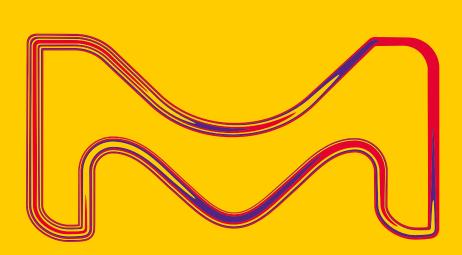
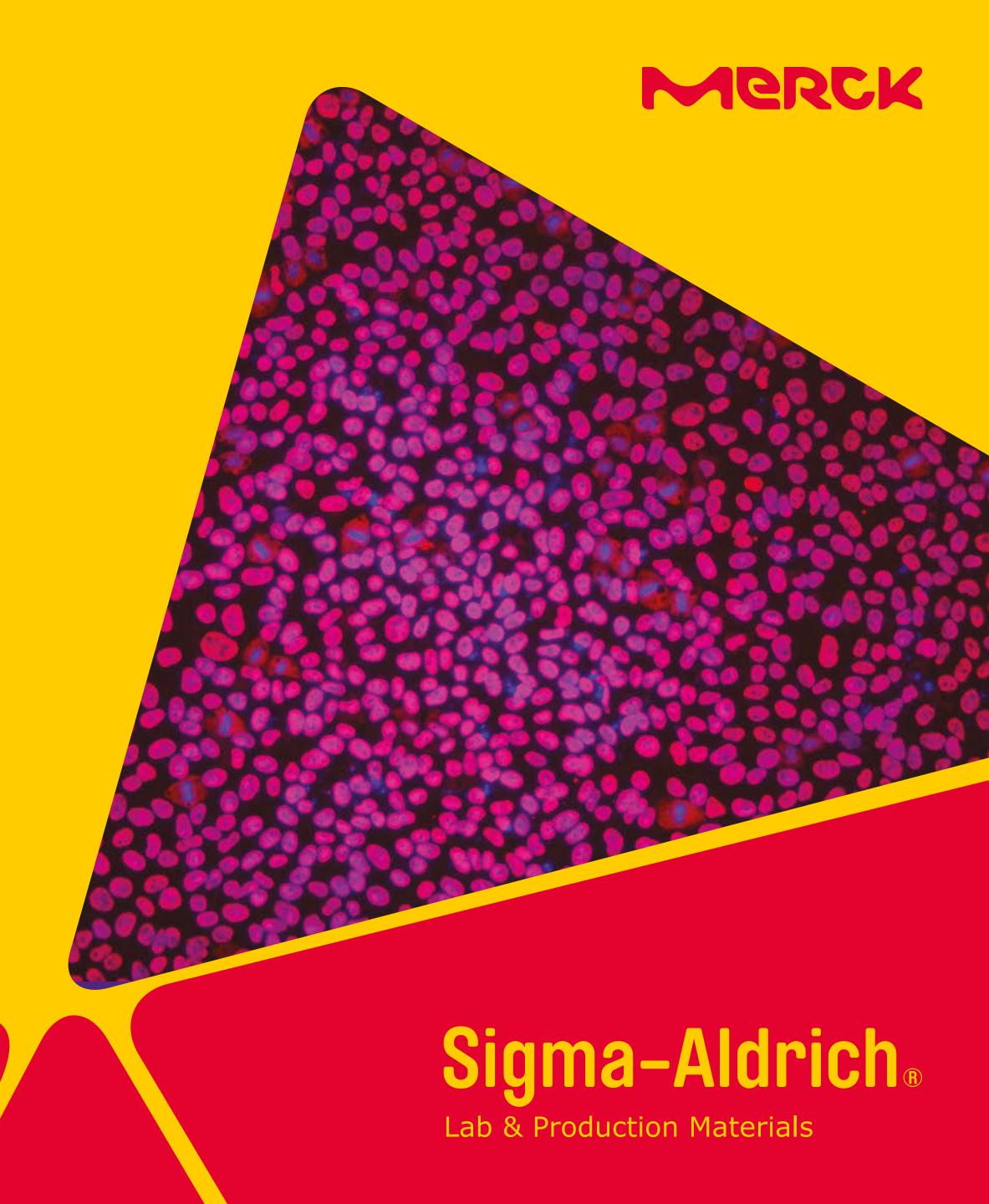
Stem Cell Product Selection Guide

Dedicated to supporting all your explorations



The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.



what are you interested in? CHOOSE ONE



A pluripotent stem cells

- Human ES Cells
 (also induced Pluripotent Stem Cells iPS)
- Mouse ES cells/Mouse Embryo

Multipotent stem cells

Neural Stem Cells

Mesenchymal Stem Cells

Hematopoietic Stem Cells

Stem Cell
Learning Center

3D Printing

3D Cell Culture

General Workflow Overview

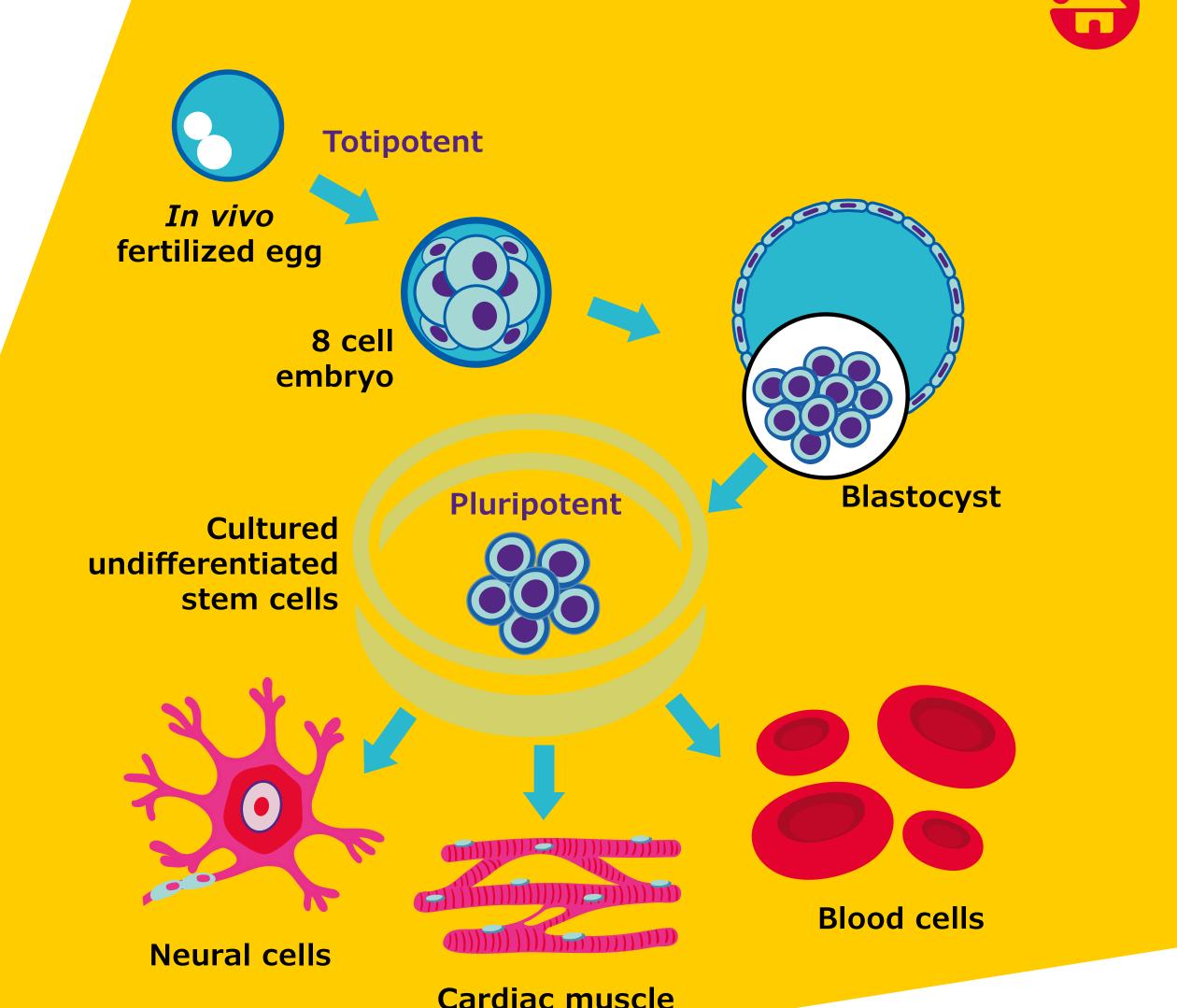


Pluripotent Stem Cells

Pluripotent stem cells, including embryonic germ (EG), embryonal carcinoma (EC), embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells, have the capacity to give rise to differentiated progeny representative of all three germ layers (ectoderm, endoderm, and mesoderm).

The ability to expand pluripotent cells *in vitro* and direct differentiation to produce specific cell types is crucial to the development of cellbased therapies to replace or restore tissue that has been damaged by disease or injury.

We offer a range of tools for human and mouse pluripotent stem cell research, including ES cell lines, iPS cells, cell culture reagents, characterization kits, and novel antibodies.



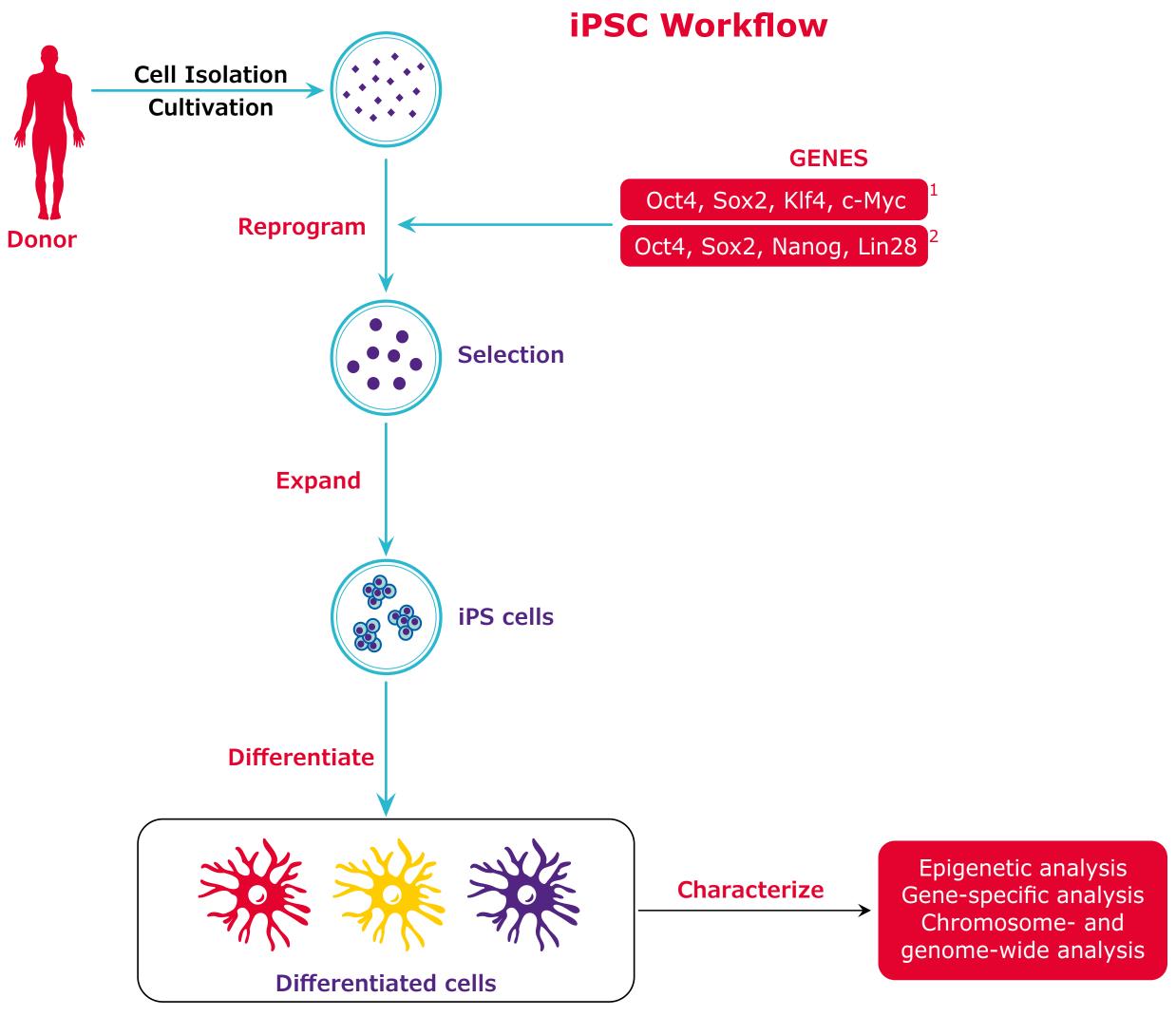


Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPS cells or iPSCs) are a type of pluripotent stem cell that can be generated from adult somatic cells such as skin fibrobalsts or peripheral blood mononuclear cells (PBMCs) by genetic reprograming or the 'forced' introduction of reprogramming genes (Oct4, Sox2, Klf4 and c-Myc). In 2006, Shinya Yamanaka produced the first iPS cells - murine ES (embryonic stem) like cell lines - from mouse embryonic fibroblasts (MEFs) and skin fibroblasts by inserting four transcription factor genes encoding Oct4, Sox2, Klf4, and c-Myc. Another group of researchers identified two other genes, Nanog, and Lin28 as a replacement of Klf4, and c-Myc to reprogram human cells. Recently, non-integrating methods of reprogramming have become popular including RNA sendai virus and RNA based reprogramming methods.

The advantage of iPS cells is that they are not derived from human embryos, which is the ethical concern in this field. Another significant benefit of iPS cell technology would permit for creation of isogenic control cell lines using CRISPR/Cas9 gene editing that are genetically tailored to model a disease phenotype.

iPS cells are similar to ES cells in morphology, teratoma formation, proliferation, expression of pluripotency markers, long telomeric zone, generation of embryoid bodies and viable chimeras as well as their ability to <u>differentiate</u> along a given lineage. They also express <u>stem cell surface markers</u> and genes that characterize ES cells such as Oct4, Sox2, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4 and Nanog.





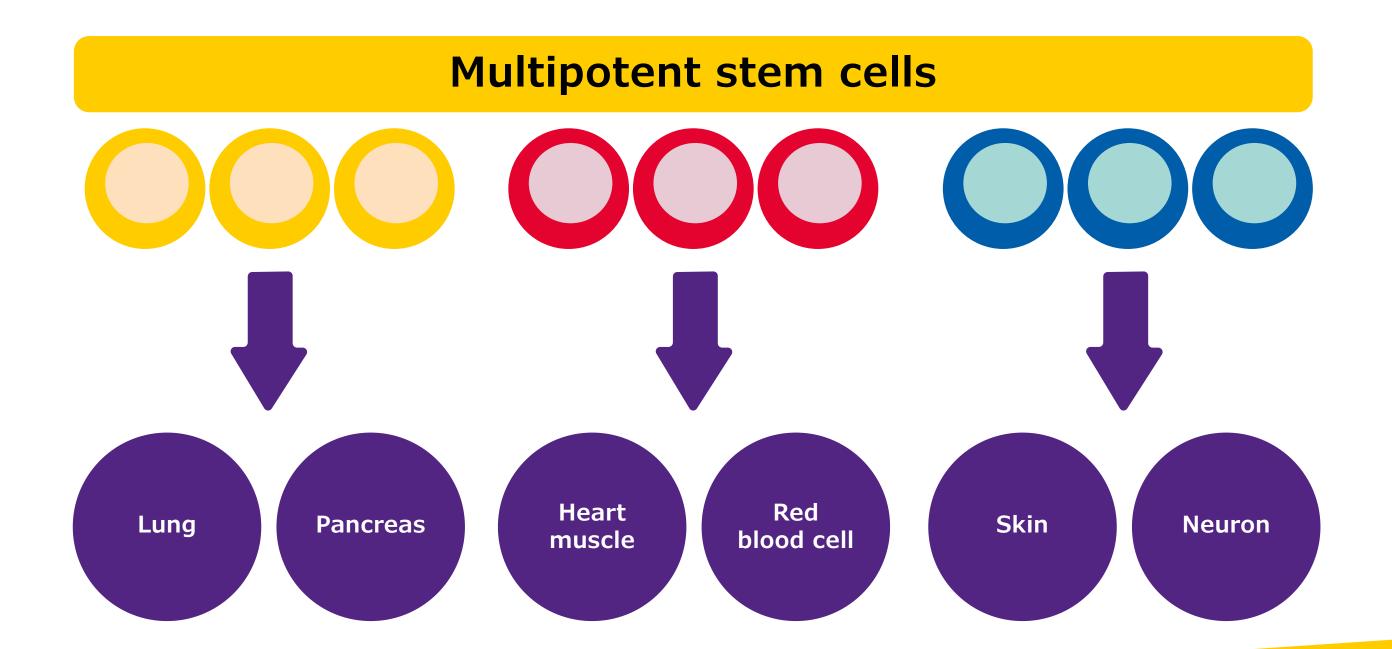
Multipotent Stem Cells

Multipotent stem cells are capable of differentiating into a variety of cellular subtypes and, as such, are highly useful in studies of stem cell differentiation.

We offer a wide selection of progenitor cell lines, differentiation kits, media, antibodies, and reagents to enhance your research.

These products fall into three main categories: neural, hematopoietic, and mesenchymal stem cells.

Popular items include our ReNcell® and ENStem™-A human neural progenitors, hundreds of antibodies, and more.



Mouse Embryo Culture

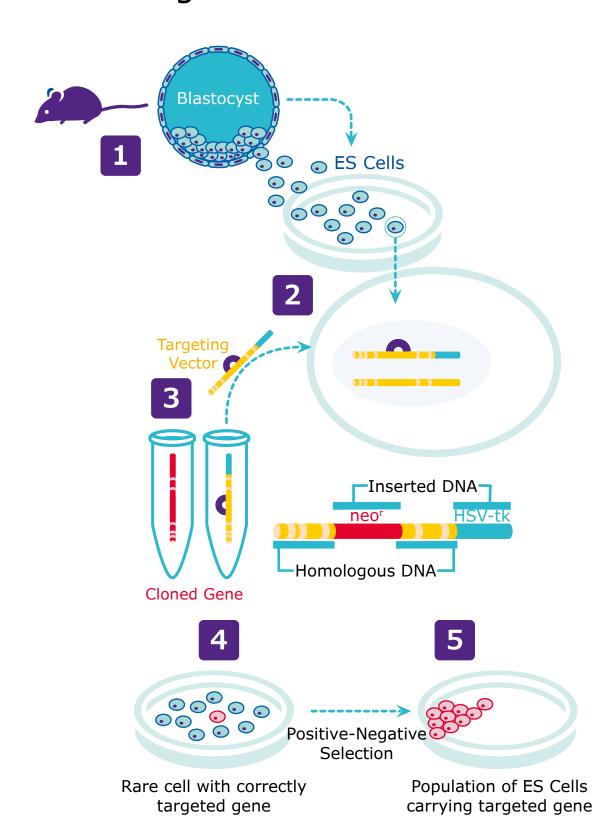
Transgenic and gene knockout technologies are powerful tools for studying gene function. A commonly used method for creating transgenic and knockout mice involves the introduction of genetically modified ES cells into early-stage mouse embryos by either blastocyst injection or aggregation techniques. These methods result in the generation of chimeric offspring; the genetic modification may be transmitted to successive generations if the ES cells contribute to the germline.

ES Cell Culture

1. Embryonic stem (ES) cells are cultured from mouse blastocysts.

Genetic Manipulation

- 2. Construction of a targeting vector (traditional or CRISPR/Cas9 system)
- 3. Transfection or transduction of targeting vector into ES cells or microinjecting zygote directly (CRISPR/Cas9 only)
- 4. Homologous recombination
- 5. Selection of targeted ES cells



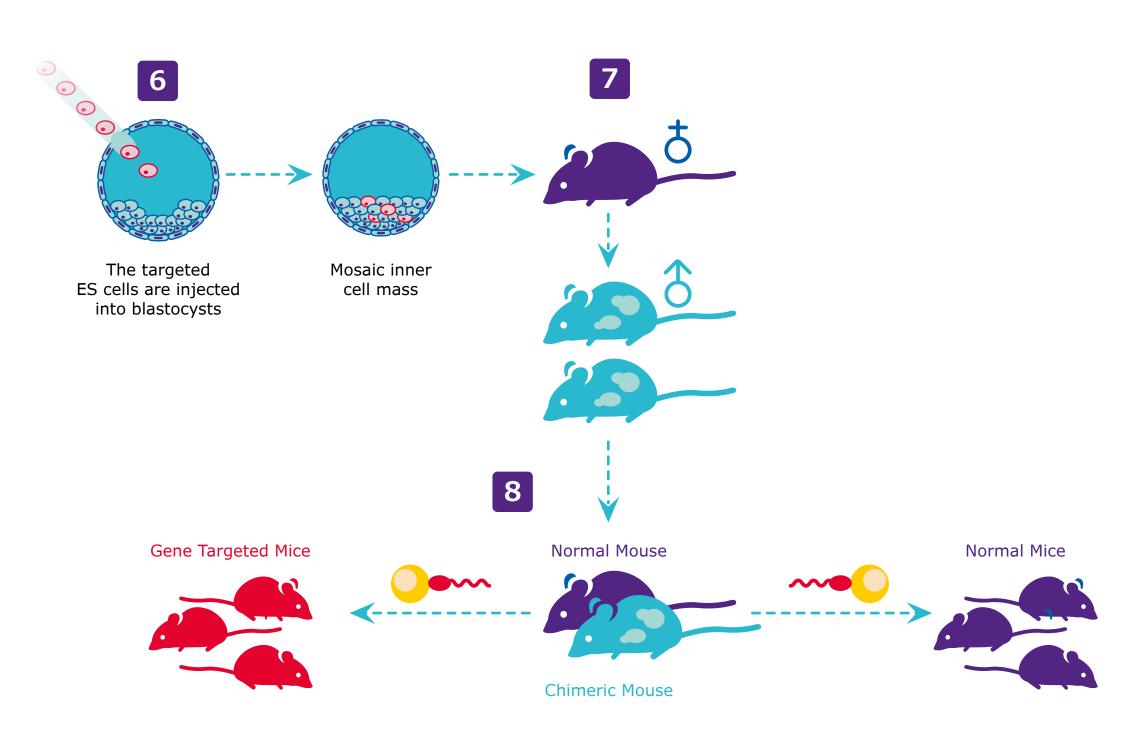
Mouse Embryo Handling and Culture

- 6. Targeted ES cells are injected into the blastocyst
- 7. Blastocyst is injected into the mouse, which acts as a surrogate mother

Mouse Birth and Breeding

- 8. Chimeric mice are produced; they are mated with normal mice
- 9. Gene-targeted mice are born

Genotyping





General Workflow Overview



Cells Prepare One of the control o

- Induced Pluripotent Stem Cells (EBiSC)
- Human Fibroblasts
- iPSC Reprogramming Kits
- Multipotent Stem Cell Lines
 - Neural
 - Mesenchymal
 - Hematopoietic
 - Cancer
- MEF Feeder Cells
- Mouse ES Cells
- CRISPR
- Transfection Reagents
- esiRNA

- Serum
- Stem Cell Media
- Buffers
- Supplements
- Antibiotics
- Growth Factors
- Cytokines
- Extracellular Matrix Proteins (ECM)
- General Labware
- Sterile Filtration devices
 - Stericup® E

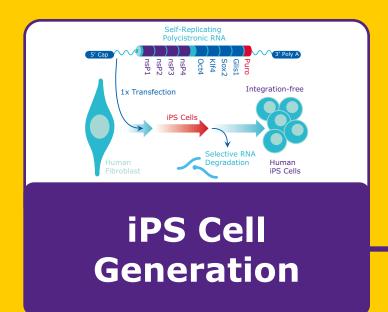
- General Labware
- Millicell[®] hanging/ standing inserts
- Millicell® 24- and 96-well plates
- Millicell[®] HY-Multilayer Flasks
- <u>Scepter™ automated</u> Cell Counter
- Dissociation Reagents
- Freezing Media
- 3D Cell Culture
 - Organoids

- Growth Factors
- Differentiation Media/Kits
- Antibodies
- Lineage Specific Antibodies
- Characterization Kits
- Small Molecule Inducers
- Millcell® EZ –slide
- <u>Live Cell Imaging Reagents</u>
 Fluorescent Dyes, Probes,
 Biosensors, AEI Dots
- Duolink[®]

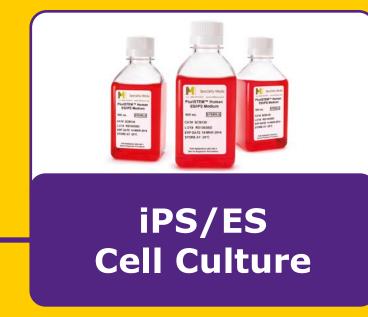


Pluripotent Human Stem Cell and iPS Workflow

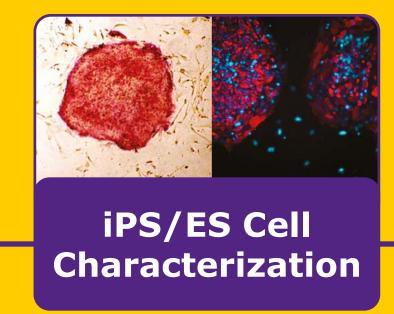




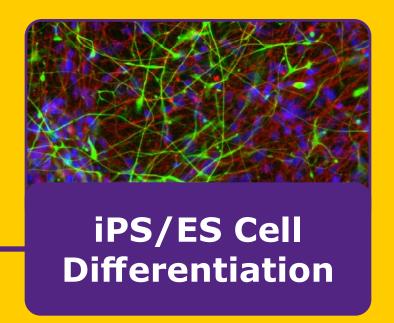
- EBISC
- Simplicon[™] (Non-Viral)
- <u>STEMCCA™ (Lentiviral)</u>
- Xeno-Free Human Fibroblast
- Reprogramming Boost Kits
- B18R Protein
- CRISPR



- PluriSTEM Human
 ES/iPS Medium
- Growth Factors (human bFGF, LIF, Activin-A)
- Dissociation Reagents
 Collagenase, Dispase,
 Accutase, Accumax, EZ-Lift
- Stericup®/Millex®
- Eco-friendly Stericup® E and Steritop® E
- General Cell
 Culture Reagents
- ECM Proteins e.g. ECMatrix



- Pluripotency Markers
- Alk Phos Detection kit
- ES Cell Characterization kit
- iPSC Selection Kit
- Germ Layer PCR Kit
- Epigenetics Antibodies and ChIP Kits



- Growth Factors
- Small Molecule Inducers
- Epigenetics Antibodies and ChIP Kits
- Lineage Antibody Markers
- Cell Characterization Kits
- <u>Differentiation</u>



♦ iPS CELL GENERATION

EBISC





European Bank of induced pluripotent Stem Cells

- Extension of the European Collection of Authenticated Cell Cultures (ECACC)
- Currently 800 patient derived IPS cells
 - Diseases (35) including Alzheimer's Disease, diabetes, prolonged QT, bi-polar disorder
 - Isogenic iPS cell lines

All cell lines are thoroughly characterized in order to sustain a collection of high quality iPSC lines.

Sterility

Adventitious contamination of cell lines can seriously affect cell identity, proliferation, morphology and behaviour. All lines processed within EBiSC undergo continuous monitoring for the presence of microbiological agents such as Mycoplasma and bacteria.

Mycoplasma testing is performed on in-process samples using endpoint PCR and cell banks for distribution are screened using the more sensitive QPCR method. All lines also undergo broth inoculation and continuous visual assessments to detect non-specific microbiological growth.

Cell Phenotype

Human pluripotent stem cells have a well characterised phenotype associated with the maintenance of self-renewal. A number of factors contribute to maintaining this phenotype including optimisation of cell culture conditions.

EBiSC >

Within EBiSC, cell line phenotype is assessed by quanti fying the expression of self-renewal markers SSEA4, TRA-1-60, OCT3/4 and a lack of SSEA1 expression using flow cytometry. Morphology and confluency checks are also carried out on a daily basis by visual inspection to assess colonies for tight, defined borders and smooth surfaces.

Pluripotent Potential

Pluripotency is defined as the ability for a cell line to form all post-embryonic lineages. This is determined within EBiSC by spontaneous differentiation of iPSC lines to early germ layer populations. Quantitative PCR is used to assess the expression levels of early germ layer markers, with up-regulation indicating functional pluripotency.

Chromosomal Stability

Chromosomal abnormalities such as deletions or insertions of genetic material may affect cell identity, proliferation and response to stimuli.

Within EBiSC, karyology by G-banding is used to visualise chromosomal structure and to detect genomic abnormalities such as inversions, deletions, duplications and translocations.

Genetic Identity

EBiSC will assess and record the genetic identity of each iPSC line to detect and prevent the spread of any cross contamination of cell lines. This will be done by recording a genetic fingerprint for each line by assessment of Short Tandem Repeats (STRs). STR loci are varied numbers of sequence repeats found in highly polymorphic stretches of DNA. They are detected by amplification of a number of these loci and sub-sequent high resolution, size-based separation of amplicons.

♦ <u>iPS CELL GENERATION</u>

Simplicon™ RNA Reprogramming Technology

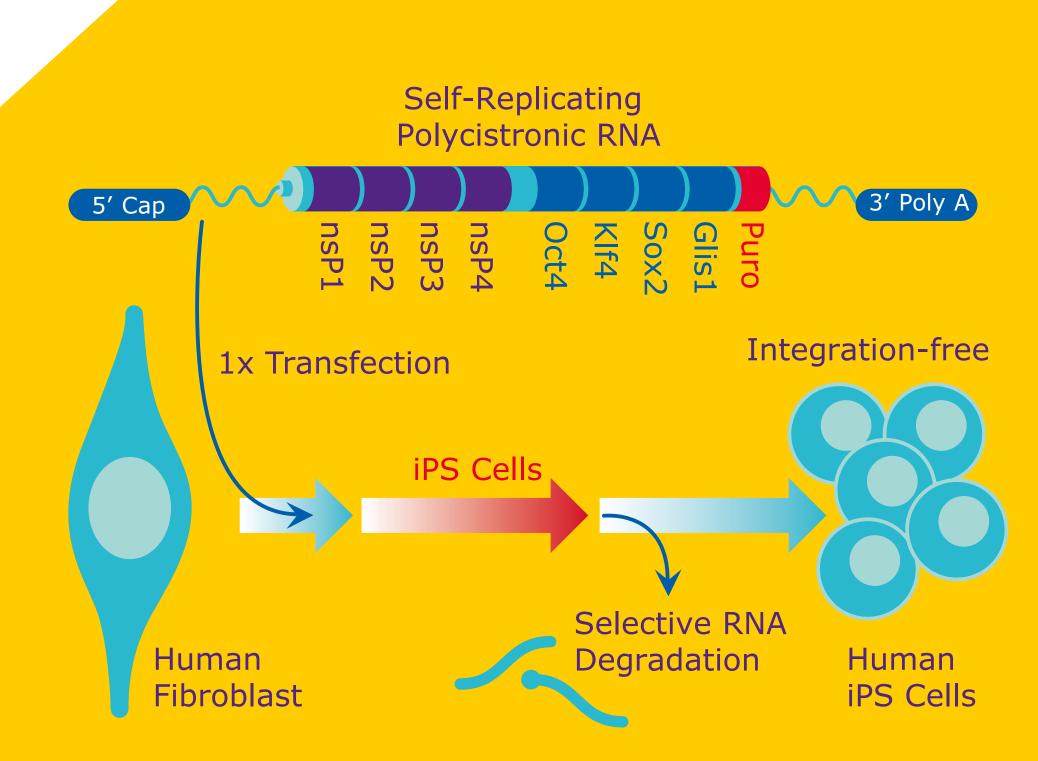


Features and Benefits

- Integration-free iPS cells. No risk of genomic integration
- 1-day transfection required. No more laborious daily transfection of multiple mRNA over a 14-day period
- Efficient, rapid reprogramming
- No screening require to ensure viral remnants are not present
- Controlled elimination of reprogrammed genes

Item Description	Cat. No.
Simplicon™ RNA Reprogramming Kit (OKSG)	SCR550
Simplicon™ OKSG-cMyc RNA (OKSGM)	SCR703
TagRFP Simplicon RNA Kit	SCR712
TagGFP2 Simplicon RNA	SCR713
Human OKSG-cMyc TagRFP Simplicon RNA	SCR714





Katze, M., He, Y. & Gale, M. Viruses and interferon: a fight for supremacy. Nat Rev Immunol 2, 675–687 (2002). https://doi.org/10.1038/nri888



♦ <u>iPS CELL GENERATION</u>

Role of B18R Protein

- Blocks cellular IFN response and has potent neutralizing activity
- Needed with RNA reprogramming to block the immune response and RNA degradation
- Other applications: Immunity, Immune Suppression, Viral Tolerance, RNA Reprogramming, Cancer, HIV and Hepatitis

OPEN @ ACCESS Freely available online

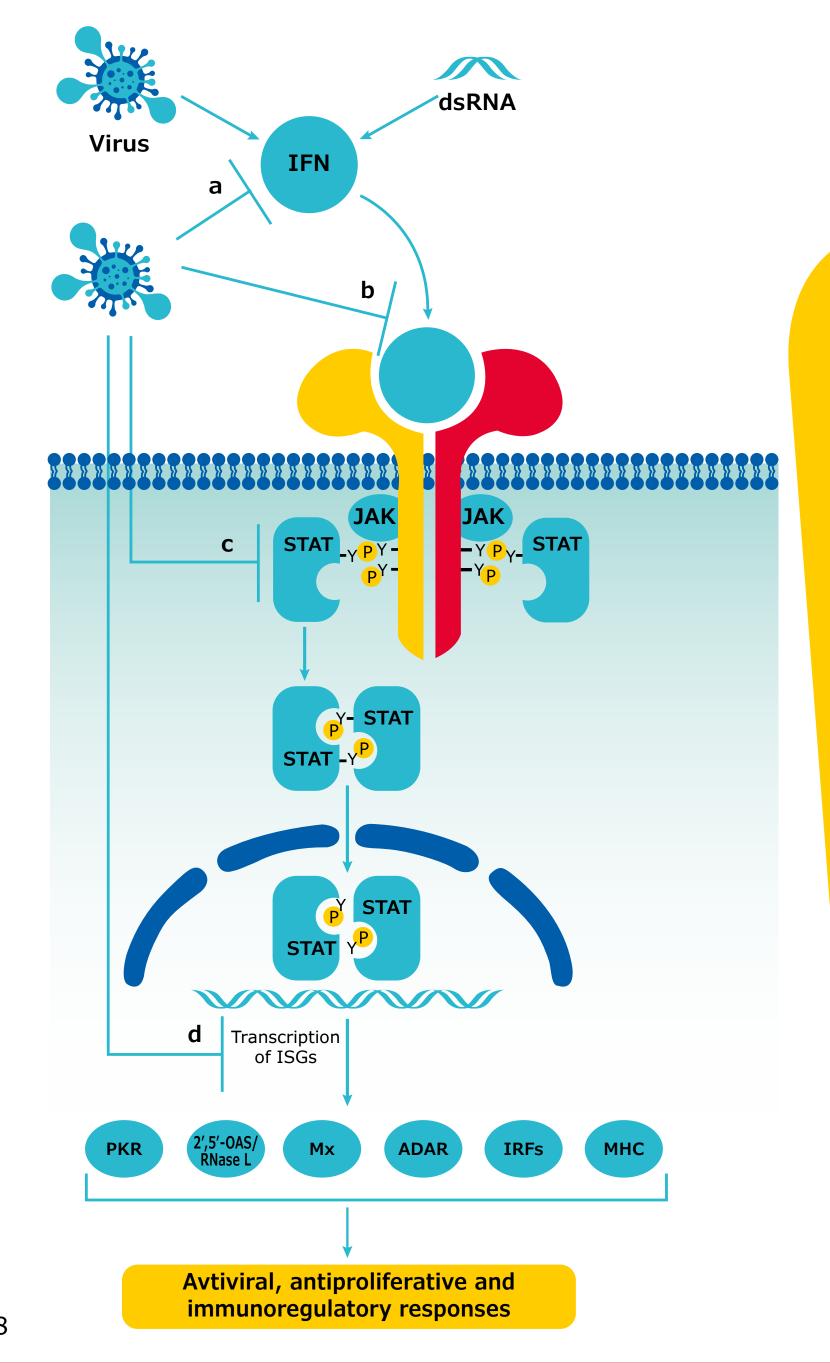


Innate Immune Suppression Enables Frequent Transfection with RNA Encoding Reprogramming Proteins

Matthew Angel¹, Mehmet Fatih Yanik^{1,2*}

1 Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America, 2 Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America

Katze, M., He, Y. & Gale, M. Viruses and interferon: a fight for supremacy. Nat Rev Immunol 2, 675–687 (2002). https://doi.org/10.1038/nri888







♦ iPS CELL GENERATION

PluriSTEM™ Human ES/iPS Medium

Features

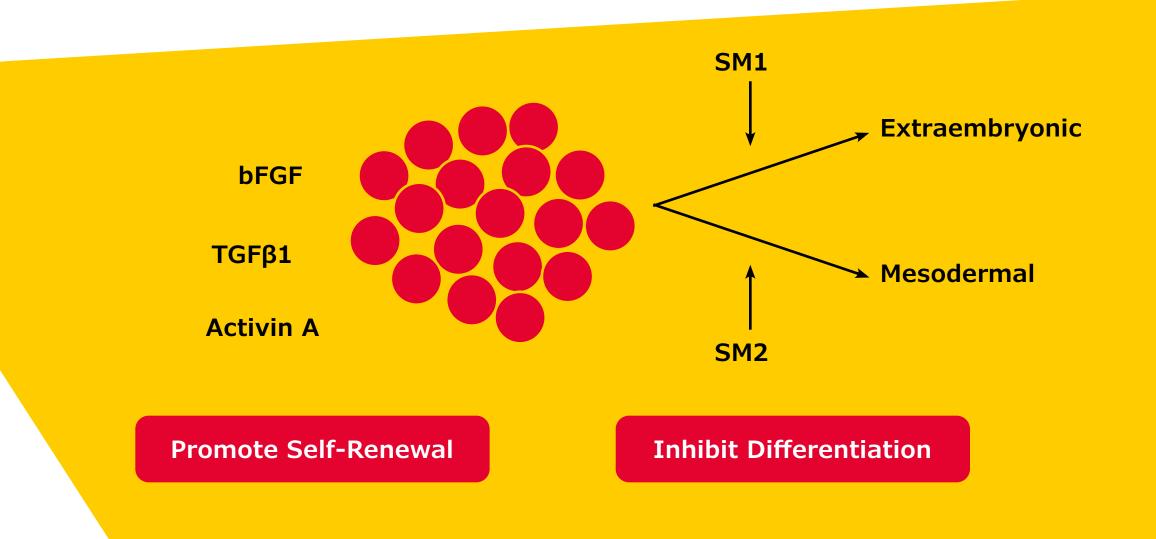
- Small molecule based defined feeder-free, serum-free medium for expansion of human pluripotent ES and iPS cells (>30 passages).
- Contains 11 components
- Lowered FGF (by 10%)
- Lowered TGFβ1 (by 25%)
- Activin A
- Small Molecule Inhibitors

Specialty Media U.S. -1886-200-4879 Www.milliparte.com PluriSTEM Im Human ES/IPS Medium 500 mL STERLE CAT# SCM130 LOT# RD1303002 EXP DATE 14-MAR-2014 STORE AT -20°C FOR RESEARCH USE ORLY INSTRUMENT PROMISES FOR RESEARCH USE ORLY Not for Chapmonic Procedures

Benefits

- Media exchanges every other day
- No weekend feeding required
- Freeze cells for the holiday and recover cells within 1 week
- Superior single cell culture, passage and selection of human pluripotent clones
- Single cell expand into colonies within 1 week.
- Simple & easy transition to PluriSTEM™ from feeder-based and other feeder-free culture systems
- No period of low cell yield

Item Description	Cat. No.
PluriSTEM™ Human ES/iPS Medium	SCM130
PluriSTEM-XF™ Human ES/iPS Medium	SCM132
PluriSTEM™ Dispase-II Solution	SCM133
PluriSTEM™ Freeze Medium	SCM134
PluriSTEM-XF™ Freeze Medium	SCM135
PluriSTEM-XF™ Recombinant Vitronectin	CC130





ECMatrix™ 511 E8 Laminin Substrates Overview

Human pluripotent stem cells express the major integrin species $\alpha 6\beta 1$, and therefore can be maintained stably and expanded efficiently in feeder-free conditions on culture vessels coated with laminin-511, the binding partner of integrin $\alpha 6\beta 1$.

Features and Benefits

- Animal-free, xeno-free formulation: Consistent from lot-to-lot, with no prescreening required
- No plate precoating required: Save time by simply adding to media while passaging cells
- Supports single-cell
 passaging without ROCKi:
 Ideal for CRISPR editing or
 clonal isolation
- Higher adhesion and growth rates: Achieve densities needed for experiments sooner
- **Easy to handle:** No chilling of cell culture consumables required

β1 φ5 γ1 β1

Laminin-511 E8 fragment

Item Description	Recommended Cell Type	Cat. No.
	Human ES/iPS Cells	CC160-350UG
ECMatrix™-511 E8 Laminin Substrate	Human ES/iPS Cells	CC160-1050UG
Lammin Substrate	Human ES/iPS Cells	CC160-100ML
ECMatrix™-511 Silk E8	Human ES/iPS Cells	CC161-1050UG
Laminin Substrate	Human ES/iPS Cells	CC161-100ML
ECMatrix™-411 E8	Endothelial Progenitors	CC162-350UG
Laminin Substrate	Endothelial Progenitors	CC162-1050UG
ECMatrix™-221 E8	Cardiomyocytes	CC163-350UG
Laminin Substrate	Cardiomyocytes	CC163-1050UG
Stem Cell Qualified	Human ES/iPS Cells	CC131-5ML
ECM Gel Matrix	Human ES/iPS Cells	CC131-10X5ML
PluriSTEM-XF™ Recombinant Vitronectin	Human ES/iPS Cells	CC130

ECMatrix

Over 200 Publications "i-Matrix 511" including Yamanaka Lab at CiRA



Human iPS Dissociation Reagents

Dispase II: Proven to be a rapid, yet gentle, enzyme for passaging human iPS cells as aggregates in both feeder and feeder-free conditions. The enzyme does not damage cell membranes as much as other enzymatic dissociation reagents.

Accutase®/Accumax®: A cell detachment solution of proteolytic and collagenolytic enzymes that can be used for the routine detachment of stem cells from standard tissue culture plasticware. When used with rho-associated kinase inhibitor (ROCKi), single cell passaging of human iPSCs with Accutase® reagent can be successfully achieved.

EZ-LiFT™ Stem Cell Passaging Reagent: A proprietary enzyme-free and chemically defined stem cell dissociation reagent that selectively passages only undifferentiated pluripotent stem cells. The reagent eliminates the need for manual colony selection or cell scraping and maintains high cell viability without the need for ROCKi. EZ-LiFT™ reagent can also rescue highly differentiated iPS cell cultures.

	Dispase II	Accutase®/Accumax™	EZ-LiFT™ Reagent
Mechanism	Enzymatic	Enzymatic	Non-Enzymatic
Composition	Bacteria Derived, Undefined	Animal Derived, Undefined	Animal-Free, Chemically Defined
Passage Method	Aggregates	Single Cell	Small Aggregates
Recommended Split Ratio	1:6*	1:30*	1:30*
Pluripotent Cell Enrichment	No	No	Yes
Cell Survival	++	++	+++
Plating Consistency	Low	High	High
Lot to Lot Variability	Med	Low	Low
ROCKi Requirement	No	Yes	No
Membrane Protein Damage	High	Med	Low
Cryopreservation (Vials/Well)	1 vial	2 vials	5 vials
Transfection Ready	No	Yes	Yes

^{*} Suggested split ratio. Optimal split ratio must be determined by end user.



♦ iPS/ES CELL CULTURE

Growth Factors for Stem Cell Research

Name	Expressed in	Function	More	Cat. No.
Activin A human	Human cells	Mesodermal induction;	A otivino	SRP6153
Activin B human	СНО	neural cell differentiation	<u>Activins</u>	A1729
BMP-4 human	HEK 293	Bone formation; induction of ventral mesoderm	BMPs	SRP6156
EGF human	E. coli	Generation of neural	ECEc	E9644, E5036
EGF human	HEK 293	progenitors	<u>EGFs</u>	SRP6253
FGF-1 human	E. coli	Embryonic development; angiogenesis		F5542
FGF-2 human	E. coli			F0291
FGF-4 human	Human cells		FGFs	SRP6160
FGF-7 human	E. coli			SRP6161
FGF-10 human	E. coli		•	F8924
HGF human	NSO			H9661
HGF human	CHO	Generation of liver progenitors	HGF	SRP6014
HGF human	HEK 293	p. ogoo.	•	SRP6166
IL-3 human	HEK 293	Generation of liver, cardiac, hematopoietic progenitors		H7166
IL-6 human	HEK 293		Interleukins	SCU0001
IL-11 human	E. coli		-	SRP3072

Name	Expressed in	Function	More	Cat. No.
Noggin human	HEK 293	Generation of pancreas progenitors	Noggins	N17001
PDGF human	E. coli	Generation of mesenchymal progenitors	PDGFs	SRP3138
SCF human	HEK 293	Generation of hematopoietic progenitors	SCFs	H8416
TGF-b1 human	СНО	Maintenance and differentiation of embryonic stem cells and somatic stem cells	TGFs	<u>T7039</u> , <u>11412272001</u>
VEGF human	E. coli	Generation of cardiac and hematopoietic progenitors	VEGFs	V7259, SRP3182
Wnt-1 human	E. coli			SRP4754
Wnt-2 human	E. coli			SRP6560
Wnt-3a mouse	E. coli	Tissue homeostasis, tissue patterning and cell fate	Wnts	GF154
Wnt-5a mouse	E. coli			GF146
Wnt-7a human	HEK 293			SRP3296



♦ iPS/ES CELL CHARACTERIZATION



Pluripotency Markers, Kits & ES Characterization Kits

Cell Surface Pluripotency Markers

Target	Clonality	Validated Application	Sequence Identity	Cat. No.
NACC1	Polyclonal	IHC, WB*, ICC-IF	Mouse/Rat	HPA062245
NANOG	Monoclonal	IHC, WB, ICC-IF	71%/73%	AMAb91393
NES	Monoclonal	IHC, WB*, ICC-IF	-	AMAb90556
OCT4/POU5F1	Polyclonal	ICC-IF	47%/42%	HPA058267
SALL4	Polyclonal	IHC*, ICC-IF	76%/76%	HPA015791
SOX2	Monoclonal	IHC, WB*, ICC-IF	74%/74%	AMAb91307
TCL1	Polyclonal	IHC*, WB	99%/99%	HPA016604
FUT4/SSEA4/CD15	Monoclonal	WB	52%/53%	AMAb91414

Induced PSCs Markers

Target	Clonality	Validated Application	Sequence Identity	Cat. No.
ANPEP/CD13	Polyclonal	IHC*, WB, ICC-IF	78%/78%	HPA004625
FUT4/SSEA4/CD15	Monoclonal	WB	-	AMAb91414
NANOG	Monoclonal	IHC, WB, ICC-IF	_	AMAb91393

Item Description	Cat. No.
ES Cell Characterization Kit	SCR001
Fluorescent Mouse ES/iPS Cell Characterization Kit	SCR077
Fluorescent Human ES/iPS Cell Characterization Kit	SCR078
Alkaline Phosphatase Detection Kit	SCR004
Quantitative Alkaline Phosphatase ES Characterization Kit	SCR066
Human, iPS Selection Kit	SCR502
AldeRed ALDH Detection Assay	SCR150
BioTracker 529 Green Pluripotent Stem Cell Dye	
Live cell imaging dye that selectively identifies undifferentiated human ES and iPS cells.	SCT029
BioTracker CyP AP Live Cell Dye	
Live cell imaging dye for alkaline phosphatase (AP) for cancer and stem cell applications.	SCT046

Stem Cell Marker >





Mouse ES cells/Mouse Embryo Workflow

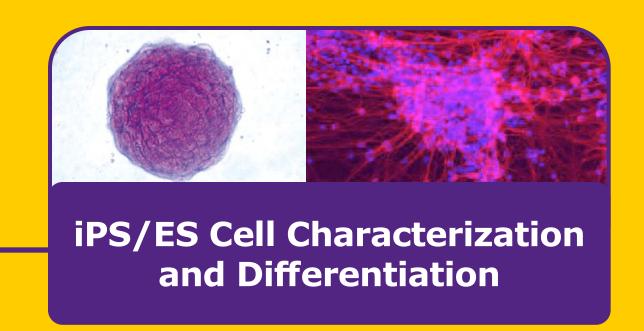


- Mouse ES cell lines
- PMEFS
- ESGRO Supplement
- ESGRO Complete: Serum-free, feeder-free
- ESGRO-2i media: Naive Culture Conditions
- PCR Reagents for Genotyping (KOD Hot Start)
- Growth Factors
- <u>ECM Proteins</u> (Laminin, Fibronectin)
- Transfection Reagents
- CRISPR



Mouse Embryo Culture

- EmbryoMax Embryo Media:
 - KSOM Media
 - M2 Media
 - M16 Media
 - FHM Media
- Human Tubial Fluid (HTF)
- Embryo Tested Mineral Oil
- BSA
- HEPES
- CRISPR
- General Embryo Tested Reagents: Gelatin, NEAA, Glutamine, bME, basal media, ES Screened FBS



- Alkaline Phosphatase Detection
- Pluripotency Antibodies
- ES Cell Characterization Kits
- ES2N medium (ES to Neural Cell)
- N21, Neuro-2 Supplement
- Mouse Embryoid Body Formation Medium
- Growth Factors
- ECM Proteins
- Small Molecule Regulators
- Lineage Specific Antibodies
- Epigenetics Antibodies and Kits



Mouse ES Cell Lines

Item Description	Qty.	Cat. No.
PluriStem™ B6-White Murine ES cell line	5 x 106 cells	SCR011
PluriStem™ 129/S6 Murine ES cell line	5 x 106 cells	SCR012
EmbryoMax® 129/SVEV (S6) Murine ES cell line	5 x 106 cells	CMTI-1
EmbryoMax® C57/BL6 Murine ES cell line	5 x 106 cells	CMTI-2
ESGRO Complete™ Adapted C57/ BL6 Mouse ES Cell Line	5 x 106 cells	SF-CMTI-2
PluriStem® C57BL/6N Murine ES Cells	5 x 106 cells	SCC050
PluriStem® DBA/2N Murine ES Cells	5 x 106 cells	SCC054
PluriStem® C3H/HeN Murine ES Cells	5 x 106 cells	SCC055



New Cell Lines are constantly being added to our website, **SigmaAldrich.com**.

Remember to check back regularly for updates.

ESGRO® Leukemia Inhibitory Factor (LIF)

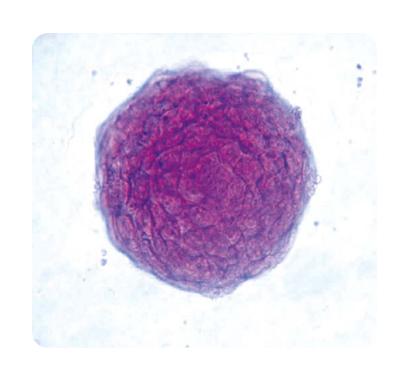
LIF is a growth factor (protein) that can be added to cell culture media to maintain pluripotency of mouse ES cells.

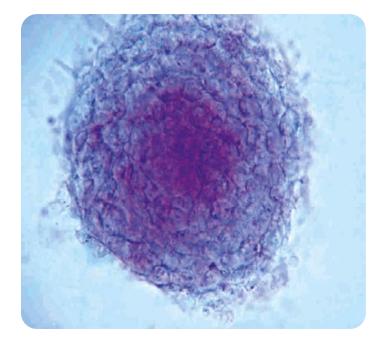
Maintaining pluripotency

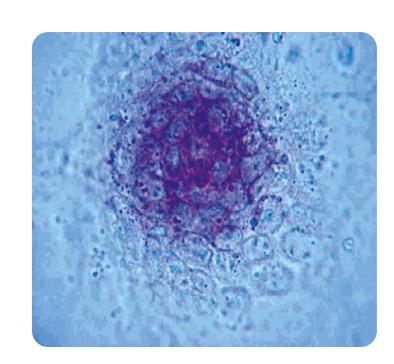
For over a decade, stem cell researchers have trusted their cultures with ESGRO® mouse LIF supplement for maintaining the pluripotent state of their mouse ES cell lines. The gold standard for undifferentiated mouse ES cell culture, ESGRO® mLIF features:

- Consistent inhibition of ES cell differentiation
- Convenient format, supplied in active units/mL
- No batch-to-batch variation
- Flexibility; supports feeder-free and feeder-based cell culture

Item Description	Cat. No.
ESGRO® Leukemia Inhibitory Factor (LIF), 1 million units/1 mL	ESG1106
ESGRO® Leukemia Inhibitory Factor (LIF), 10 million units/1 mL	ESG1107
Leukemia Inhibitory Factor Protein, Recombinant mouse	L5158
Leukemia Inhibitory Factor Protein, Recombinant human	L5283
Leukemia Inhibitory Factor human, animal component free	L9545







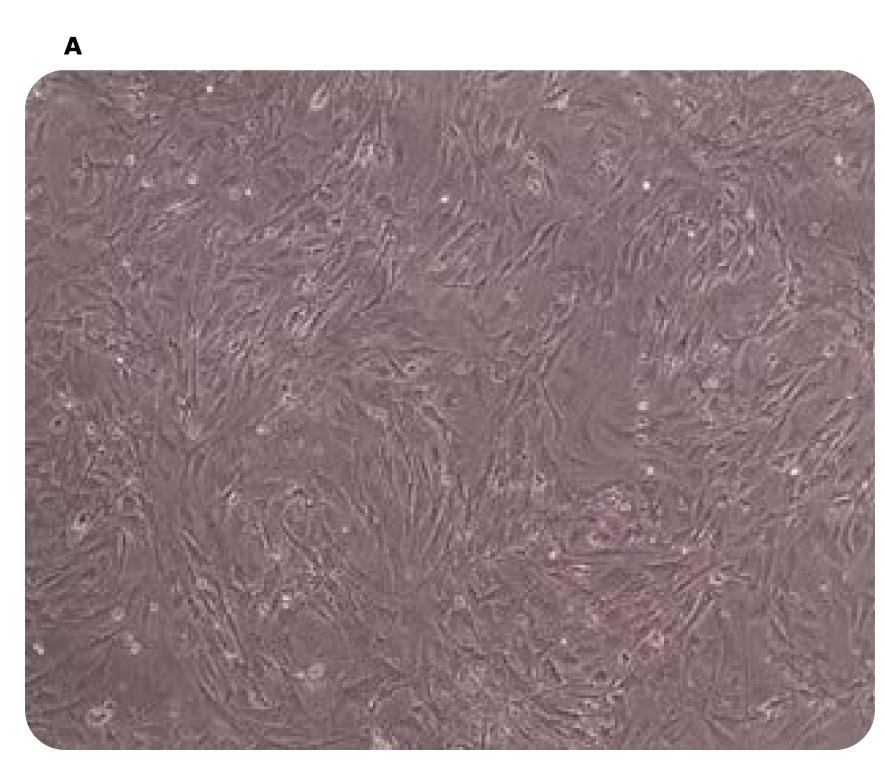
Alkaline Phosphatase staining of ES cells.

- A. Undifferentiated ES cells (mouse MBL.5 cell line) cultured for five days in media containing CHEMICON's LIF/ESGRO®. A concentration of 103 Units/mL is used for inhibition of differentiation.
- B. Differentiated ES cells cultured at low-medium density for three days in media without any LIF/ESGRO®.
- C. Differentiated ES cells cultured at low-medium density for six days in media without any LIF/ESGRO®.

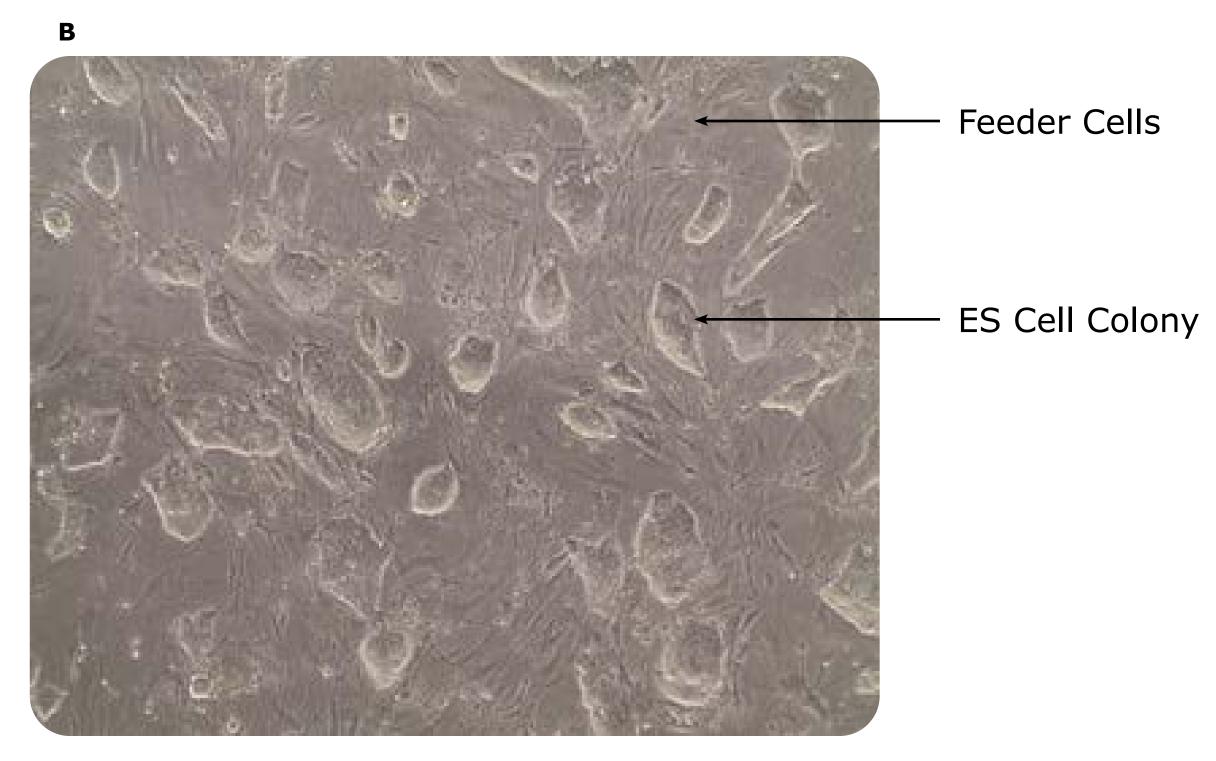


EmbryoMax® MEF feeder cells

Highly Published Mouse Embryonic Fibroblasts Cells that can support the growth of Mouse and Human ES Cells in a co-culture method.



A. PMEF-CF-C Mouse Embryonic Fibroblasts (MEFs) seeded at a density of 5X10^4 cell/cm2 onto 0.1% gelatin coated plates in FBS containing MEF media 24hrs after seeding.



B. CMTI-1 mouse ES cells grown on MEFs in mouse ES cell media maintain correct pluripotent morphology (clumped colonies with clear/bright borders).

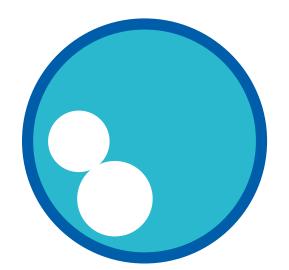
✓ MOUSE EMBRYO CULTURE

KSOM Embryo Culturing Media

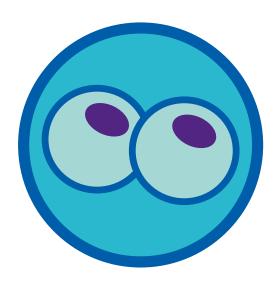
KSOM is a unique mouse embryo culture media that improves the development of mouse preimplantation embryos in vitro.

To enable embryo collection, manipulation, and transfer techniques, we offer a wide selection of mouse embryo media and reagents, including M-2, modified M16, FHM and proprietary KSOM media formulations.

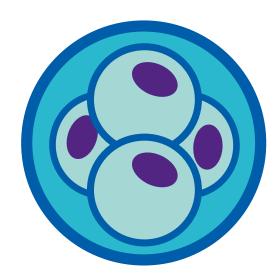
Stages of Embryo Development



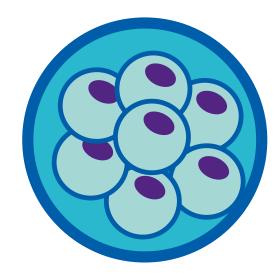
Zygote Mouse Day 0



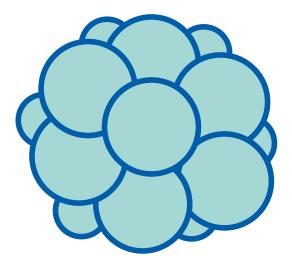
2-cell Day 1



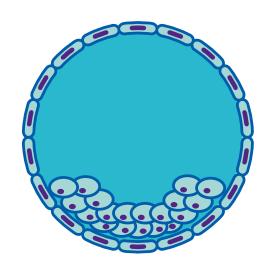
4-cell Day 1-2



Multi-cell Day 2



Morula
Day 2-3



Blastocyst Day 3-4

New Differentiated Product: Advanced KSOM (MR-101-D) single media for both handling and growth of mouse embryos (Exclusively Licensed from Harvard- KSOM Inventor)

EmbryoMax® Liquid Mouse Embryo Media is produced on a bi-monthly basis. For regular users, we encourage standing orders to ensure that backorders do not occur.



◆ MOUSE EMBRYO CULTURE

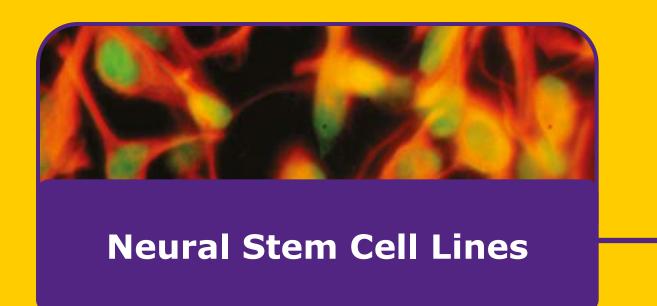


EmbryoMax® Mouse Embryo Culture Reagents

Item Description	Qty.	Cat. No.
EmbryoMax® M2 Medium (1x), Phenol Red	50 mL	MR-015-D
EmbryoMax® M2 Medium (1x), Phenol Red & hyaluronidase	10 mL	MR-051-F
EmbryoMax® M2 Medium (1x), Powdered Media Kit	1 x 50 mL	MR-015P-D
EmbryoMax® M2 Medium (1x), Powdered Media Kit	5 x 50 mL	MR-015P-5D
EmbryoMax® M2 Medium (1x), Powdered Media Kit	5 x 10 mL	MR-015P-5F
EmbryoMax® Modified M16 Medium (1x), w/o Phenol Red	50 mL	MR-010-D
EmbryoMax® Modified M16 Medium (1x), Phenol Red	50 mL	MR-016-D
EmbryoMax® Modified M16 Medium (1x), Powdered Media Kit , w/o Phenol Red	1 x 50 mL	MR-010P-D
EmbryoMax® Modified M16 Medium (1x), Powdered Media Kit , w/o Phenol Red	5 x 50 mL	MR-010P-5D
EmbryoMax® Modified M16 Medium (1x), Powdered Media Kit , w/o Phenol Red	5 x 10 mL	MR-010P-5F
EmbryoMax® FHM Mouse Embryo Media,(1X), Liquid, w/o Phenol Red and BSA	50 mL	MR-122-D
EmbryoMax® FHM Mouse Embryo Media,(1X), Liquid, with Phenol Red & Hyaluronidase	10 mL	MR-056-F
EmbryoMax® FHM Mouse Embryo Media,(1X), Liquid, w/o Phenol Red	50 mL	MR-025-D
EmbryoMax® FHM Mouse Embryo Media,(1X), liquid, with Phenol Red	50 mL	MR-024-D

Neural Stem Cells

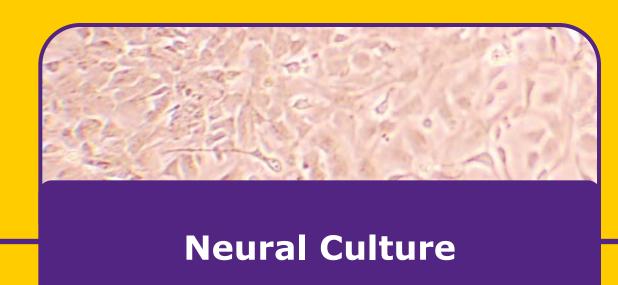




Human Neural Stem Cell Lines

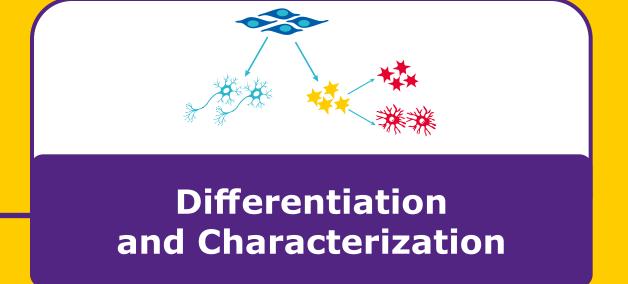
- ReNcell® VM
 Neural Progenitors derived from the ventral mesencephalon brain region
- ReNcell® CX
 Neural Progenitors derived from the cortical brain region
- ENStem-A™ Neural Progenitors
 H9 human embryonic stem cells
 derived NSCs that proliferate as an
 adherent monolayer
- XCell Sciences Neural Cells
 Gene-edited isogenic knockout neural cells
- Oligodendrocyte Progenitors

Mouse Neural Stem Cell LinesRat Neural Stem Cell Lines



Media & Supplements

- ReNcell® NSC Maintenance Media
- ENStem-A[™] Neural Expansion Medium
- Neural Stem Cell Basal Medium
- Stemline® Neural Stem Cell Expansion Medium
- Rat Neural Stem Cell Basal Medium
- Rat Neural Stem Cell Growth Medium
- Neural Stem Cell Freezing Medium (1X)
- ReNcell® VM Neural Stem Cell Freezing Medium
- NDiff Neuro-2 Medium Supplement (200x)
- NDiff Neuro-27 Medium Supplement (100x)
- N21 Medium Supplement (50X)



Differentiation

- Human ES/iPS Neural Induction Medium
- Human ES/iPS Neuronal Differentiation Medium
- Human ES/iPS Neurogenesis Kit
- Human Dopaminergic Neurogenesis Kit

Characterization

- Progenitor Markers: Nestin, Sox-2, Pax-6, Musashi, Neuro-D
- Neuronal Markers: ß-tubulin, MAP2, NeuN, Vglut
- Glial Markers: GFAP, Olig1, MBP, Gal-C, MOG, S100-beta
- Human Neural Stem Cell Characterization Kit



ReNcell® Neural Progenitor Cells

Cell Lines derived from brain tissue from 10 week old fetal tissue.

There are 2 cell types:

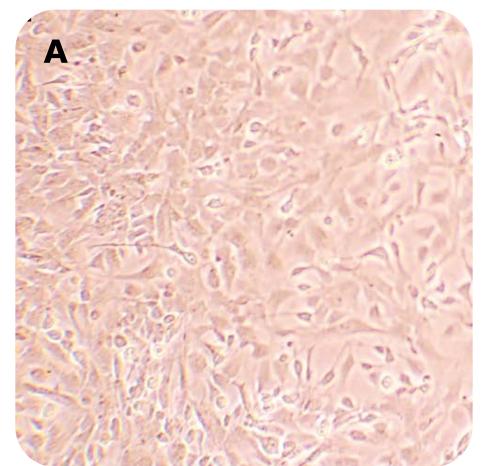
- ReNcell® VM isolated from ventral mesencephalon
- ReNcell® CX isolated from cortex

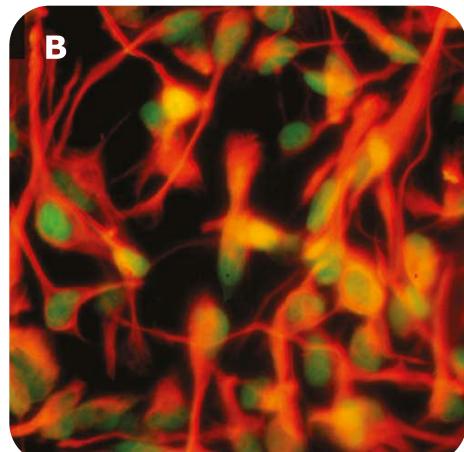
Cell lines are immortalized

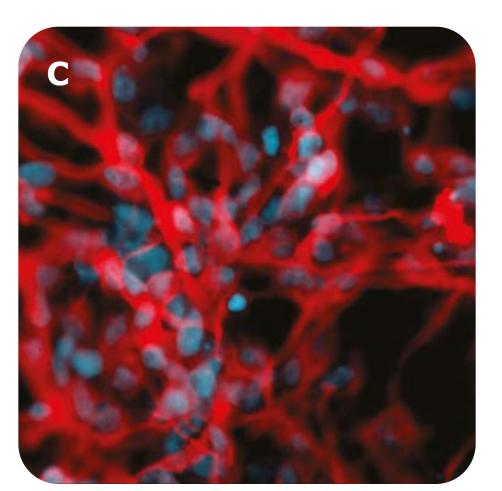
• Transduced with oncogene (myc) which promotes continuous growth and maintains a stable genotype

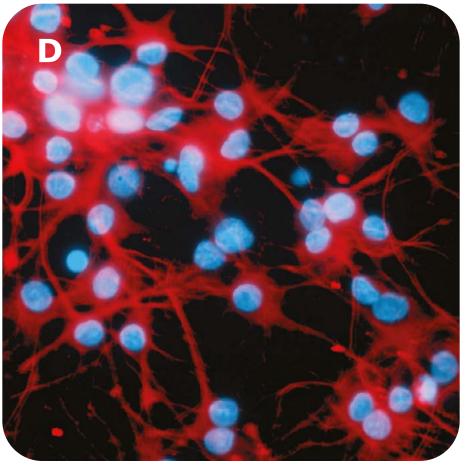
Cell Culture

- Both cell lines grow as monolayers on laminin
- Optimized, Serum-free, defined medium (ReNcell[®] Maintenance Medium, SCM005)
- Maintain multipotency for >45 passages (morphology, karyotype, Sox-2 and Nestin expression)









ReNcell VM cells (CHEMICON® Cat. No. SCC008) are grown as monolayers (A) and express NSC markers, Nestin (B, red, MAB5326) and Sox-2 (B, green, AB5603). ReNcell VM cells are able to differentiate into neurons (βΙΙΙ-tubulin, MAB1637; C) and glial cells (GFAP, AB5804; D).



ReNcell® Neural Progenitors



Features & Benefits

Derived from human fetal tissue

Convenient source of neural progenitors from single source

Cells are immortalized

Can be passaged indefinitely, maintaining karyotype stability

Maintain stability for up to >45 passages

Extended lifespan

Optimized, serum-free Maintenance media available

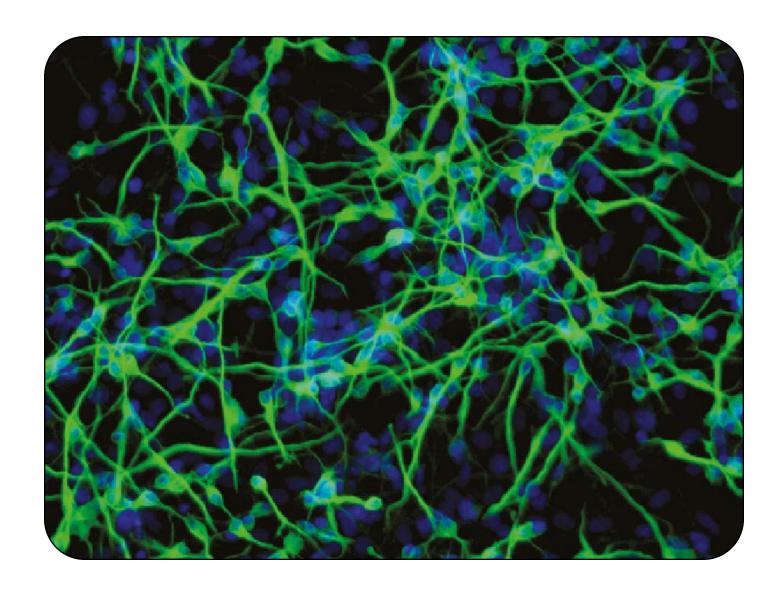
Defined, pre-optimized media for guaranteed performance



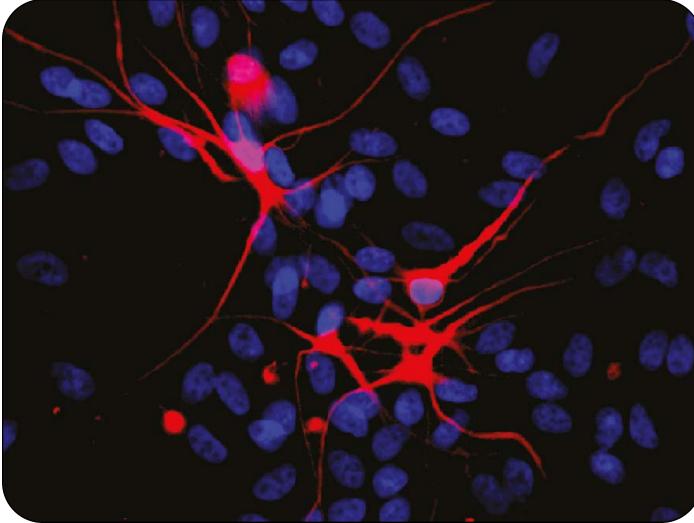
ReNcell® Neural Stem Cells

Both CM and VX can differentiate into all 3 neural phenotypes

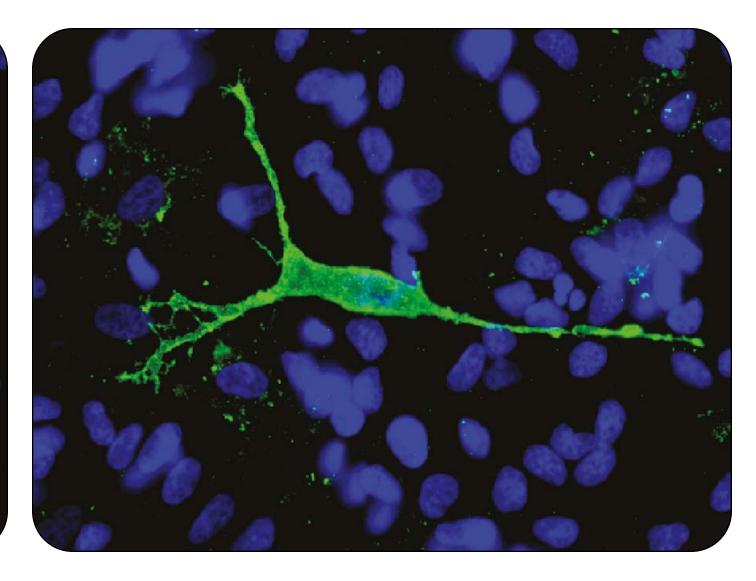
Differentiation of ReNcell® VM Cells



Neurons as shown by BIII Tubulin expression (Green)



Astrocytes as shown by GFAP expression (Red)



Oligodendrocytes as shown by Gal C expression (Green)

All samples counterstained with Hoechst Nuclear Stain (Blue)



3D Stem Cell Culture for Alzheimer's Disease



Experimental Workflow

Human Neural Progenitor Cells **Viral transfection of FAD** gene mutations

FACS enrichment

3D Culture

Neural Differentiation

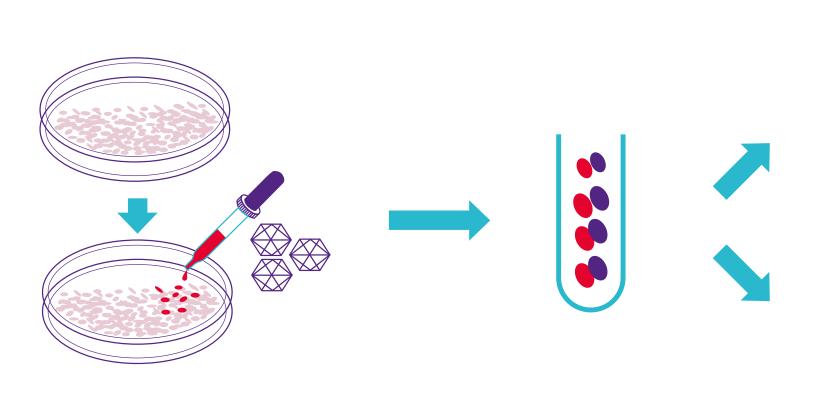
Characterize

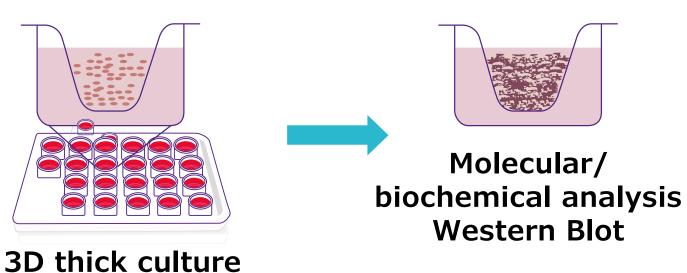
Differentiation Media

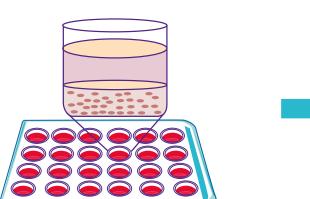
- Human iPS/ES Induction Medium (SCM110)
- Human iPS/ES Expansion Medium (SCM004)
- Human iPS/ES Differentiation Medium (SCM111)

ReNcell® **Immortalized Human** Neural Progenitor cells



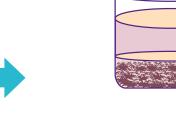






3D thin culture





IF, IHC **Analysis**

Molecular/

Western Blot

NSC/Neuron Characterization

- hNSC characterization kit (SCR060)
- Abs against neuronal Markers:
 - β-tubulin, MAP2, Tau, NeuN, VGlut, Tyrosine Hydroxylase, Synapsin, DCX, ChAT

APPSL-GFP Lentiviral Particle (SCR526)

PSEN1-RFP Lentiviral Particle (SCR527)

Extracellular Matrix

- ECM Gel (E1270)
- TrueGel3D™ (TRUE1)

Culture plates:

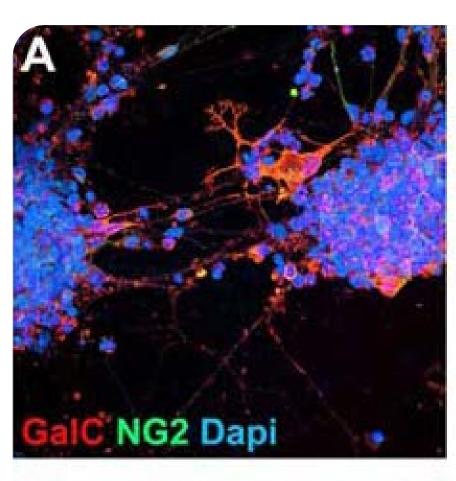
- Corning® 96 well Clear bottom microplate (CLS3370)
- Millicell® CM culture plate inserts (PICM01250)
- Millicell®-24 Cell culture insert plate (PSHT010R5)

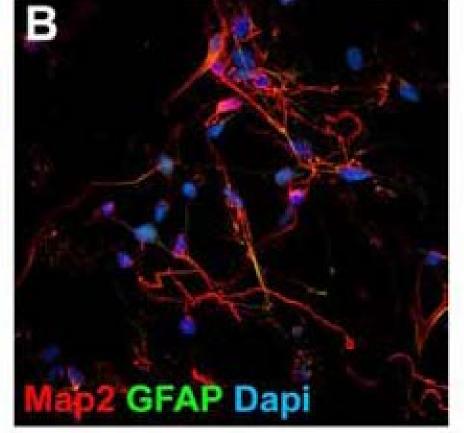


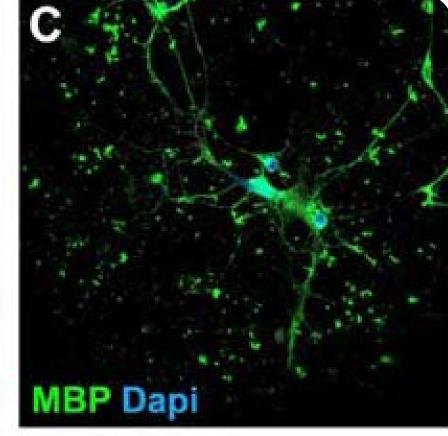


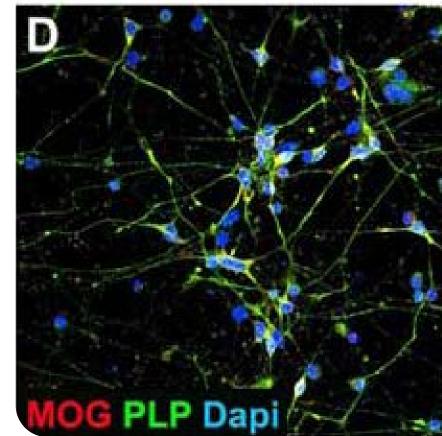
Human Oligodendrocyte Progenitor Cells (OPC)

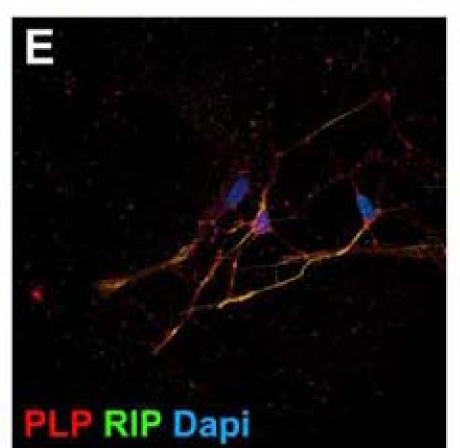
- Problem: Differentiation protocols to make Oligodendrocytes from ES cells takes >100 Days
- Solution: Highly Pure Cryopreserved Oligodendrocyte Progenitors (OPCs) derived from ES-NSCs that will expand for 3-5 passages and differentiate into mature oligodendrocytes in 2-3 weeks (ability to myelinate neurons)
- Serum-Free and Defined Media (Expansion and Differentiation)











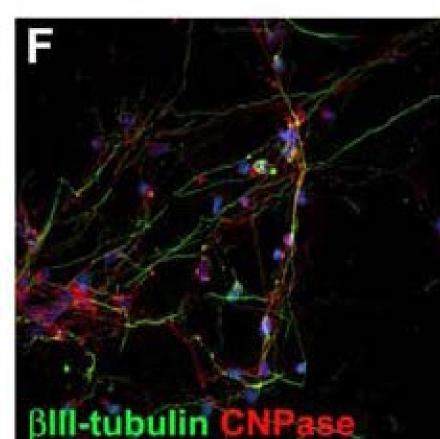


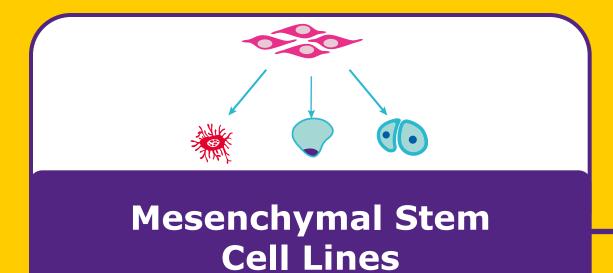
Figure 4. Mature Oligodendrocyte Characterization. After 2 weeks of spontaneous differentiation, Human OPCs generate approximately 30% mature oligodendrocytes (MBP, MOG, PLP, RLP, RIP) and ~50% neurons (Tubulin, MAP2). Human OPCs were plated at 104 cells/cm² onto poly-Lornithine and laminin coated 24 well plates in Human OPC Expansion Complete Media. Twenty-four hours post-seeding, spontaneous differentiation was initiated by media exchange with Human OPC Spontaneous Differentiation Complete Media.

Derivation of Functional Oligodendrocyte Progenitor Cells (OPCs) from Human Neural Stem Cell Lines



Mesenchymal Stem Cells





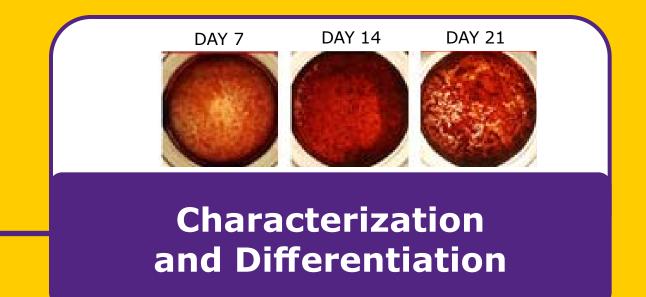
Human MSC Lines

- Bone Marrow
- Adipose Tissue
- ES Cell Derived



MSC Expansion Media

- Xeno-Free/animal free supplement
- Mesenchymal Stem Cell Validated FBS
- PLTMax® Human
 Platelet Lysate



MSC Differentiation Media

- OsteoMAX-XF (SCM121)
- AdipoMAX (SCM122)
- ChondroMAX (SCM123)

MSC Characterization

- CD105, CD73, CD90, STRO-1
- Human Mesenchymal Stem Cell Characterization Kit

MSC Stains

- Oil-Red-O
- Alizarin Red
- Safranin-O/Alcian Blue



OsteoMAX-XFTM Differentiation Media

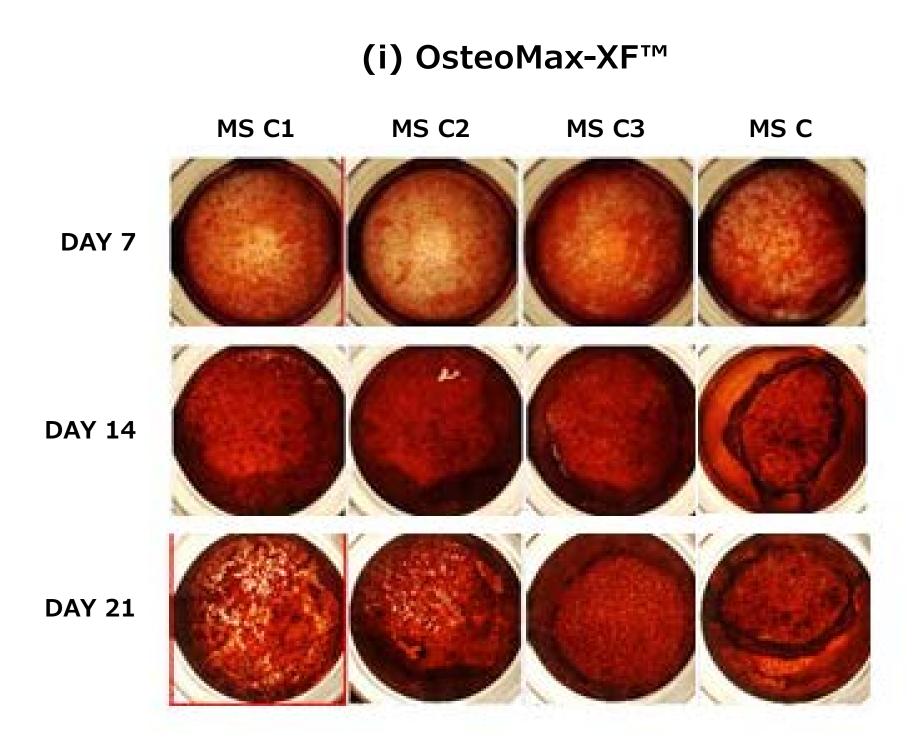
Optimal Complete Media for Accelerated Differentiation of MSCs into Osteocytes

Features and Benefits

- Serum-free, xeno-free, defined medium formulation
- Rapid, robust, and highly efficient mineralization and bone nodule formation in standard tissue culture system by as early as 7 days of differentiation.
- Proven to work in 3D
 BioPrinting applications

"OsteoMAX-XF produces tissue of impressive osteogenic potential, including key bonespecific and mineralization markers. We found we could rapidly fabricate functional, fully human tissue derived from MSCs and the ECM proteins they produce using the NovoGen MMX 3D-Bioprinter"

Eric Michael David MD JD, Chief Strategy Officer, Organovo



(ii) Competitor's Osteogenesis Kit

Alizarin Red staining of representative wells at day 7, 14 and 21



▼ MESENCHYMAL CULTURE

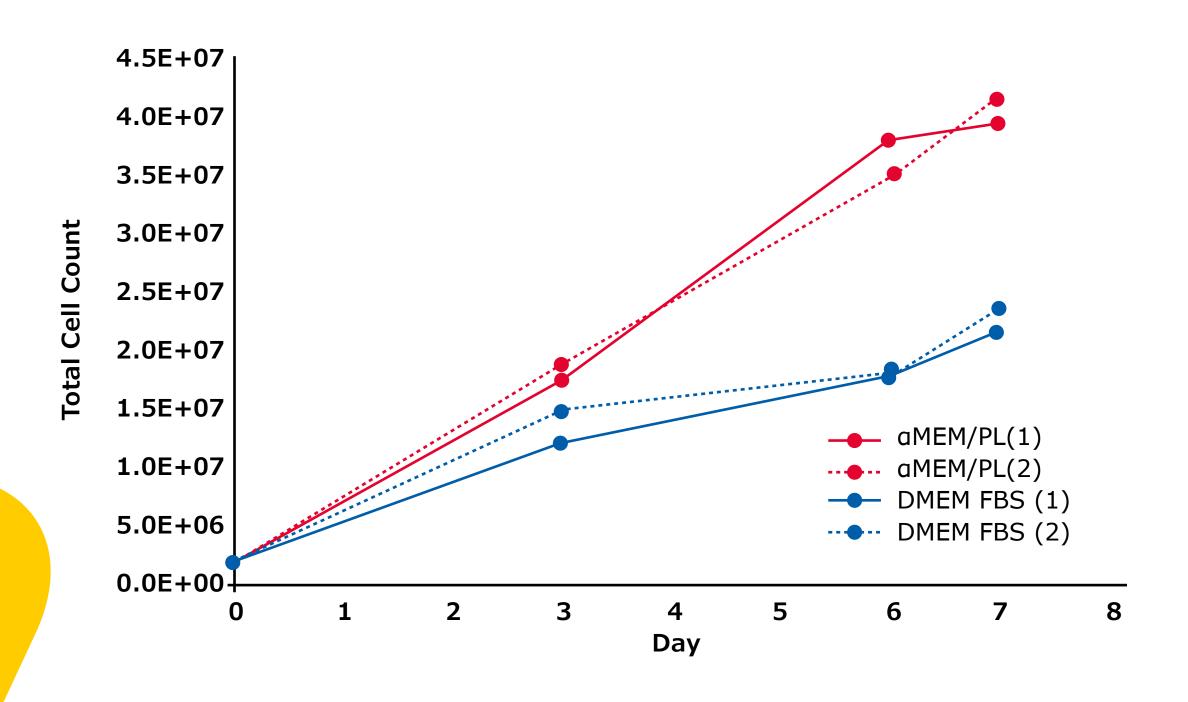
PLTMax Human Platelet Lysate

A Superior Alternative to FBS for Human MSC Cultures

Features and Benefits

- Increased cell growth kinetics vs. FBS supplemented media
- Cost effective alternative to serum free media
- Manufactured to reduce lot-to-lot variation
- Human derived material applicable for translational research applications







◆ MESENCHYMAL CULTURE

PLTGold™ >

PLTGold™ Human Platelet Lysate

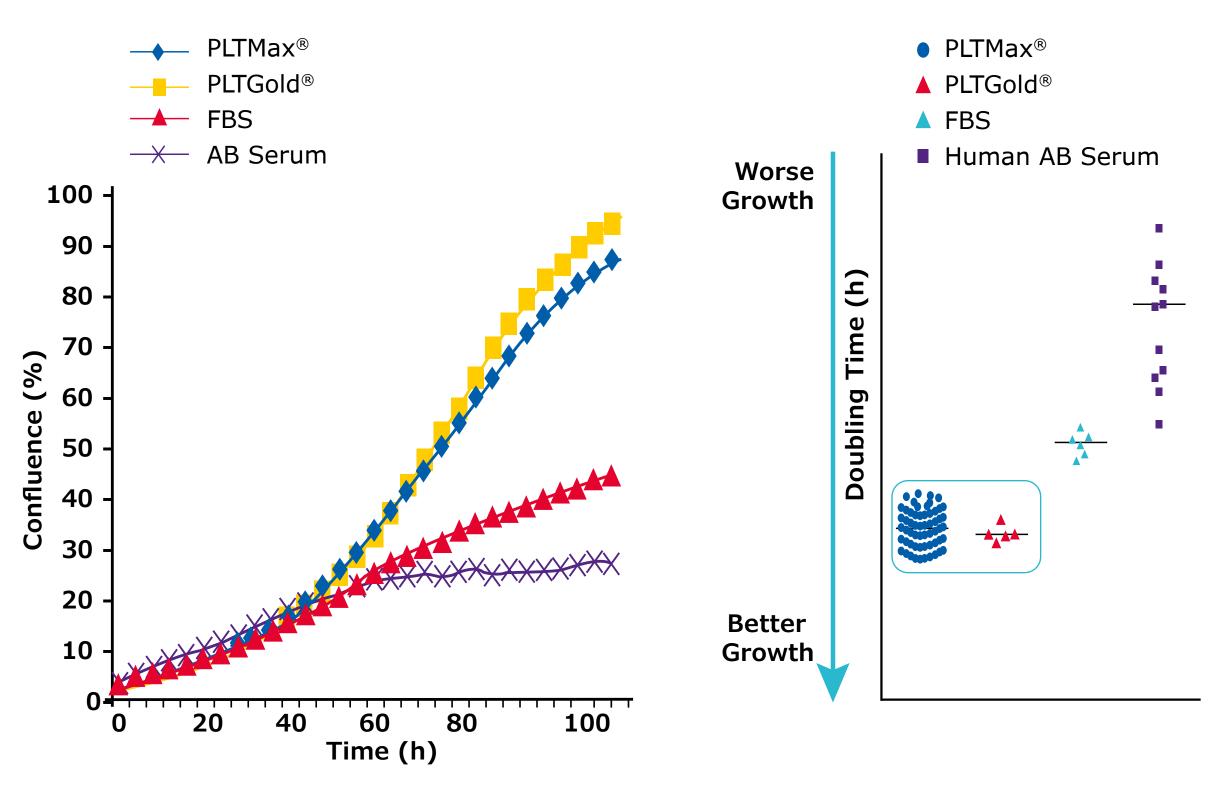


Completely Xeno-Free/animal free supplement that is a superior alternative to fetal bovine serum (FBS) for use in human mesenchymal stem cell cultures.

PLTGold is an unfractionated product derived from human platelets that does not require the addition of heparin.



PLTGold remains clot-free without the addition of Heparin

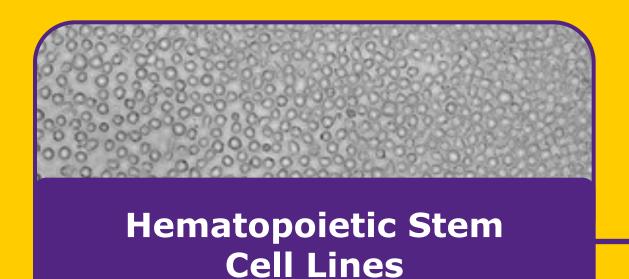


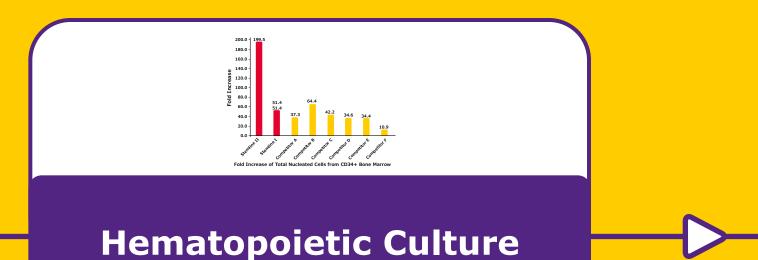
PLTGold has similar performance to PLTMax



Hematopoietic Stem Cells (HSCs)







Stem Cell lines & Immune Cells

- CD34+ Hematopoietic Progenitor Cells
- Human Monocytes
- Human Mononuclear Cells (PBMCs)
- Human Macrophages
- Human Dendritic Cells

Expansion Media

- Hematopoietic Progenitor Expansion Medium DXF
- Hematopoietic Progenitor Medium
- Stemline® II Hematopoietic Stem Cell Expansion Medium

Cytokines and Growth Factors

Cytokines/Growth

Factors/Markers

• Interleukins, GM-CSF, G-CSF, M-CSF, EPO, TPO, SFC, Flt-3 Ligand

Markers:

- CD34, CD38, CD90, CD133, CD105, CD45, c-Kit, SCA-1, Stem Cell Factor, ALDH
- AldeRed™ ALDH Detection Kit

Stromal Cells for T-Cell Differentiation





To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**Technical Service: **SigmaAldrich.com/techservice**

Safety-related Information: SigmaAldrich.com/safetycenter

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