.5 cm^2/well	0.5 x 10^6/well	0.7 x 10^6/well
T25 flask	25 cm^2	1-1.5 x 10^6	2 x 10^6
10cm dish	55 cm^2	3 x 10^6	4 x 10^6
T75 flask	75 cm^2	3- 4 x 10^6	4-6 x 10^6
T225 flask	225 cm^2	12-15 x 10^6	18-21 x 10^6

Final densities can vary significantly based on user experience, the strain of MEFs and species of stem cells. These recommendations are based on what is commonly used by scientists for stem cell proliferation and research.

amsbio

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V2.0

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