Stem Cell Research

Cells, media, reagents and tools for stem and specialty cell culture

Merck Millipore is a division of MERCK

Platforms, Technologies, and Services

As a tools provider and partner in research, Merck Millipore is committed to the advancement of life science research and drug discovery. This guide includes a number of new and existing novel products for the stem cell workflow including reprogramming kits, cell culture media, and characterization tools. These products provide proven solutions for a range of applications and are backed by extensive technical support.

STEM CELLS AND PRIMARY CELLS

Merck Millipore offers an extensive range of embryonic, neural, and mesenchymal stem cells for both human and rodent studies. This includes novel human neural and mesenchymal stem cells, and a complete line of mouse embryonic stem cells. Endothelial and epithelial cells are also available.

www.merckmillipore.com/stemcells

CELL CULTURE MEDIA AND REAGENTS

Merck Millipore provides media designed for virtually all types of stem cells, including human and rodent embryonic, mesenchymal and neural stem cells. Many of these optimized media are available as serum-free, feeder-free formulations, validated specifically for stem cells. Supporting the full range of expansion and differentiation media are feeder cells, supplements, passaging and cryopreservation reagents, cell cultureware and sterile filtration devices.

www.merckmillipore.com/stemcells
www.merckmillipore.com/cellculture

ANTIBODIES AND IMMUNOASSAYS

Merck Millipore offers an extensive, focused portfolio of antibodies, immunoassays and novel tools for improving the Western blotting workflow. With the expertise of Upstate® and Chemicon®, Merck Millipore provides validated products with breadth and depth in major research areas backed by excellent service and support. Our extensive portfolio of antibodies for stem cell research includes widely published stem cell targets as well as recently discovered markers. Characterization kits are also available with panels of antibodies to comprehensively analyze multiple differentiation pathways.

www.merckmillipore.com/antibodies

CELL-BASED ASSAYS AND LIVE CELL IMAGING

Our portfolio of live cell, whole-cell, and cell-based activity assays and reporter systems enable direct and indirect detection of cellular processes. These technologies, including new SmartFlare™ probes for detecting specific RNAs in live cells, facilitate protein target validation, identify cellular pathways, and determine mechanism of action for lead optimization environments. Also available are microfluidic systems that advance stem cell culture by mimicking *in vivo* conditions, providing coculture options, and facilitating live cell imaging.

Cell-based assays:
www.merckmillipore.com/antibodies
Live cell RNA detection:
www.merckmillipore.com/smartflare
Microfluidic culture and live cell imaging:
www.merckmillipore.com/cellasic

FLOW CYTOMETRY ASSAYS AND SYSTEMS

Flow cytometry is essential for simultaneous measurement of multiple parameters and for in-dpeth cell analysis. Our Amnis® imaging flow cytometers, which combine the speed, sensitivity, and phenotyping abilities of flow cytometry with the imagery and functional insights of microscopy, take stem cell research to higher levels of discrimination and discovery. Our easyCyte™ flow cytometers provide precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. Validated FlowCellect® assay kits, Milli-Mark® conjugated antibodies, and application-specific software modules provide a complete solution for flow cytometry.

www.merckmillipore.com/flowcytometry

MILLIPLEX® MAP MULTIPLEX ASSAYS

Isolated biomarkers are often inadequate to distinguish differentiated cells from progenitors or one lineage from a closely related lineage. Multiplexed detection of protein biomarkers can accelerate studies of differentiation, development and stem cell biology. With our MILLIPLEX® MAP magnetic bead-based stem cell assay panels, Merck Millipore is the first to provide multiplexed protein immunoassays for stem cell characterization. Based on Luminex xMAP® technology our MILLIPLEX® MAP portfolio is backed by a complete spectrum of Luminex® instrumentation, software, service and support.

www.merckmillipore.com/milliplex

PROTEIN PURIFICATION AND PREPARATION

For every step of the molecular biology and protein workflow, from cloning DNA targets to purifying and concentrating recombinant proteins, Merck Millipore provides reagents, kits, cells and tools that are specifically designed to meet your scientific and technical goals. For protein quantitation, the infrared-based Direct Detect® spectrometer distinguishes proteins and peptides from interfering sample components, providing more accurate results without the pitfalls of colorimetric assays.

Molecular biology:
www.merckmillipore.com/mobio
Protein purification and preparation:
www.merckmillipore.com/psp
Direct Detect® spectrometer:
www.merckmillipore.com/directdetect

CALBIOCHEM® COMPOUNDS

Small molecule compounds, including inhibitors, activators, and other pathway modulators, are critical tools for researchers studying cell signaling and other intracellular mechanisms that control cell fate, function, and phenotype. From libraries and pathway panels to individual reagents, the Calbiochem® line of products offers the widest and most cited selection of inhibitors and activators worldwide.

www.merckmillipore.com/calbiochem

Table of Contents

1 Cellular Reprogramming
pages 6–15

2 Pluripotent Stem Cells
pages 16–24

3 Neural Stem Cells
pages 25–34

4 Mesenchymal Stem Cells
pages 35–40

5 General Cell Culture
pages 41–42

Introduction

Stem cells have the unique ability to self-renew and generate additional stem cells or to differentiate into various progenitor cells in response to appropriate signals. These properties provide stem cells with unique capabilities for tissue repair, replacement, and regeneration.

Stem cells can be classified as either embryonic stem cells (ESC) or adult stem cells. ESCs are derived from the inner cell mass of preimplantation embryos and are the most pluripotent type of stem cells. They can undergo infinite, undifferentiated proliferation *in vitro* and can also differentiate into a wide variety of somatic and extraembryonic tissues. Adult stem cells, found in differentiated tissues, can self-renew and differentiate into mature cell types of the specific tissue. In contrast to ESCs, adult stem cells can proliferate only for a limited number of cycles, and their response to differentiation signals declines with each cell division.

Embryonic Stem Cells

- Pluripotent
- Highly proliferative
- Potentially tumorigenic
- Ethical concerns
- Non-autologous

Adult Stem Cells

- Multipotent
- Limited proliferative capacity
- Non-tumorigenic
- Less controversial
- Autologous

Merck Millipore is committed to providing the tools you need to advance stem cell research. This guide outlines our comprehensive selection of stem cells, media, supplements, growth factors, cultureware, and tools for stem cell characterization. These proven solutions cover a broad spectrum of stem cell and specialty cell culture areas and are backed by our knowledgeable technical support.

For more information, visit:
www.merckmillipore.com/stemcells

1

Cellular Reprogramming

The successful reprogramming of adult human cells was first reported in 2007 by Shinya Yamanaka and colleagues, who introduced different combinations of pluripotency-associated genes into somatic cells. Since then, many different reprogramming techniques have been reported. Common to all these techniques are certain key stages of the reprogramming workflow: iPS cell generation, iPS Cell Expansion and iPS cell characterization.

iPS Cell Generation

Successful reprogramming of adult human cells is traditionally accomplished by transducing different combinations of pluripotency-associated genes. First, adult tissue is isolated (e.g., dermal fibroblasts), and these cells are expanded *in vitro*. Then, reprogramming factors are introduced into these cells via viral, episomal or protein vectors. Because this process is sensitive to multiple variables, Merck Millipore supports the entire iPS cell generation workflow with resources including fibroblasts validated for efficient reprogramming, optimized growth media and reagents for iPS cells and our STEMCCA™ lentiviral based reprogramming technology.

iPS Cell Expansion

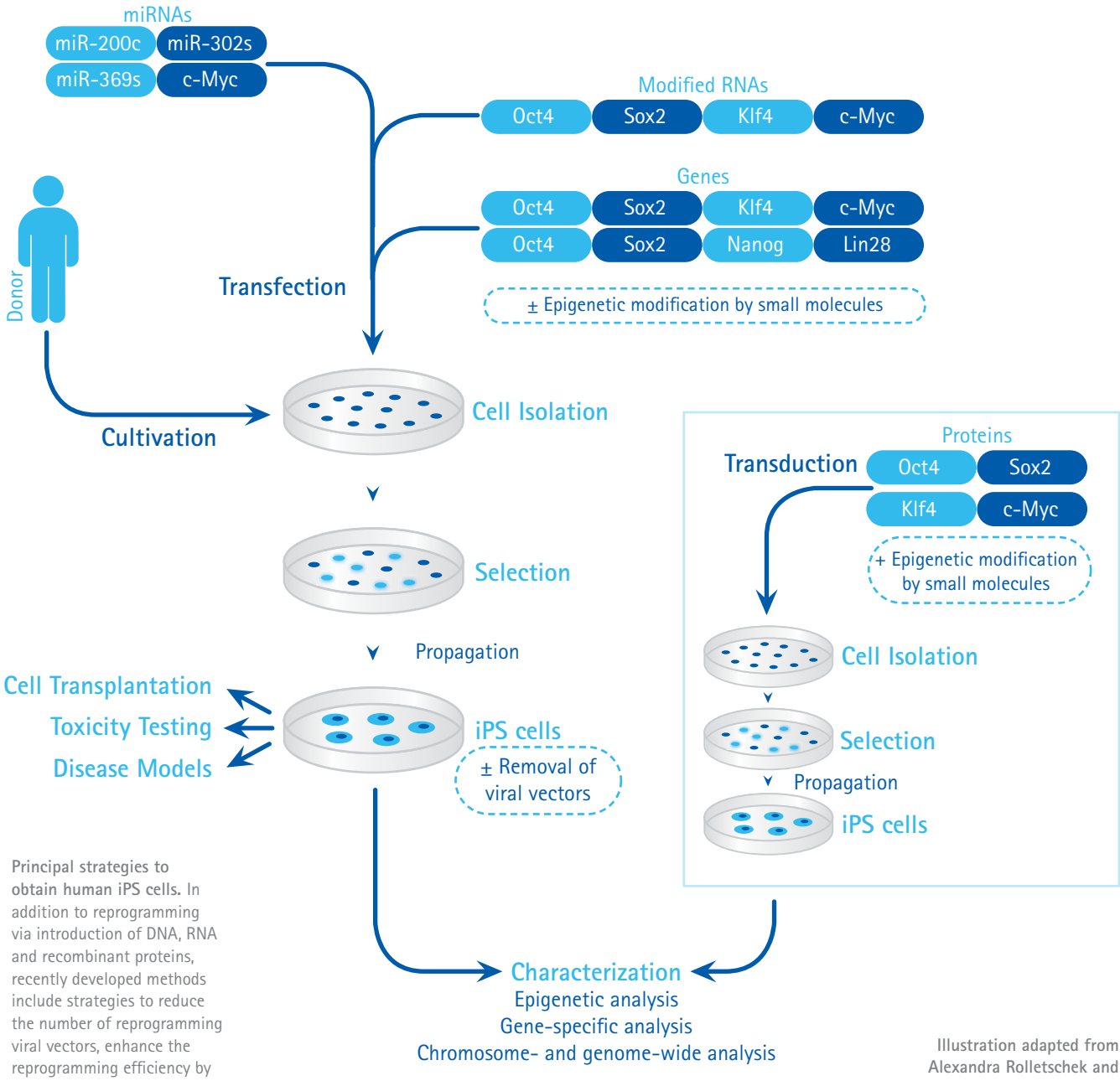
The success of iPS cell generation and subsequent redifferentiation protocols is highly dependent upon the media, growth factors, and extracellular matrix (ECM) environments of the developing colonies. Cells must remain stable enough to survive virus or small molecule treatments, construct excision, ESC expansion conditions, and redifferentiation. Implementing consistency in reprogramming protocols has been challenging due to the nascent state of iPS cell research, in which different laboratories are currently using a wide variety of protocols and conditions. Merck Millipore provides validated high-quality media and supplements that reduce variation and increases success of target terminal differentiation.

iPS Cell Characterization

During the reprogramming process, cells undergo dynamic, but gradual changes, with fully reprogrammed cells showing the most ES cell-like patterns of gene expression and partially reprogrammed cells showing intermediate phenotypes. Thus, classical markers for the pluripotent embryonic state, such as alkaline phosphatase activity or TRA expression, are critical for tracking cell reprogramming and for gaining confidence in the dedifferentiated status of the reprogrammed cells.

Comparing ES and iPS cells continues to be an active area of investigation. Specifically, recent studies have shown differences in gene expression[1], protein phosphorylation[2], gene copy number[3], chromosome number[3] and aberrant DNA methylation[4] between iPS cells and ES cells. Therefore, all iPS cell clones have to be carefully characterized before they can be applied to diagnostic or therapeutic uses. Following characterization of ES-like state, the iPS cells can be guided down distinct differentiation pathways using various growth factors, small molecules or by other extracellular microenvironment manipulations.

1. Chin MH et al. Cell Stem Cell. 2009 Jul 2;5(1):111-23.
2. Phanstiel DH et al. Nat Methods. 2011 Sep 11;8(10):821-7.
3. Mayshar Y et al. Cell Stem Cell. 2010 Oct 8;7(4):521-31.
4. Lister R et al. Nature. 2011 Mar 3;471(7336):68-73.



Principal strategies to obtain human iPS cells. In addition to reprogramming via introduction of DNA, RNA and recombinant proteins, recently developed methods include strategies to reduce the number of reprogramming viral vectors, enhance the reprogramming efficiency by small molecules, and remove viral vectors after successful reprogramming.

Illustration adapted from Alexandra Rolletschek and Anna M. Wobus Biol. Chem., Vol. 390, pp. 845–849, September 2009

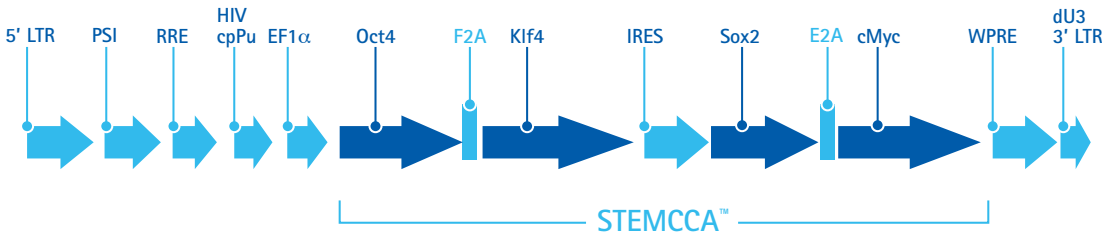
STEMCCA™ Reprogramming Kits

For efficient iPS cell generation with a single vector, use STEMCCA™ lentivirus reprogramming kits. Unlike traditional iPS cell generation, which requires simultaneous co-infection by four separate expression vectors, the STEMCCA™ kits improve efficiency using a single polycistronic lentiviral vector to reduce the number of viral integrations.

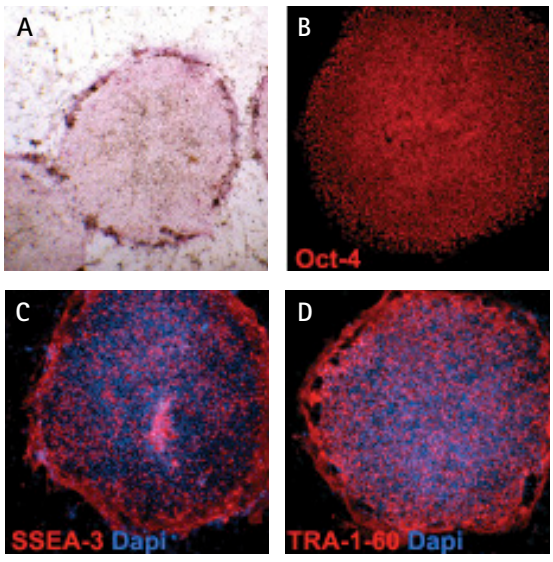
STEMCCA™ kits are available for reprogramming either rodent or human cells, and include lentivirus that express the (human or mouse) OKSM factors from a single polycistronic transcript. Both human and mouse STEMCCA™ lentivirus kits are available in constitutive and Cre/LoxP-regulated formats.

STEMCCA™ Advantages

- **Efficient**: uses a single vector with four transcription factors rather than co-transducing four separate expression vectors.
- **Minimizes viral integrations**: single vector reduces the risks of insertional mutagenesis and viral reactivation.
- **Excisable**: Cre/LoxP-regulated version enables removal of reprogramming transgenes.
- **Inducible**: Dox-inducible version enables enhanced control of viral transgene expression.
- **Ready to use**: convenient, pre-packaged lentiviral particles provided with detailed protocols.



The STEMCCA™ vector is comprised of the transcription factors Oct-4, Klf4, Sox2, and c-Myc (OKSM), separated by the self-cleaving 2A peptide and IRES sequences driven by the EF-1α constitutive promoter. It is also available with flanking LoxP sites incorporated for Cre-mediated excision of the exogenous reprogramming transgenes.



Successful generation of iPS cells from human foreskin fibroblasts after infection with single-vector Human STEMCCA™ Cre-Excisable Lentivirus (Cat. No. SCR545), as indicated by expression of characteristic pluripotency markers. Resulting passage 3 human iPS cells exhibit high alkaline phosphatase activity (A), Oct-4 expression (B), SSEA-3 expression (C), and TRA-1-60 expression (D). Nuclei are stained with DAPI (blue).

Description	Cat. No.
Human STEMCCA™ Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR544
Human STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR545
Human STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM/L-Myc) Lentivirus Reprogramming Kit	SCR548
Mouse STEMCCA™ Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR510
Mouse STEMCCA™ Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR530
Mouse STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR511
Mouse STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR531
Mouse STEMCCA™ Dox-Inducible Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR512
Mouse STEMCCA™ Cre-Excisable Dox-Inducible Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR513

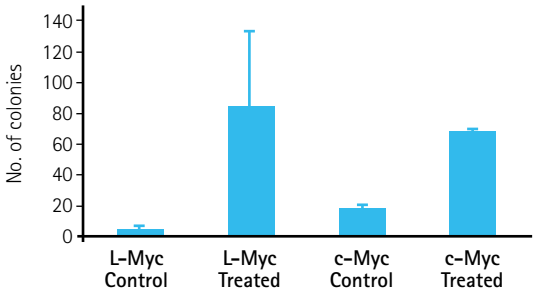
References:
1. Sommer CA, et al. iPS cell generation using a single lentiviral stem cell cassette. Stem Cells. 2009 Mar;27(3): 543-9.
2. Sommer CA, et al. Excision of Reprogramming Transgenes Improves the Differentiation Potential of iPS Cells Generated with a Single Excisable Vector. Stem Cells. 2010 Jan;28(1): 64-74.
3. Merling RK, et al. Transgene-free iPSCs generated from small volume peripheral blood non-mobilized CD34+ cells. Blood. 2013 Apr 4;121(14):e98-e107.

Featured Products for Cellular Reprogramming

Human iPS Reprogramming Boost Supplement II

(Cat. No. SCM094)

Containing three proprietary small molecules (TGF-β RI Kinase Inhibitor IV, Sodium Butyrate and PS48) in amounts sufficient to supplement 350 mL of human ES cell maintenance medium, this cocktail can be used in conjunction with the Human STEMCCA™ lentivirus reprogramming kits to enhance the efficiency of human iPS colony formation by 10-15 fold and shorten the time to establish fully reprogrammed colonies by 50%.



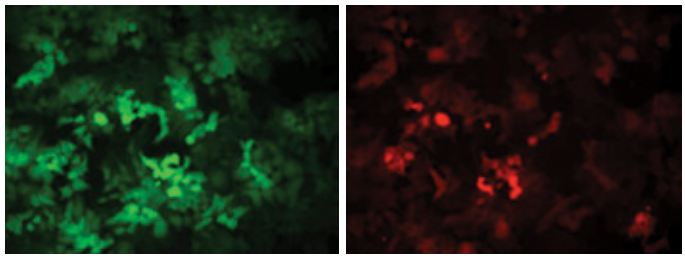
Human iPS Cell Boost Supplement II significantly enhanced colony formation by 28-fold and 4-fold when used in combination with EF1α-hSTEMCCA-LoxP (OKS/L-Myc) and EF1α-hSTEMCCA-LoxP (OKSM) lentivirus kits (Cat. Nos. SCR548 and SCR545), respectively. Results may vary between users and experimental constructs.

Description	Cat. No.
Mouse iPS Reprogramming Boost Supplement	SCM087
Human iPS Reprogramming Boost Supplement	SCM088
Human iPS Reprogramming Boost Supplement II	SCM094

TAT-Cre Recombinase

(Cat. No. SCR508)

Excise reprogramming transgenes using a cell permeable Cre recombinase, which catalyzes the site-specific recombination event between two loxP DNA sites. Merck Millipore's TAT-Cre Recombinase is a fusion protein containing the cell-penetrating peptide sequence TAT (Trans-activator of transcription) along with NLS (nuclear localization sequence) to efficiently excise loxP-flanked genes from live cells. TAT-Cre Recombinase has been validated to efficiently excise STEMCCA™-loxP transgenes from both human and mouse iPS cells with up to 75% efficiency.

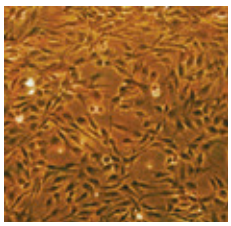


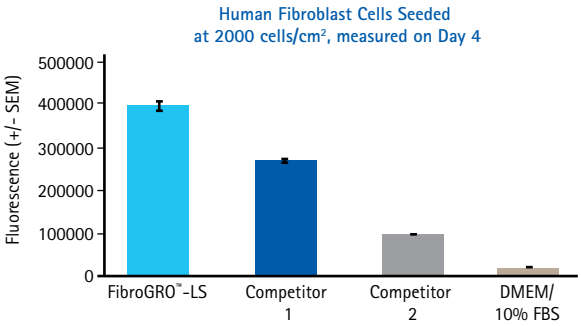
A HEK293 LoxP-GFP reporter cell line was used to monitor the efficiency of Cre recombinase excision using TAT-Cre Recombinase. Non-treated cells express RFP (right) while Cre recombinase-treated cells express GFP (left). TAT-Cre Recombinase has also been validated to function at high efficiencies on STEMCCA™-LOXP-generated human and mouse iPS cells.

Description	Cat. No.
TAT-Cre Recombinase	SCR508
Human STEMCCA™/TAT-Cre Bundle	SCR545-CRE
Anti-Cre Recombinase, clone 2D8	MAB3120
STEMCCA™ Viral Gene Detection qPCR Kit (Human)	SCR580
STEMCCA™ Viral Gene Detection qPCR Kit (Mouse)	SCR581

Xeno-Free Human Fibroblast Feeder Cells and FibroGRO® Medium

FibroGRO® Xeno-Free human foreskin fibroblasts (HFF) are derived from normal human foreskin and have been isolated and propagated under xeno-free conditions, proliferating rapidly in any FibroGRO® Medium (Cat. Nos. SCMF001, SCMF002, SCM037). Rapid proliferation of HFF enables efficient reprogramming of the cells to iPS cells. FibroGRO® Xeno-Free Human Foreskin Fibroblasts have been tested and validated to generate iPS cells using STEMCCA™ Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kits.





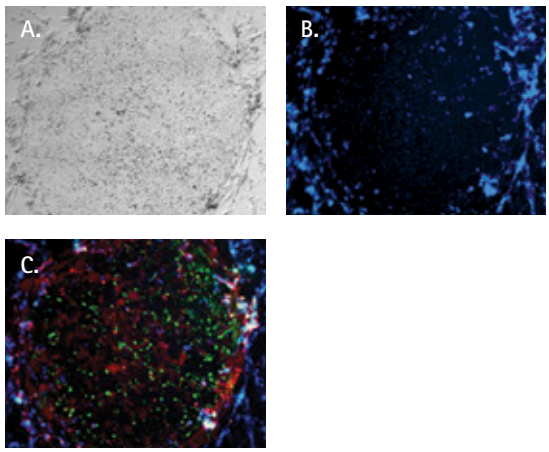
FibroGRO® Xeno-Free Human Fibroblasts display normal fibroblast morphologies (left) for extended passages and expand rapidly in FibroGRO® media. FibroGRO® media provides extremely fast proliferation rates when compared with competitor or standard culture medium (right).

Description	Cat. No.
FibroGRO® Xeno-Free Human Foreskin Fibroblasts	SCC058
FibroGRO® Inactivated Xeno-Free Human Foreskin Fibroblasts	SCC057
FibroGRO® Complete Media Kit for Culture of Human Fibroblasts	SCMF001
FibroGRO®-LS Complete Media Kit for Culture of Human Fibroblasts	SCMF002
FibroGRO® Xeno-Free Human Fibroblast Expansion Medium	SCM037

Human iPS Cell Selection Kit

(Cat. No. SCR502)

Choose this quick, easy and non-invasive method to monitor the pluripotent state of live human ESC and fully reprogrammed human iPS cells using immunocytochemistry (ICC). The Human iPS Cell Selection Kit allows live cell imaging and identification of fully reprogrammed human iPS cell colonies from a heterogeneous population of reprogramming intermediates using conjugated antibodies and enables the live selection of human iPS cells that can be further passaged and expanded for downstream applications. The selected live stained human iPS clones maintain normal ESC morphology and can proliferate and be passaged normally.

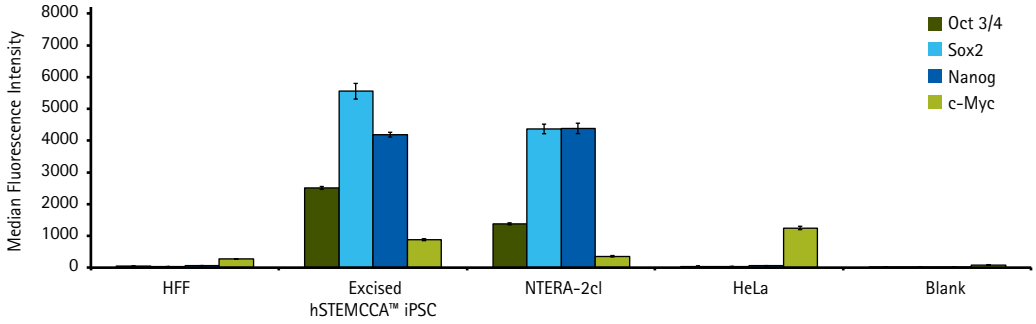


Fully reprogrammed human iPS cells express human pluripotent markers, TRA-1-60 FITC (C, green) and SSEA-4 PE (C, red) while downregulating the fibroblast marker, CD13 PE (data not shown) as visualized with the Human iPS Cell Selection Kit. Cells were stained with cell-permeable Hoechst nuclear dye (B, blue). Fully reprogrammed human iPS cells exhibit Hoechst dim phenotype (see colony center in B) while non-iPS and differentiated cells exhibit a Hoechst bright phenotype (see the periphery of the colony in B, which is surrounded by fibroblast cells and is Hoechst bright).

MILLIPLEX® MAP Human Stem Cell Pluripotency Magnetic Bead Panel 1 and 2

(Cat. Nos. 48-617MAG, 48-620MAG)

Monitoring pluripotency of stem cells is crucial for obtaining consistent experimental results. However, traditional methods to detect pluripotency markers, such as flow cytometry and PCR techniques, have some limitations. In contrast, MILLIPLEX® MAP assays, based on trusted Luminex® xMAP® bead-based technology, offer a quick, simple and accurate way to detect a larger number of pluripotency markers in a single measurement, providing fast feedback on the potency status of a cell culture by measuring protein expression levels. The MILLIPLEX® MAP Human Stem Cell Pluripotency Magnetic Bead Panel 1 is used to simultaneously detect total protein levels of Oct-3/4, Sox2, Nanog, and c-Myc in cell lysates. Panel 2 contains multiplexed immunoassays to detect seven more markers, including LIN-28, TRA-1-60, and E-cadherin.



Differentiated human foreskin fibroblasts (HFF P6, Cat. No. SCC058) were reprogrammed by transduction with the STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (Cat. No. SCR511) resulting in hSTEMCCA™ iPSC and virus-excised hSTEMCCA™ iPSC. HFF and iPS cells were assayed with the 4-plex MILLIPLEX® MAP Human Stem Cell Pluripotency Kit 1 using NTERA-2cl.D1 and HeLa as control cell lines.

Epigenetics Kits and Asssays

www.merckmillipore.com/epigenetics

Epigenetics describes heritable changes in gene expression caused by nongenetic mechanisms without any alterations in DNA sequence. Histone modifications, positioning of histone variants, chromatin and nucleosome remodeling, DNA methylation, and small and non-coding RNA-mediated epigenetic regulation are some of the more frequently studied epigenetic modifications. Given the reported epigenomic differences between differentiated cells, ES cells and iPS cells, identifying differential patterns of epigenetic modifications can be useful in characterizing iPS cells and may help elucidate the mechanisms by which reprogramming confers pluripotency. Merck Millipore offers a complete line of epigenetics research products including:

- Chromatin immunoprecipitation kits
- Bisulfite modification kits and assays
- 5mC and 5hmC enrichment and quantitation antibodies and kits
- DNA methylation standards and kits
- ChIP-validated antibodies
- Histone deacetylase and acetyltansferase inhibitors

Browse all products for epigenetic analysis:
www.merckmillipore.com/epigenetics

Key Products for Cellular Reprogramming

Cells, Reagents and Kits

Description	Cat. No.
EmbryoMax® Primary Mouse Embryo Fibroblasts, Neo Resistant, Not Mitomycin C-treated, Strain FVB, passage 1	PMEF-NL-P1
EmbryoMax® Primary Mouse Embryo Fibroblasts, Not Mitomycin C-Treated, Strain CF1, passage 1	PMEF-CFL-P1
Polybrene® Transfection Reagent	TR-1003-G
Fast-Trap® Lentivirus Purification and Concentration Kit	FTLV00003, FTAV00003
STEMCCA™ Viral Gene Detection qPCR Kit	SCR580, SCR581
Alkaline Phosphatase Detection Kit	SCR004
Quantitative Alkaline Phosphatase ES Characterization Kit	SCR066
ES Cell Marker Sample Kit	SCR002
SmartFlare™ Oct4 Hu-Cy3 RNA Detection Probe	SF-438
SmartFlare™ NANOG Hu-Cy5 RNA Detection Probe	SF-875
SmartFlare™ MYC Hu-Cy5	SF-702

Antibodies

Description	Cat. No.
Anti-Oct-4	MAB4401, MAB4419
Anti-Sox2	MAB4423, MAB4343, AB5603, 17-656
Anti-Lin28	MABD102, 07-1385
Anti-c-Myc	MAB8864, CBL434, AB3419, CBL430
Anti-NANOG	MABD24, AB5731, AB9220
Anti-Klf4	AB4138 , 09-821, MABC631
Anti-SSEA1, clone MC-480	MAB4301
Anti-SSEA-3, clone MC-631	MAB4303
Anti-SSEA-4, clone MC-813-70	MAB4304
Anti-SSEA-5, clone 8e11	MABD88
Anti-TRA-1-60, clone TRA-1-60	MAB4360
Anti-TRA-1-81, clone TRA-1-81	MAB4381
Anti-Nuclei, clone 235-1	MAB1281
Anti-Podocalyxin-like protein I (Cytotoxic), clone 84	MAB4414
Anti-SOX17	09-038
Anti-DPPA-2, clone 6C1.2	MAB4356
Anti-UTF-1, clone 5G10.2	MAB4337
TG343, clone TG343	MAB4346
Anti-HESCA-1, clone 051007-4A5	MAB4407
Anti-TERT (human), clone 2C4	MABD55

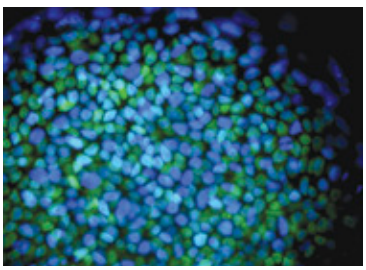
Skip the Secondary With Fluorescently Conjugated Stem Cell Antibodies

Immunocytochemistry Validated Antibodies

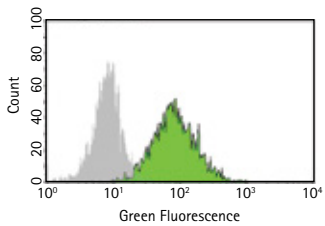
Description	Cat. No.
Anti-Oct-4, clone 10H11.2, Alexa Fluor® 488 conjugate	MAB4401A4
Anti-Oct-4 [POU5F1], clone 7F9.2, Cy3 conjugate	MAB4419C3
Anti-NANOG, clone 7F7.1, Alexa Fluor® 488 conjugate	MABD24A4
Anti-Sox2, clone 10H9.1, Cy3 conjugate	MAB4423C3
Anti-TRA-1-60, clone TRA-1-60, Alexa Fluor® 488 conjugate	MAB4360A4
Anti-TRA-1-81, clone TRA-1-81, Cy3 conjugate	MAB4381C3
Anti-SSEA1, clone MC-480, Cy3 conjugate	MAB4301C3
Anti-Nuclei, clone 235-1, Alexa Fluor® 488 conjugate	MAB1281A4
Fluorescent Mouse ES/iPS Cell Characterization Kit	SCR077
Fluorescent Human ES/iPS Cell Characterization Kit	SCR078

Flow Cytometry Validated Antibodies

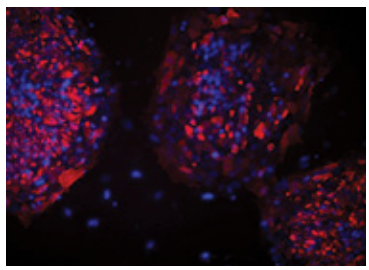
Description	Cat. No.
Anti-Oct-4, clone 10H11.2, Alexa Fluor® 488 conjugate	FCMAB113A4
Milli-Mark® Anti-Nanog-Alexa Fluor® 488, N-terminus	FCABS352A4
Milli-Mark® Anti-Human-Nuclei-PE, clone 235-1	FCMAB306P
Milli-Mark® Anti-SSEA-4-PE, clone MC-813-70	FCMAB116P
Anti-SSEA-1, clone MC-480, PE conjugate	FCMAB117P
Milli-Mark® Anti-SSEA3 Alexa Fluor® 488, clone MC-631	FCMAB141A4
Anti-Sox2, clone 6G1.2, FITC conjugate	FCMAB112F
Anti-TRA-1-60, clone TRA-1-60, FITC conjugate	FCMAB115F
Milli-Mark® Anti-TRA-1-81, clone TRA-1-81, FITC conjugate	FCMAB132F
FlowCellect® Human iPS Cell Characterization Kit	FCSC100107
FlowCellect® Mouse ESC Nuclear Marker Characterization Kit	FCMEC25110
FlowCellect® Human ESC (HESCA-1) Surface Marker Characterization Kit	FCHEC25104



Epifluorescent analysis of H9 hESCs stained with Anti-Nanog Antibody, clone 7F7.1, Alexa Fluor® 488 conjugate(MABD24A4). Cells Counterstained with DAPI.



Flow cytometric analysis of 2102 Ep cells stained with anti-SSEA 3 (green histogram) or isotype control (grey histogram).



Epifluorescent analysis of H9 hESCs stained with anti-TRA-1-81, clone TRA-1-81, Cy3 conjugate (MAB4381C3). Cells counterstained with DAPI.

StemSelect® 384-Well Small Molecule Regulators and InhibitorSelect™ Protein Kinase Inhibitor Libraries

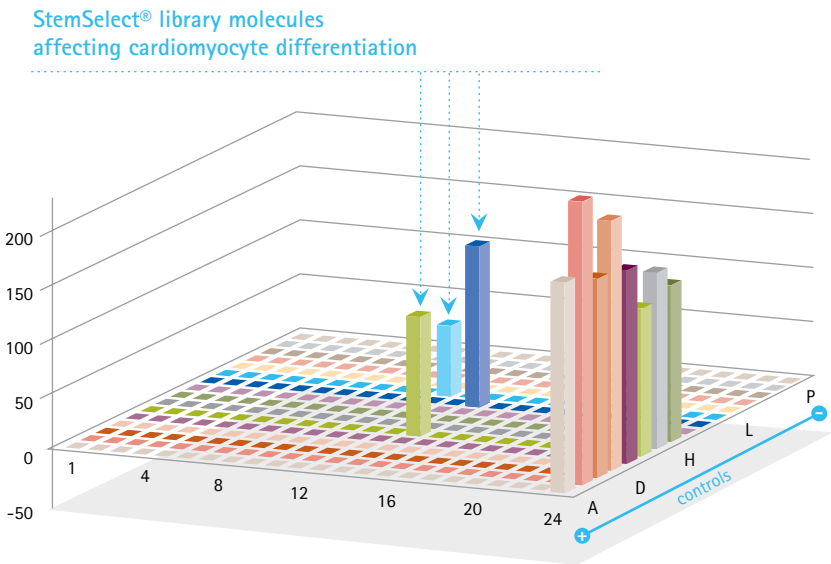
The use of small molecule inhibitors, activators, and regulators have become increasingly important in the study of stem cell biology. Small molecule-based medium supplementation has emerged as a popular method to increase iPS reprogramming efficiencies. Small molecule-based cell fate modulation protocols have many advantages over protein-based protocols including lower cost of reagents, more consistent supply and biological activity, slower degradation of inducers and more selective biological activities. Merck Millipore makes small molecule investigation easy by offering convenient panels of potent, selective and pharmacologically active small molecule inhibitors, activators and regulators in various 96- or 384-well formats.

For a complete table of inhibitor targets included in each panel, visit: **www.merckmillipore.com/inhibitorselect**

StemSelect® 384-Well Small Molecule Regulators Library

(Cat. No. 569744)

This library of 303 pharmacologically active, well-documented, structurally diverse small molecules includes extracellular domain-targeting reagents as well as cell-permeable compounds that effectively regulate intracellular targets. These molecules are useful for studying the survival, migration, proliferation, differentiation, signaling, and other functions of normal or cancer stem cells as well as non-stem cells.



StemSelect®, a library of highly targeted, well-characterized compounds, provide a high hit rate when screening for modulators of cardiomyocyte differentiation. With three molecules (indicated) showing activity, this library proved more effective than doing a large-scale, untargeted compound screen. Data courtesy of Dr. Mark Mercola, Sanford|Burnham Institute.

InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I

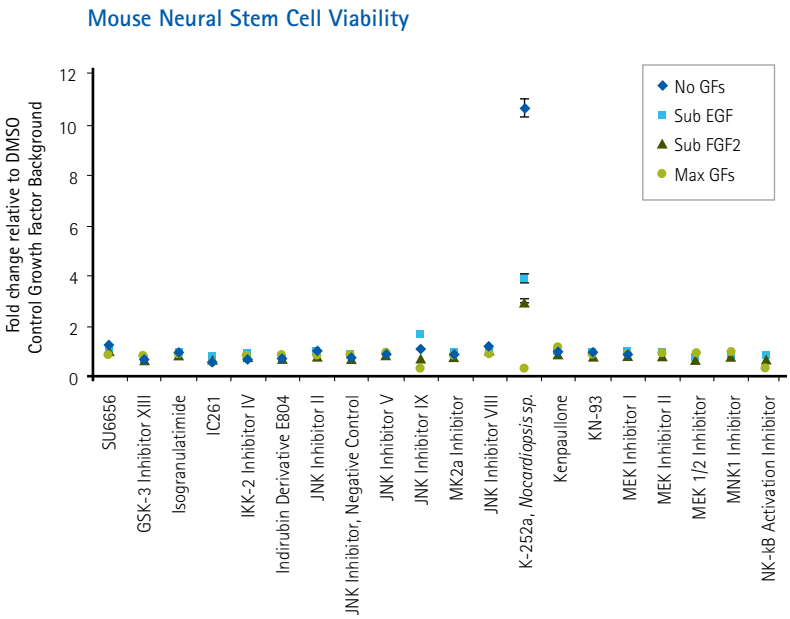
(Cat. No. 539744)

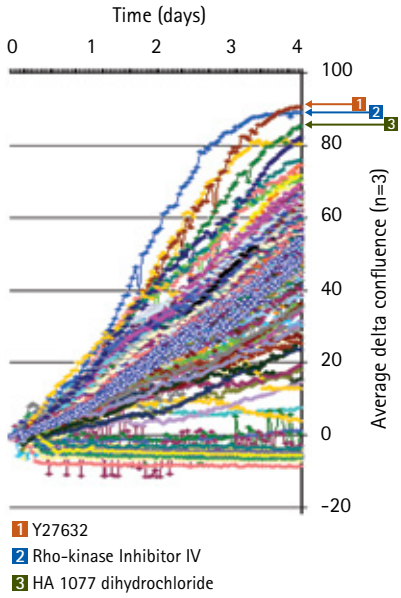
Analyze cell signaling networks with this panel of 80, well-characterized protein kinase inhibitors targeting mainly tyrosine, AGC, and atypical families of kinases, the majority of which are cell-permeable and ATP-competitive.

InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library II

(Cat. No. 539745)

This panel of compounds consists of 80, well-characterized, cell permeable, potent and reversible protein kinase inhibitors targeting mainly CMGC and CaMK families of kinases; the majority of which are ATP-competitive.





Modulation of mouse neural stem cell viability. InhibitorSelect™ 96-Well Protein Kinase Inhibitor Libraries I & II (160 inhibitors; Cat. Nos. 539744 and 539745) were screened for influence on proliferation and survival of mouse neural stem cells (mNS) in a cell viability assay under 4 conditions: (A) No GFs – No Growth Factors (to identify survival/proliferation factors) (B) Sub EGF – Sub-optimal EGF (to identify inhibitors/potentiators) 20 pg/mL EGF (C) Sub FGF2 – Suboptimal FGF2 (to identify inhibitors/potentiators) 500 pg/mL FGF2 (D) Max GFs – Maximal EGF + FGF2 (to identify inhibitors/ potentiators) 20 ng/mL EGF + 20 ng/mL FGF2 The presence of inhibitor K-252a, Nocardiopsis sp. (Cat. No. 420297) alone in the culture medium resulted in a 10-fold mNS cell viability. Data courtesy of Donna McLaren, Stem Cell Sciences, Cambridge, UK.

Modulation of neural embryonic stem cell expansion. Screening of 160 kinase inhibitors included in InhibitorSelect™ libraries I and II. Data show Delta Confluence Values, corresponding to the change in relative cell number for twelve mock-treated wells and 160 kinase inhibitors. Three compounds, all affecting Rho kinases, were selected as primary hits for their effect on expansion of Neural Embryonic Stem Cells and are detailed in the top right. Data courtesy of D Danovi, Wellcome Trust Centre for Stem Cell Research, University of Cambridge, UK.

Description	Cat. No.
StemSelect® Small Molecule Regulators 384-Well Library I	569744-1EA
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library I	539744-1EA
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library II	539745-1EA
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library III	539746-1EA
InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library IV	539747-1EA

2

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 subject them to direct differentiation to produce specific cell types is crucial to the development of cell-based therapies to replace or restore tissue that has been damaged by disease or injury.

Another important application of pluripotent stem cells is in the development of transgenic and knockout mice for the analysis of gene function. These targeting experiments commonly use murine embryonic stem (ES) cells, cultured *in vitro*. As a result, efficient procedures for the *in vitro* culture and maintenance of pluripotent ES cells have been vital to the success of this research.

Merck Millipore offers a range of tools for human and mouse pluripotent stem cell research, including ES cell lines, cell culture reagents, characterization kits, and novel antibodies. Merck Millipore's range of ES cell-qualified products provides researchers with convenient and cost-effective solutions for the reliable culture of ES cells.

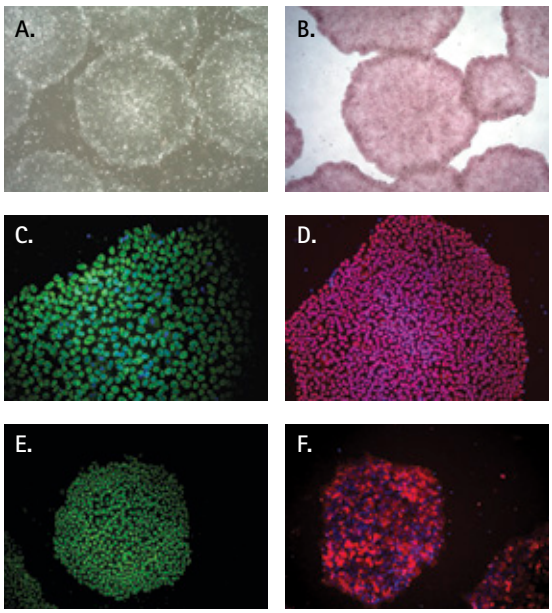
PluriSTEM™ Human ES/iPS Cell Medium

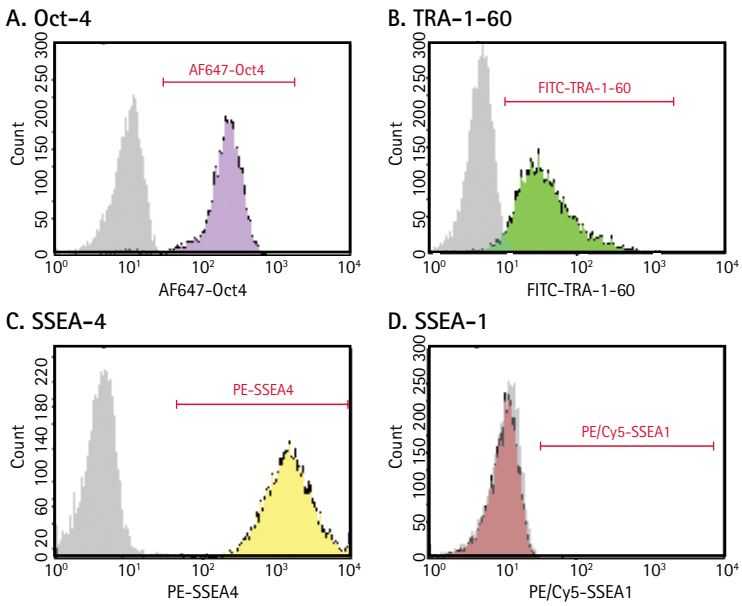
This specially formulated, defined medium enables maintenance of human pluripotent stem cells in feeder-free and serum-free conditions with less frequent feeding and hands-on cell culture time. The proprietary formulation uses Activin-A, TGFβ1, and b-FGF to promote stem cell self-renewal and potent small molecule combinations to inhibit unwanted spontaneous differentiation, along with human serum albumin (HSA) to improve overall colony morphology.

PluriSTEM™ Benefits

- Feeder-free, serum-free, defined cell culture system for human ES/iPS cells.
- Less frequent cell feeding (every other day, weekend-free).
- Small molecule-based formulation. Low total protein and growth factor concentrations.
- High viability and proliferation in single cell passaging.

Human pluripotent stem cells maintain proper marker expression when cultured in PluriSTEM™ Human ES/iPS Cell Medium

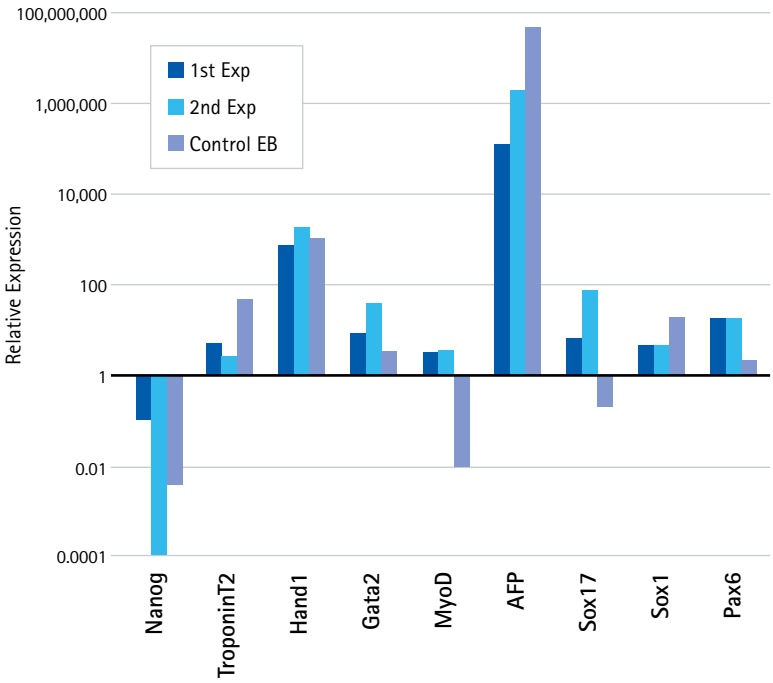




H9 human ES cells grown in PluriSTEM™ medium for 22 passages with feeding 3 days per week express high levels of Alkaline Phosphatase (B, Purple), Oct-4 (C, Green), Nanog (D, Red), Sox-2 (E, Green) and TRA-1-60 (F, Red). Cells were counterstained with Dapi. Cells were also analyzed for Oct-4, TRA-1-60, SSEA-4 and SSEA-1 expression by flow cytometric analysis.

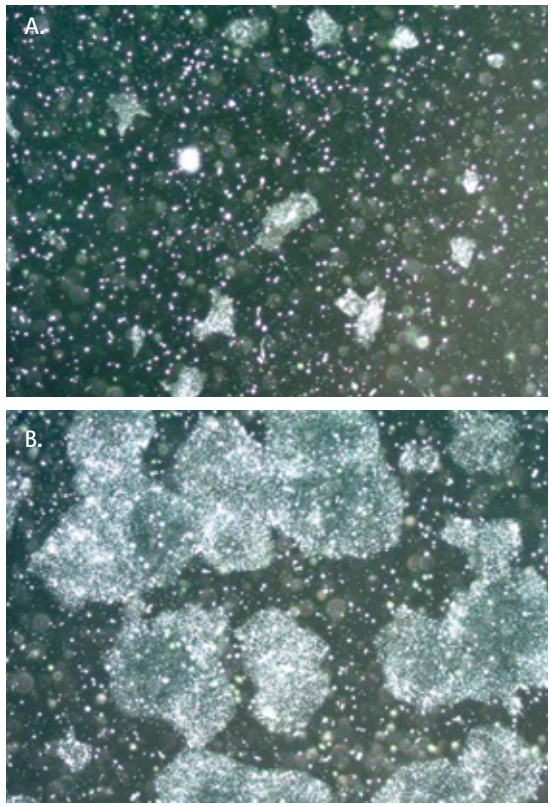
PluriSTEM™ Human ES/iPS Cell Medium (continued)

Cells grown in PluriSTEM™ medium maintain the ability to differentiate into all three germ layers



Human RT-PCR analysis of the expression of the pluripotency gene Nanog, Mesodermal genes TROPONINT2, HAND1, GATA2, and MYOD; endodermal genes AFP and SOX17 and neuroectodermal genes SOX1 and Pax6 in D31 EB derived from H9 P50 (10 passages cultured in PluriSTEM™ medium).

PluriSTEM™ culture enables single-cell dissociation and passaging of ES cells



H9 hESCs P62(P22) were dissociated into single cells with Accumax™ reagent. 10^5 cells/well were then seeded into 6-well-plate on Matrigel® matrix in a competitior's feeder-free human ES cell medium (A) or PluriSTEM™ medium (B) and cultured for an additional 6 days. Rock inhibitor was added one hour before harvest and 24 hrs post-plating. Cells proliferated much faster in PluriSTEM™ medium compared to the same cells cultured in the competitor's feeder-free human ES cell medium when passaged into single cells.

Description	Cat. No.
PluriSTEM™ Human ES/iPS Medium	SCM130
PluriSTEM™ Dispase-II Solution	SCM133
PluriSTEM™ Freeze Media	SCM134

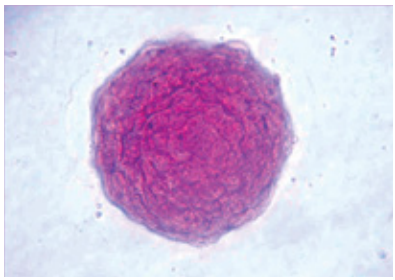
Featured Products for Pluripotent Stem Cells

ESGRO® and LIF Medium Supplements

With Merck Millipore's gold-standard ESGRO® medium supplement, you can trust that your mouse embryonic stem (ES) and induced pluripotent stem (iPS) cells will remain pluripotent even during the long-term culture required for successful gene targeting experiments and production of germline chimeras. ESGRO® medium supplement contains potent, pure, HPLC-grade recombinant mouse leukemia inhibitory factor (LIF). Each lot of ESGRO® supplement is formulated at an optimized concentration and activity for reproducible results.

Benefits

- Consistent inhibition of cell differentiation for reliable results.
- No batch-to-batch variation for better reproducibility.
- Enables feeder-free cell culture for greater control and time savings.

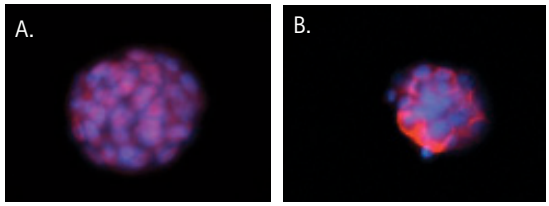


Alkaline phosphatase staining shows that ESGRO® supplement inhibits mES cell differentiation.

Description	Cat. No.
ESGRO® (LIF) Supplement	ESG1106, ESG1107
Rat ESGRO® Supplement	ESG2206, ESG2207
Leukemia Inhibitory Factor, recombinant human	LIF1005, LIF1010, LIF1050
Leukemia Inhibitory Factor, recombinant mouse	LIF2005, LIF2010, LIF2050
Leukemia Inhibitory Factor, recombinant rat	LIF3005, LIF3010

ESGRO®-2i Medium & Supplement Kits

Merck Millipore provides ESGRO® mLIF supplement with 2i inhibitor combinations in both media and supplement formats. The ESGRO®-2i medium is a defined, LIF containing medium, supplied with GSK3β and MEK inhibitors. The ESGRO®-2i supplement kit contains ready to use aliquots of ESGRO® mLIF supplement, MEK and GSK3β inhibitors that can be used to supplement 500mL or 5L of complete medium.

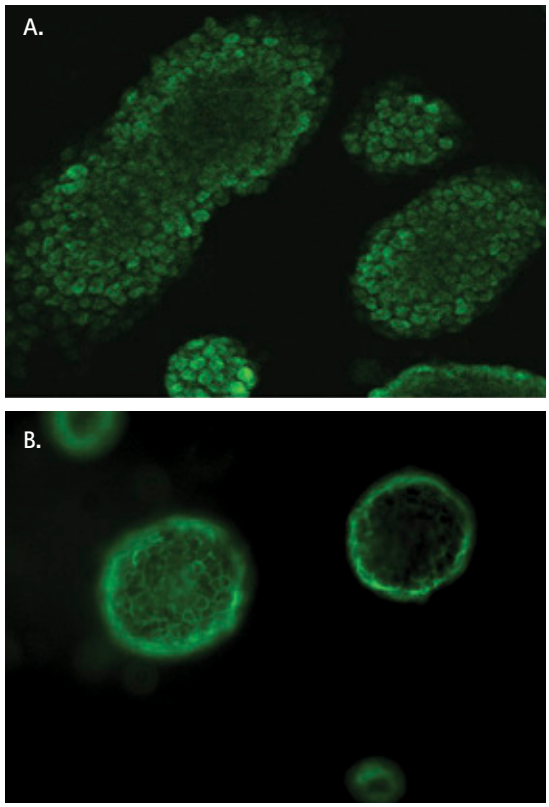


ESGRO®-2i-adapted mouse ES cell colonies were stained with anti-Oct4 (A) and anti-SSEA-1 (B) antibodies, both shown as red staining with blue DAPI nuclear staining.

Description	Cat. No.
ESGRO®-2i Supplement	ESG1120, ESG1121
ESGRO®-2i Medium	SF016-100, SF016-200

ESGRO Complete™ PLUS Media and Reagents

The ESGRO Complete™ system is the first to enable serum-free and feeder-free culture of mouse ES cells. The cornerstone of this system is the ESGRO Complete™ clonal grade medium, which supports the self-renewal of mouse ES cells by providing the basic nutrients normally supplied by serum and feeders in the traditional culturing method. These nutrients include hormones, vitamins, the growth factors mLIF and BMP4, as well a selective GSK3β inhibitor for enhanced mouse ES cell growth and viability at clonal densities in serum-free conditions.



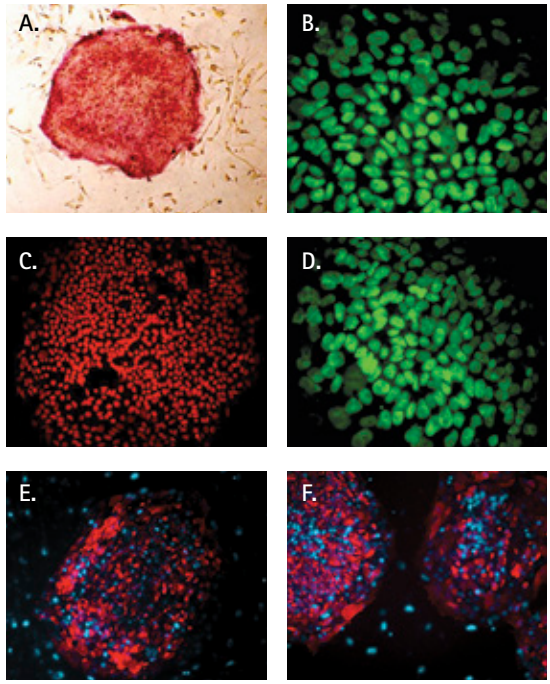
To confirm pluripotency of ES cells after culturing in ESGRO Complete™ PLUS medium, cells were immunostained for Oct-4 (A) and SSEA-1 (B) after 3 passages.

Description	Cat. No.
ESGRO Complete™ PLUS Clonal Grade Medium	SF001-500P, SF001-100P
ESGRO Complete™ Basal Medium	SF002-500, SF002-100
ESGRO Complete™ Serum-Free Cell Culture Freezing Medium	SF005
ESGRO Complete™ Accutase	SF006
ESGRO Complete™ Gelatin Solution	SF008
ESGRO Complete™ Trypsin Solution	SF007
ESGRO Complete™ Adapted C57/BL6 Mouse ES Cell Line	SF-CMTI-2

Fluorescent Human ES/iPS Cell Characterization Kit

(Cat. No. SCR078)

This kit contains a range of sensitive tools for the phenotypic assessment of the pluripotent status of human ES/iPS cells. Included in the kit is an enzymatic assay to measure alkaline phosphatase activity in the cells, directly conjugated antibodies to pluripotent transcription factors, Oct-4, Sox-2 and Nanog, and directly conjugated antibodies to cell surface epitopes TRA-1-60 and TRA-1-81. Together, these reagents enable rapid immunocytochemical marker analysis. The DAPI nuclear stain is included to aid in cell quantification. Fluorescent ES/iPS Cell Characterization Kits are available with both human and mouse specificities. Non-fluorescent ES cell antibody characterization kits also available.



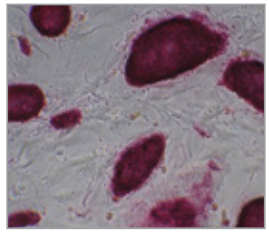
Pluripotent hES/iPS cells express pluripotent markers, alkaline phosphatase (40x, A), Oct-4 Alexa® 488 (400x, B), Sox-2 Cy3 (100x, C), Nanog Alexa® 488 (400x, D), TRA-1-60 Cy3 (100x, E), and TRA-1-81-Cy3 (100x, F).

Description	Cat. No.
Fluorescent Mouse ES/iPS Cell Characterization Kit	SCR077
Fluorescent Human ES/iPS Cell Characterization Kit	SCR078
ES Cell Characterization Kit	SCR001
ES Cell Marker Sample Kit	SCR002

Primary Mouse Embryonic Fibroblasts (PMEFs)

Feeder cells, including PMEFs, support ES cell growth by secreting important growth factors that help maintain pluripotency and by providing a cellular matrix. EmbryoMax® PMEFs are ideal for both human and mouse ES and iPS cell culture and conveniently eliminate the need for time-consuming embryonic feeder cell isolation and preparation. Several varieties are available, including actively dividing, growth-arrested (mitomycin-C and Irradiated), low passage (P1) and drug-resistant feeder cells.

Description		Cat. No.
EmbryoMax® Primary Mouse Embryo Fibroblasts, Neo-Resistant, Strain FVB	Non-treated, passage 1	PMEF-NL-P1
	Non-treated, passage 3	PMEF-NL
	Irradiated, passage 3	PMEF-NX
	Mitomycin C treated, passage 3	PMEF-N
EmbryoMax® Primary Mouse Embryo Fibroblasts, Strain CF1	Non-treated, passage 1	PMEF-CFL-P1
	Non-treated, passage 3	PMEF-CFL
	Irradiated, passage 3	PMEF-CFX
	Mitomycin C treated, passage 3	PMEF-CF
EmbryoMax® Primary Mouse Embryo Fibroblasts, Hygro Resistant, Strain C57/BL6	Non-treated, passage 3	PMEF-HL
	Mitomycin C treated, passage 3	PMEF-H

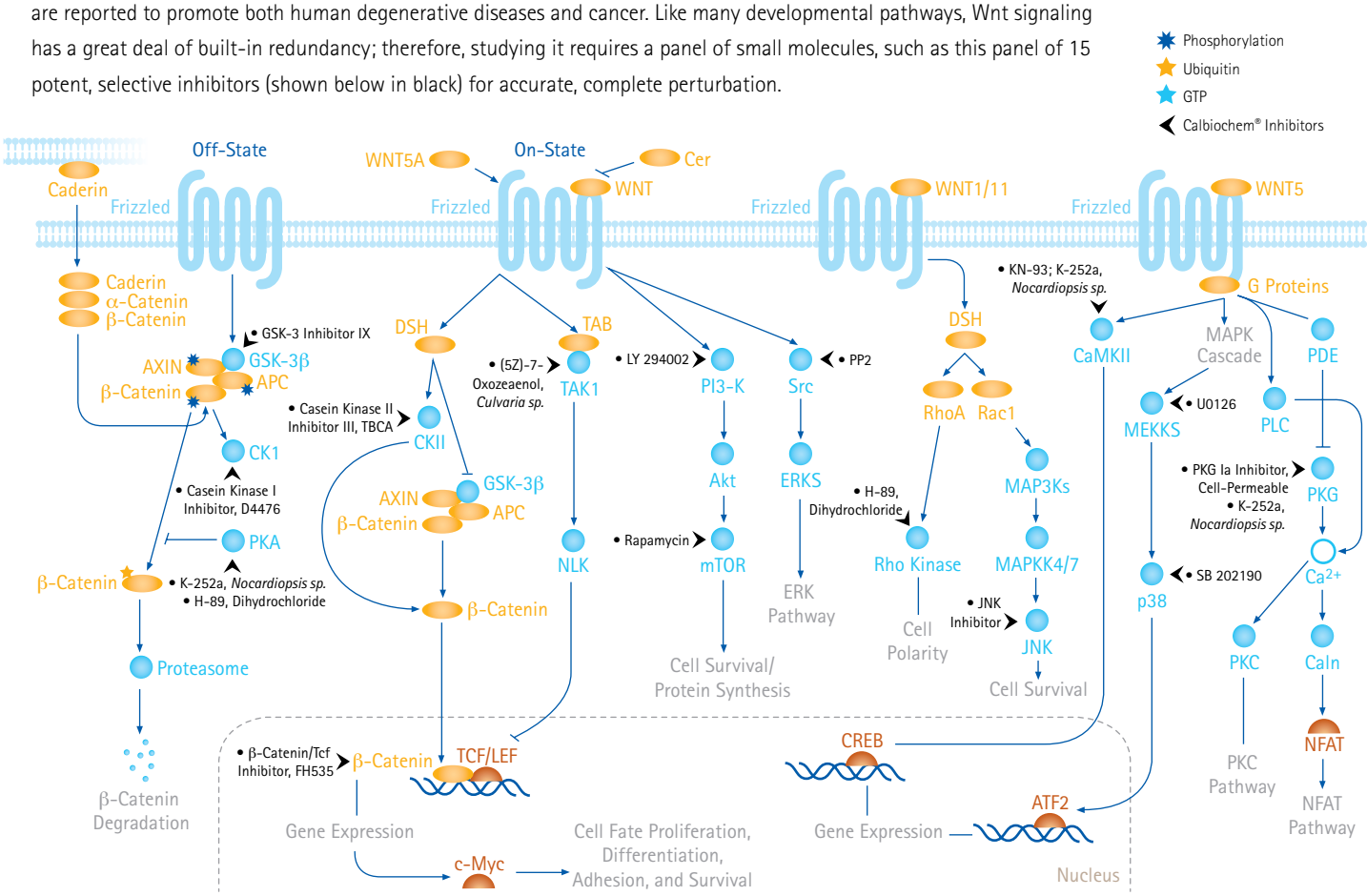


Mouse embryonic fibroblasts (PMEF-CFL) infected with the STEMCCA™ lentivirus display characteristic ES cell morphology and marker expression. These passage 3 mouse iPS cells express high levels of alkaline phosphatase as determined using the alkaline phosphatase detection kit (Cat. No. SCR004).

InhibitorSelect™ Wnt Signaling Pathway Inhibitor Panel

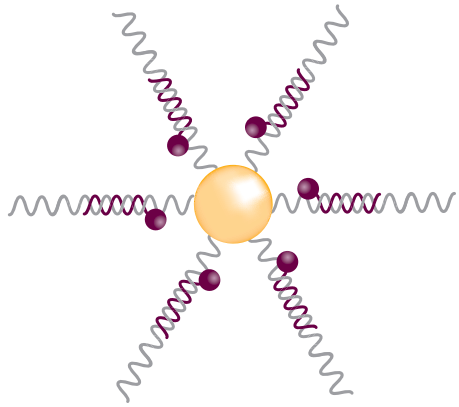
(Cat. No. 681666)

The Wnt signaling pathway is an evolutionarily-conserved pathway involved in fate specification, development, cell proliferation, cell migration, and polarity, and migration of cells. Wnt genes encode a large family of secreted, cysteine-rich proteins that are also important in development and in maintenance of adult tissues. Abnormalities in Wnt signaling are reported to promote both human degenerative diseases and cancer. Like many developmental pathways, Wnt signaling has a great deal of built-in redundancy; therefore, studying it requires a panel of small molecules, such as this panel of 15 potent, selective inhibitors (shown below in black) for accurate, complete perturbation.



SmartFlare™ detection of pluripotency-associated miRNAs in live cells

MicroRNAs (miRNAs) play crucial roles in determining cell fate during development, and they are integral parts of the transcriptional and post-transcriptional regulatory network determining pluripotency. In 2011, two independent studies reported reprogramming of mouse and human cells via introduction of pluripotency-associated miRNAs, to generate iPS cells that shared gene expression profiles, phenotypes and pluripotent properties with iPS cells reprogrammed using traditional (OKSM) techniques. SmartFlare™ RNA Detection Assays, by enabling quantitation of miRNAs in live cells, may help understand the mechanisms by which miRNAs regulate pluripotency that had been impossible until now. After fluorescence detection or cell sorting, SmartFlare™ probes exit the cells, enabling researchers to perform downstream analyses using those same, unperturbed cells.



Some SmartFlare™ probes targeting stem cell-associated miRNAs are listed here:

Description	Cat. No.
SmartFlare™ miR-200c-3p Hu-Cy5	SF-450
SmartFlare™ miR-21-5p Hu-Cy3	SF-471
SmartFlare™ miR-22-3p Hu-Cy3	SF-701
SmartFlare™ miR-24-3p Hu-Cy5	SF-726
SmartFlare™ miR-17-5p-Hu-Cy5	SF-180
SmartFlare™ miR-222-3p Hu-Cy5	SF-432

Browse all SmartFlare™ probes and learn m ore about SmartFlare™ technology at: **www.merckmillipore.com/smartflare**

Key Products for Pluripotent Stem Cells

Cells, Reagents and Kits

Description	Cat. No.
PluriSTEM® 129/S6 Murine ES cells	SCR012
MilliTrace™ Nanog GFP Reporter Mouse Embryonic Stem Cell Kit	SCR089
Human Embryonic Progenitor Cell Line 4SKEL20 and Expansion Medium Kit	SCR221
SmartFlare™ OCT4 Hu-Cy3 and Cy5 RNA Detection Probes	SF-438, SF-460
RESGRO™ Culture Medium	SCM001, SCM002
COMPLETE ES Cell Medium W/ 15% FBS Serum AND LIF	ES-101-B
EmbryoMax® KSOM Medium (1X) w/ 1/2 Amino Acids & Phenol Red	MR-121-D
EmbryoMax® M2 Medium (1X), Liquid, with phenol red	MR-015-D
EmbryoMax® FHM HEPES Buffered Medium (1X), liquid, w/ Phenol Red	MR-024-D
EmbryoMax® ES Cell Qualified Fetal Bovine Serum	ES-009-B
EmbryoMax® DMEM/F12, with L-Glutamine, without HEPES	DF-042-B
EmbryoMax® ES DMEM (1X), liquid, With 4,500 mg/L Glucose, 2.25 g/L Sodium Bicarb, without L-Glut and Sodium Pyruvate	SLM-220-B
EmbryoMax® L-Glutamine Soultion (100X), 200 mM	TMS-002-C
EmbryoMax® MEM, Non Essential Amino Acids (100X)	TMS-001-C
EmbryoMax® 0.1% Gelatin Solution	ES-006-B
EmbryoMax® Ultra Pure Water, sterile H_2O	TMS-006-A, TMS-006-B, TMS-006-C

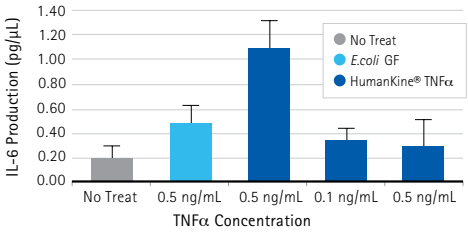
Growth Factors and Cytokines

Description	Cat. No.
Fibroblast Growth Factor basic, human animal-free recombinant	GF003-AF, GF003AF-MG
Fibroblast Growth Factor basic, human recombinant	GF003
EGF, human recombinant animal-free	GF316
EGF, human recombinant	01-107, GF144
WNT-1, human recombinant	GF175
Wnt-5a, recombinant mouse	GF146
DKK-1, human recombinant	GF170
Transforming Growth Factor-β1, human recombinant	GF111
Activin A, human recombinant animal-free	GF300
BMP-4, human recombinant	GF167
NOGGIN, human recombinant	GF173

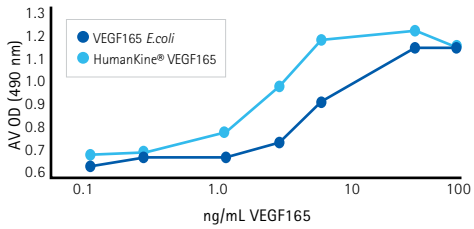
HumanKine® Xeno-Free Growth Factors

HumanKine® growth factors are produced in xeno-free conditions using proprietary engineered human cells, expression vector, and cell culture media to ensure high-yield production of recombinant proteins with native human post-translational modifications, such as disulfide bonds and glycosylation. HumanKine® growth factors are available for many areas of cell biology including stem cell biology, immunology, and other disciplines.

For a complete listing, visit: **www.merckmillipore.com/growthfactors**



HumanKine® TNF-α produces higher biological activity vs. *E.coli* protein



HumanKine® VEGF165 produces higher biological activity vs. *E.coli* protein.

Description	Cat. No.
HumanKine® Activin A, Human Recombinant Xeno-Free	GF400
HumanKine® BMP-4, Human Recombinant Xeno-Free	GF403
HumanKine® FGF basic, Human Recombinant Xeno-Free	GF407
HumanKine® IL-6, Human Recombinant Xeno-Free	GF430
HumanKine® LEFTY-1, Human Recombinant Xeno-Free	GF432
HumanKine® Noggin, Human Recombinant Xeno-Free	GF434
HumanKine® TGFβ 1, Human Recombinant Xeno-Free	GF439
HumanKine® VEGF165, Human Recombinant Xeno-Free	GF445

Bulk size quantites are available for all HumanKine® growth factors.
Request a quote today by visiting: **www.merckmillipore.com/partnerships**

ECM Proteins

Description	Cat. No.
Human Laminin (pepsinized) Purified Protein	AG56P
Laminin, mouse purified	CC095
Human Vitronectin	CC080, 08-126
Human Fibronectin	FC010, 08-102
Human Collagen Type IV	CC076
Collagen Type I, rat tail	08-115
ECL Cell Attachment Matrix	08-110
Rat Laminin-5	CC145

Small Molecule Modulators

Description	Cat. No.
BMP Inhibitor II, DMH1	203646
GSK-3β Inhibitor IX, BIO	361550
GSK-3β Inhibitor XII, TWS119	361554
GSK-3 Inhibitor IV, SB-216763	361566
ID-8	405210
iPSC Induction Enhancer, Thiazovivin	420220
MDM2 Antagonist IV, Nutlin-3a	444152
Pro-survival Compound, DDD00033532.	529659
Pluripotin	540020
Pyrintegrin	544049
Valproic acid	676380
Y-27632	688000, SCM075

3

Neural Stem Cells

Neural stem cells (NSCs) are self-renewing, multipotent cells that generate the basic cell types of the nervous system. NSCs primarily differentiate into neurons, astrocytes, and oligodendrocytes, depending on environmental cues. The use of neural stem cells in research and medicine is becoming increasingly widespread. The discovery that neurons, astrocytes, and oligodendrocytes arise from neural stem cells located in specific regions of the brain reveals the potential of using NSCs to treat central nervous system diseases, including Parkinson's and Alzheimer's disease. Neural stem cells hold great promise in regenerative therapy for damaged central nervous system (CNS) components, including the brain, spinal cord, and retina.

Merck Millipore offers a comprehensive range of tools for both human and rodent neural stem cell research, including novel human neural progenitor cell systems, serum-free cell culture media, and kits for differentiation and characterization of neural stem cells.

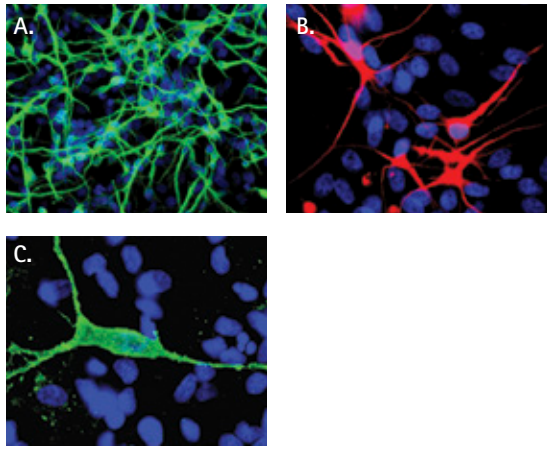
ReNcell® Human Neural Stem Cells

Breakthrough studies have recently rejected the long-standing belief that neuronal tissue is incapable of regeneration. Successful engraftment of NSCs following implantation into the brain of rodent models has demonstrated the potential of this cell type in the development of regenerative therapeutic strategies. However, neural stem cells have historically proven to be difficult to isolate and culture *in vitro* for an extended period of time. Merck Millipore offers novel, ready-to-use, neural progenitor cells isolated from both human and rodent model systems, including serum-free cell culture expansion media, and kits for differentiation and characterization.

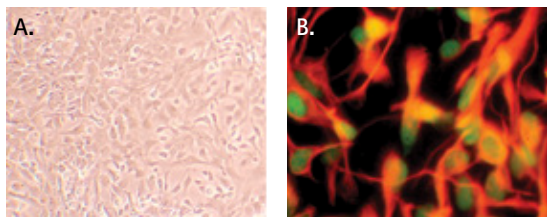
ReNcell® immortalized human neural stem cells can readily differentiate into neurons and glial cells. Two lines are available:

- ReNcell® VM (derived from the ventral mesencephalon region of human fetal brain tissue).
- ReNcell® CX (derived from the cortical region of human fetal brain tissue).

Immortalized by retroviral transduction with the myc oncogene, these robust cell lines grow rapidly as a monolayer on laminin with a doubling time of 20-30 hours. They retain a normal diploid karyotype even after prolonged passage. ReNcell® cell lines may be used for a variety of research applications such as studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitter, and receptor functions.



Multipotentiality of ReNcell® cells. Both ReNcell® CX and ReNcell® VM cell lines spontaneously differentiate into: neurons (βIII-tubulin, green, 20X, A), astrocytes (GFAP, red, 40X, B) and oligodendrocytes (Gal C, green, 60X, C); all counterstained with Hoechst nuclear stain (blue).



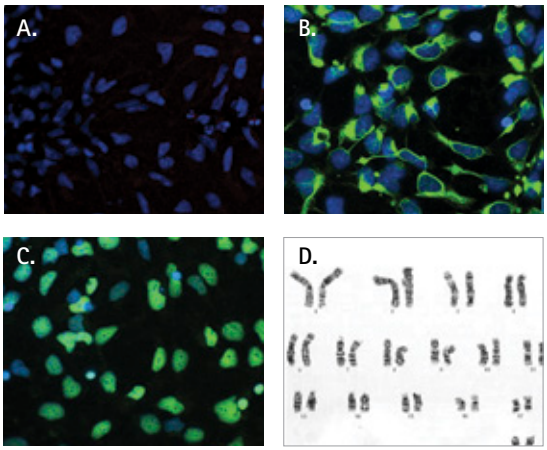
ReNcell® CX cells (Cat. No. SCC007) are grown as monolayers (A) and express NSC markers, Nestin (B, red) and Sox-2 (B, green).

Description	Qty/Pk	Cat. No.
ReNcell® CX Human Neural Progenitor Cell Line	1 x 10^6 cells	SCC007
ReNcell® CX Human Neural Progenitor Kit	1 x 10^6 cells and 500 mL of media	SCC009
ReNcell® VM Human Neural Progenitor Cell Line	1 x 10^6 cells	SCC008
ReNcell® VM Human Neural Progenitor Kit	1 x 10^6 cells and 500 mL of media	SCC010
ReNcell® Human NSC Maintenance Media	500 mL	SCM005
ReNcell® Human NSC Freezing Media	50 mL	SCM007
MilliTrace™ CX Constitutive GFP Reporter Human Neural Stem Cell Kit	1 kit	SCR095
MilliTrace™ CX Nestin GFP Reporter Human Neural Stem Cell Kit	1 kit	SCR096

Featured Products for Neural Stem Cells

ENStem™-A Human Neural Progenitor Expansion Kit

ENStem™-A human neural progenitor cells are derived from WA09 (H9) human embryonic stem cells (hESCs). These hESC-derived neural progenitors proliferate as an adherent monolayer and can readily differentiate into different neuronal subtypes. ENStem™-A human neural progenitor cells may be used for a variety of research applications such as studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitters, and receptor functions. ENStem™-A neural expansion medium is a defined, serum-free formulation that has been optimized for the culture and expansion of ENStem™-A human neural progenitors. When used in conjunction with FGF-2 (provided in the kit), cells can be maintained in an undifferentiated, multipotent state for at least 10 passages. They can be differentiated into neuronal populations with the ENStem™-A Neuronal Differentiation Medium.

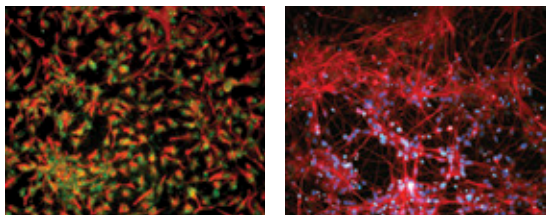


ENStem™-A cells demonstrate the expected immunoreactivity and chromosome number. (A) ENStem™-A cell line are Oct-4-negative. (B) ENStem™-A cell line labeled for nestin immunoreactivity. (C) ENStem™-A cell line labeled for Sox2 immunoreactivity. (D) Karyotype from ENStem™-A cell line.

Description	Qty/Pk	Cat. No.
ENStem™-A Human Neural Progenitor Expansion Kit	1 vial of cells & 500 mL of media	SCR055
ENStem™-A Expansion Medium	500 mL	SCM004
ENStem™-A Neuronal Differentiation Medium	100 mL	SCM017

Human iPSC-Derived Neural Progenitor Cells

These neural progenitors are derived from normal human foreskin fibroblast-derived iPS cells that were generated using STEMCCA™ technology. The OKSM viral transgenes have been removed from the genome to aid in cellular differentiation and function. These cells are supplied at passage 3 and are a convenient model for studying the relationship between iPSCs and developmental neurobiology. Cells can be purchased separately or with validated neural stem cell expansion medium.



iPS cell-derived neural progenitor cells express Sox-2 and nestin (left) can be differentiated to a high percentage of end-stage neuronal cells expressing βIII-tubulin (right).

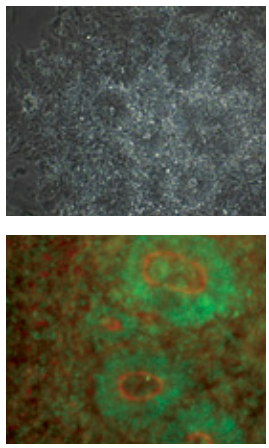
Description	Cat. No.
Human iPSC-Derived Neural Progenitors	SCC035
Human iPSC-Derived Neural Progenitor Kit	SCR131
ENStem™-A Neural Expansion Medium	SCM004

Human ES/iPS Neurogenesis Kit

(Cat. No. SCR603)

Generate expandable neural progenitor cells and terminally differentiated neurons from pluripotent human ES and iPS cells using this robust and user-friendly kit that contains all the necessary media and reagents. The media contained in this kit rely on both small molecule neural inducers and supplements to generate a highly enriched population of expandable neural progenitors and end-stage neurons based on established protocols. Neural progenitors are generated 10 days from starting cultures of traditional feeder-based and/or feeder-free cultures of undifferentiated human ES/iPS cells and can be expanded for over 3-5 passages, resulting in at least 20-fold expansion.

Description	Cat. No.
Human ES/iPS Neural Induction Medium	SCM110
Human ES/iPS Neuronal Differentiation Medium	SCM111
Human ES/iPS Neurogenesis Kit	SCR603

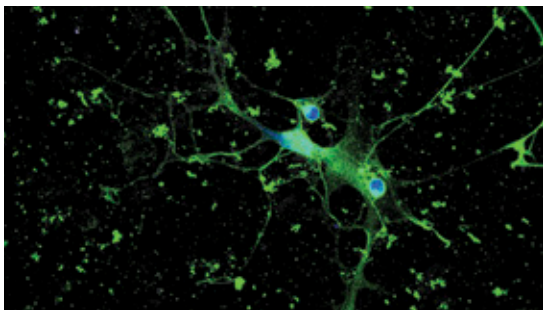


Neural induction of human pluripotent iPS cells. Human iPS cells were differentiated into >80% Pax6 positive and polarized N-cadherin (N-Cad) positive neural rosettes using Neural Induction Medium (Cat. No. SCM110).

Human Oligodendrocyte Differentiation Kit

(Cat. No. SCR600)

Easily generate enriched (>30%) populations of mature human oligodendrocytes in just 2-3 weeks with well characterized oligodendrocyte progenitor cells (OPCs) and cell culture media for expansion and spontaneous differentiation. Included OPCs are guaranteed >70% GalC positive and Sox10 positive; mature oligodendrocytes are positive for MBP, PLP and MOG. Human OPCs can be used for studies of neurotoxicity, coculture applications and screening for inducers or inhibitors of preferential differentiation to mature oligodendrocytes.

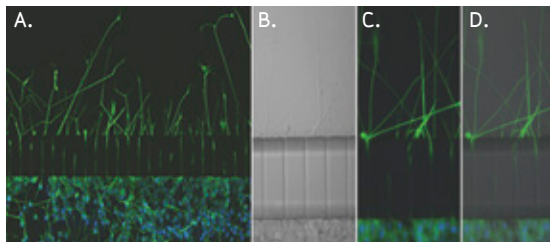


Human oligodendrocyte progenitors differentiated into oligodendrocytes stain positive for MBP (Myelin Basic Protein antibody, Cat. No. AB980).

Description	Cat. No.
Human Oligodendrocyte Differentiation Kit	SCR600
Human OPC Spontaneous Differentiation Media Kit	SCM106
Human OPC Expansion Media Kit	SCM107
Human Oligodendrocyte Characterization Kit	SCR601

AXIS® Axon Investigation System

The AXIS® system is the most advanced tool for the study of neurite outgrowth, somas, axonal development, and synaptic formation. This slide-mounted, microfluidic neuronal culture system limits neurite outgrowth to narrow microgrooves, so you can easily visualize and measure axonal extension or collapse in your newly differentiated neural cells. The fluidic isolation of the chambers and channels allows for further experimentation on neuronal response to growth factors, toxins, or other modulators.



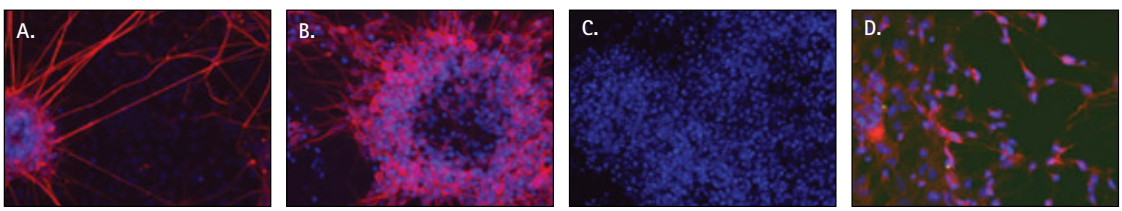
N1E-115 cells grown on an AXIS® device (Cat. No. AX150). For A-D, N1E-115 cells were loaded in the lower channel and cultured for 5 days in differentiation media. The cells were then fixed and stained with DAPI (blue) and with the neuronal cell stain MAB2300X (green). (A) fluorescent image of cells differentiating into neurons and sending neurites through the microgrooves of the AXIS® device. Cell bodies (somas) are entirely contained on one side of the device and only the neurites are extended through the microgrooves into the other channel. (B) Higher resolution bright field image of the cells and device. (C) Corresponding fluorescent image. (D) Overlay of images B and C to verify that the neurites extend through the microgrooves only.

Description	Cat. No.
AXIS® Axon Isolation Device, 150 µm	AX15010
AXIS® Axon Isolation Device, 450 µm	AX45010
AXIS® Axon Isolation Device, 500 µm	AX50010
AXIS® Axon Isolation Device, 900 µm	AX90010

ES2N Complete Medium Kit

(Cat. No. SCM082)

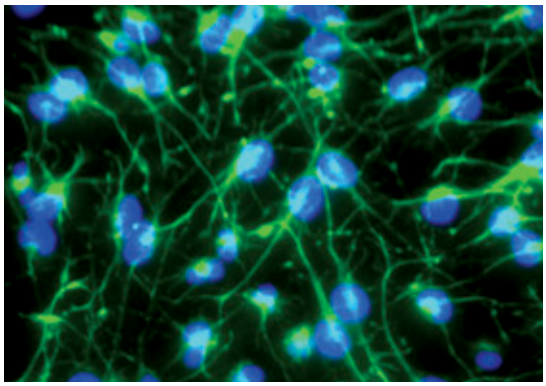
ES2N complete medium is a defined, serum-free formulation that efficiently differentiates mouse ES and iPS cells into functional neurons. Traditional neuronal differentiation involves embryoid body (EB) formation in serum-containing medium. With the ES2N complete medium, cells readily differentiate into neuron monolayers within 9-12 days on gelatin-coated culture dishes, without the formation of EBs.



Immunocytochemistry analysis of mature neurons at day 12 differentiated from 129S6 mouse ES cells. (A) Mature neurons stained with tubulin antibody. (B) Mature neurons stained with MAP-2 antibody. (C) Antibody staining of the Oct4 pluripotency marker shows no Oct4 expression. (D) Antibody staining with the astrocyte marker GFAP. All stainings are overlayed with blue DAPI nuclear staining.

Nestin Antibodies

Nestin, a large intermediate filament protein (class Type VI), is expressed during development and in myotendinous and neuromuscular junctions. Nestin expression is restricted, typically disappearing by E18. Nestin identifies the primitive neuroepithelium and many other embryonic tissues. Merck Millipore offers a variety of human and rodent nestin antibodies, including monoclonal, polyclonal, and conjugated versions.



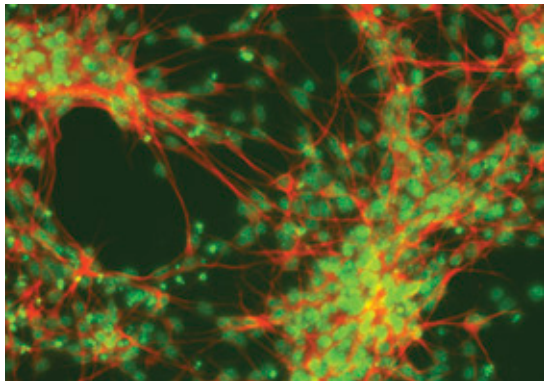
Epifluorescent analysis of rat hippocampal neural stem cells (Cat. No. SCR022) using anti-nestin, clone rat-401, Alexa Fluor®488 conjugate shows positive staining of rat nestin filaments (green). Nuclei are stained with DAPI (blue).

Description	Cat. No.
Anti-Nestin, Human Antibody	ABD69
Anti-Nestin Antibody, clone rat-401	MAB353
Anti-Nestin, clone rat-401, Alexa Fluor® 488 conjugate	MAB353A4
Anti-Nestin, clone rat-401, Cy3 conjugate	MAB353C3
Anti-Nestin, clone rat-401, Biotin conjugate	MAB353B
Anti-Nestin Antibody, clone 10C2	MAB5326
Anti-Nestin, clone 10C2, Alexa Fluor® 488 conjugate	MAB5326A4
Anti-Nestin, clone 10C2, Cy3 conjugate	MAB5326C3
Anti-Nestin, clone 10C2, Biotin conjugate	MAB5326B
Anti-Nestin, clone rat-401, Alexa Fluor® 488 conjugate	MAB353A4
Anti-Nestin, clone rat-401, Cy3 conjugate	MAB353C3
Anti-Nestin, clone 10C2, Alexa Fluor® 488 conjugate	MAB5326A4
Anti-Nestin, clone 10C2, Cy3 conjugate	MAB5326C3

N21 Medium Supplement (50X)

(Cat. No. SCM081)

This defined, serum-free supplement supports the survival and maturation of cultured primary neurons and exhibits low lot-to-lot variability. N21 supplement can also be added to other culture media that normally require B27. These include media formulations for the feeder-free, serum-free culture of mouse ESCs, for the differentiation of mESCs to neurons and for the propagation and differentiation of human ESC-derived neural progenitor cells.

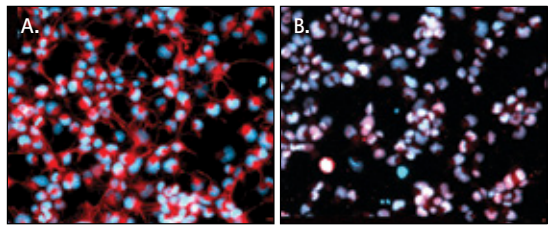


Mouse ES cells (129S6, Cat. No. SCR012) in a neuronal monolayer differentiation assay. Cells differentiated in ES2N medium supplemented with N21 medium supplement show high levels of neuronal precursor marker, nestin (red) and the neuronal differentiation marker, MAP2 (green).

Synthetic Laminin Peptide

(Cat. No. SCR127)

Laminin, an ECM protein, mediates cell adhesion, proliferation, migration, invasion, and differentiation. Merck Millipore's synthetic laminin peptide is a practical, convenient replacement for hard-to-purify native laminin, and is optimized to support adhesion, proliferation, and differentiation of rat NSCs.



Rat NSCs cultured on synthetic laminin peptide-coated tissue cultureware express multipotent NSC markers, nestin (A, red) and Sox-2 (B, red). Cells were cultured for over 10 passages on synthetic laminin peptide-coated T-25 flasks (A,B).

Description	Cat. No.
Adult Rat Hippocampal Neural Stem Cells	SCR022

Key Products for Neural Stem Cells

Cells, Reagents and Kits

Description	Cat. No.
MilliTrace™ CX Nestin GFP Reporter Human Neural Stem Cell Kit	SCR096
MilliTrace™ VM Constitutive GFP Reporter Human Neural Stem Cell Kit	SCR092
NDiff Neuro-2 Medium Supplement (200x)	SCM012
Mouse Embryonic Stem Cell Neurogenesis Kit	SCR101
Dopaminergic Differentiation Growth Factor Sampler	SCR128
Human Dopaminergic Neurogenesis Kit	SCR135
Neural Stem Cell Basal Medium	SCM003
Mouse Neural Stem Cell Expansion Medium	SCM008
ReNcell® NSC Maintenance Media	SCM005
ENStem™-A Neural Expansion Medium	SCM004
Neural Stem Cell Freezing Medium (1X)	SCM014
EmbryoMax® DMEM/F12, with L-Glutamine, without HEPES	DF-042-B
N-Base Medium	N014-B
Human Neurogenesis CELISA Assay (Colorimetric)	SCR109
Human Neural Stem Cell Characterization Kit	SCR060
FlowCellect® Rodent NSC Characterization Kit (Neural)	FCRNC25112
Rodent Neuron Differentiation Kit	SCR035

Antibodies

Description	Cat. No.
Anti-Nestin, Human	ABD69
Anti-Nestin, clone rat-401	MAB353
Anti-Nestin, clone 10C2	MAB5326
Anti-Sox2	MAB4423, MAB4343, AB5603
Anti-Mitrochondria	MAB1273, AB3598
Anti-Nuclei	MAB4383
Anti-NeuN	ABN78, ABN90, ABN91
Anti-PAX6	AB2237
Anti-β III Tubulin	AB9354, MAB1637
Anti-MAP2, clone AP20	MAB3418
Anti-Glial Fibrillary Acidic Protein (GFAP)	AB5804
Anti-CD133	MAB4310, MAB4399
Anti-BCRP	MAB4145, MAB4146

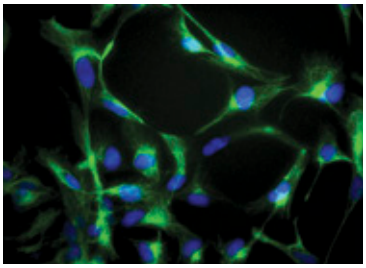
Neuroscience Antibody Conjugates

Immunocytochemistry Validated Antibodies

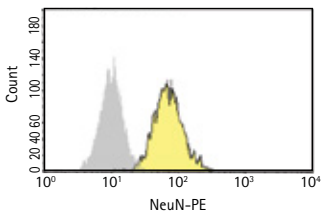
Description	Cat. No.
Anti-Nestin, clone rat-401, Alexa Fluor® 488 conjugate	MAB353A4
Anti-Nestin, clone rat-401, Biotin conjugate	MAB353B
Anti-Nestin, clone 10C2, Cy3 conjugate	MAB5326C3
Anti-Sox2, clone 10H9.1, Cy3 conjugate	MAB4423C3
Anti-Nuclei, clone 235-1, Alexa Fluor® 488 conjugate	MAB1281A4
Anti-Nuclei, clone 235-1, Biotin conjugate	MAB1281B
Anti-NeuN, clone A60, Alexa Fluor®488 conjugated	MAB377X
Anti-Tubulin β-III, clone TU-20, Alexa Fluor®488 Conjugated	CBL412X
Anti-GFAP, Cy3 conjugate	MAB3402C3
Anti-BCRP1, clone 5D3, Alexa Fluor® 488 conjugate	MAB4155A4
Anti-CD133, clone 13A4, Alexa Fluor® 488 conjugated	MAB4310X
NeuroChrom™ Pan Neuronal Marker	NS420
NeuroChrom™ Pan Neuronal Marker-OMC	NS330
NeuroChrom™ Pan Neuronal Marker-ORC	NS340

Flow Cytometry Validated Antibodies

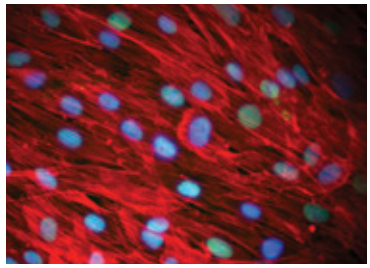
Description	Cat. No.
Milli-Mark® Anti-Nestin-PE, clone 10C2	FCMAB313PE
Anti-Sox2, clone 6G1.2, FITC conjugate	FCMAB112F
Milli-Mark® Anti-Human-Nuclei-PE, clone 235-1	FCMAB306P
Milli-Mark® Anti-NeuN-PE, clone A60	FCMAB317PE
Anti-BCRP, clone 5D3, Phycoerythrin conjugated	MAB4155P
FlowCellect® Rodent NSC Characterization Kit (Neural)	FCRNC25112
FlowCellect® Rodent NSC Characterization Kit (Astrocyte)	FCRNC25114



Epifluorescent analysis of human neural stem cells ReNcell® CX (SCC007) using Anti-Nestin, clone 10C2, Alexa Fluor®488 Conjugate (Green). Nuclei are stained with DAPI counterstain (Blue).



FC analysis of U251 cells stained with anti-NeuNPE (yellow) or isotype control (grey).



Epifluorescent analysis of human bone marrow mesenchymal stem cells using Anti-Nuclei, clone 235-1, Alexa Fluor® 488 conjugate. Cells were counterstained with DAPI (blue) and Cy3 phalloidin (red).

Growth Factors and Cytokines

Description	Cat. No.
Fibroblast Growth Factor basic, Human Animal-Free recombinant	GF003-AF, GF003AF-MG
Fibroblast Growth Factor basic, Human recombinant	GF003
Epidermal Growth Factor, Human Recombinant	GF144, 01-107
Glial-Derived Neurotrophic Factor, Human Recombinant	GF030
BDNF, Human Recombinant Animal Free	GF301
Neurotrophin 3	GF031
Neurotrophin 4/5	GF032
β-NGF, Human Recombinant Animal Free	GF307
Sonic HedgeHog (Shh), Human Recombinant	GF174

ECMs

Description	Cat. No.
Human Laminin (pepsinized) Purified Protein	AG56P
Laminin, mouse purified	CC095
Synthetic Laminin Peptide for Rat Neural Stem Cells	SCR127
Poly-D-Lysine Solution, 1.0 mg/mL	A-003-E
Poly-L-Lysine Solution (0.01%)	A-005-C
Poly-L-Ornithine Solution (0.01%)	A-004-C

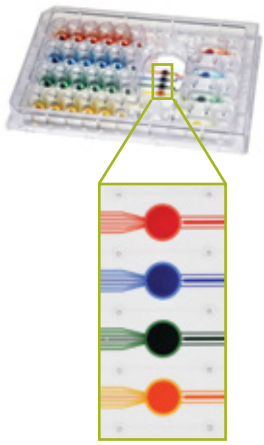
Small Molecule Modulators

Description	Cat. No.
Neurogenesis Enhancer, P7C3A20	480744
Neurogenesis Inducer V, KHS101	480747
Neuronal Differentiation Inducer, Isobavachin	480748
InSolution™ TGF-β RI Kinase Inhibitor VI, SB431542	616464
InSolution™ AMPK Inhibitor, Compound C	171261

CellASIC™ ONIX Microfluidic Platform for Neural Stem Cell Microenvironment Control and Live Cell Imaging

The easy-to-use CellASIC™ ONIX Microfluidic Platform delivers precise control for live cell imaging experiments by facilitating long-term perfusion cell culture. The system integrates with your existing microscope to enable dynamic time-lapse experiments never before possible. Cutting-edge microfluidics technology enables you to program automated changes to culture conditions while maintaining optical access to cells through your microscope, providing an improved cell culture microenvironment and exceptional quality for high magnification microscopy.

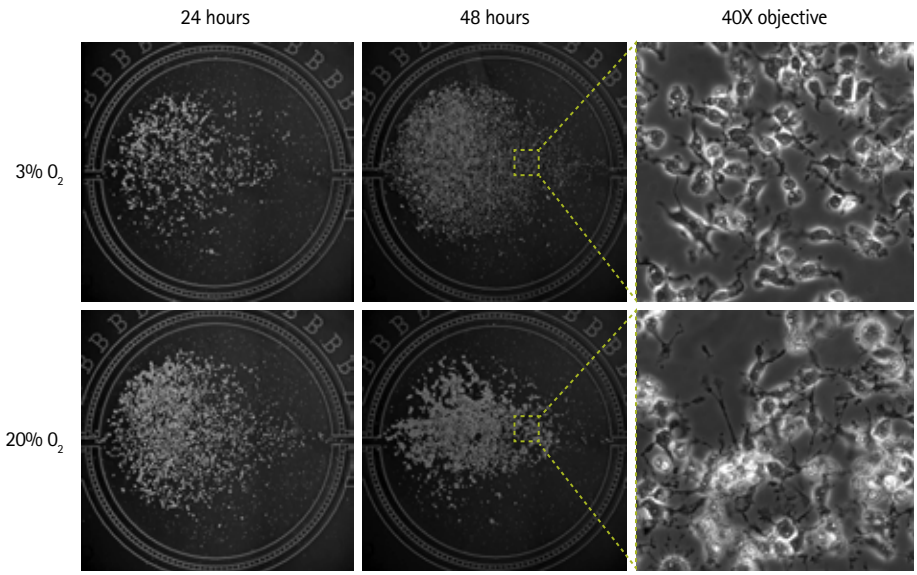
For details, visit: **www.merckmillipore.com/cellasic**



(Above) CellASIC™ ONIX Microfluidic Platform with the microfluidic system, the microincubator controller and microincubator manifold. (Left) Layout of M04S plate (four independent units and eight wells per unit).

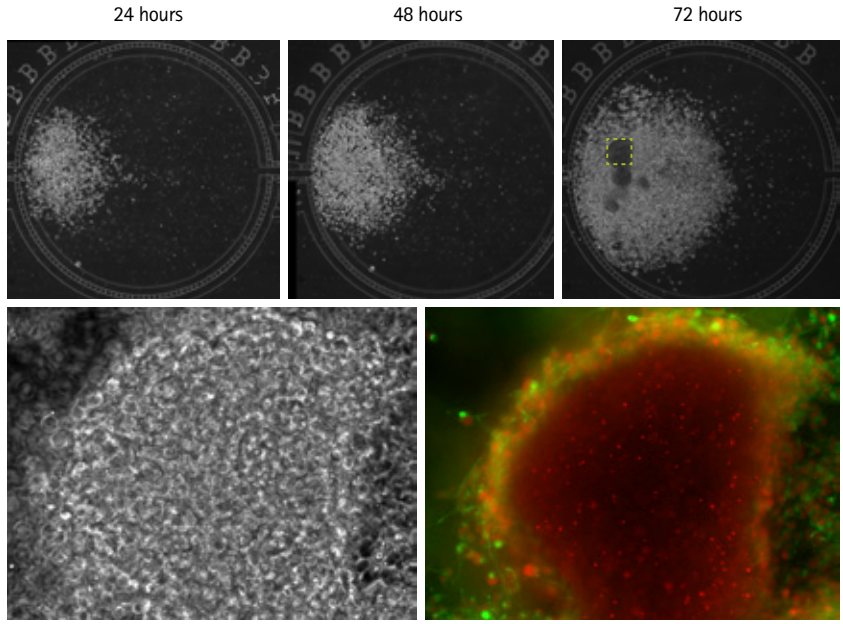
Neural stem cells (NSCs) are sensitive to microenvironmental cues, including cell-cell contact, cell-ECM interaction, nutrient and waste transport, as well as environmental oxygen composition. However, how these parameters in the microenvironment affect the stem cells' morphology, proliferation, and differentiation remains an open area for research. In this study, we demonstrated how the CellASIC™ ONIX Microfluidic Platform, with its microfluidic cell culture devices, are capable of multi-parametric microenvironment control for NSC studies.

Specifically, we were able to test the effect of gas composition on cultured rodent NSCs by exposing cells to severe hypoxia, mild hypoxia or normoxia. Cells get detached and washed away, presumably due to cell death, 24 hours after exposure to severe hypoxia. At low cell density, cells become disaggregated under mildly hypoxic conditions, which promotes single-layer cellular growth. In contrast, under normoxic conditions, the cells are aggregated into multilayer cellular masses.



Rat NSCs cultured at low densities under mildly hypoxic conditions (top) and under normoxic conditions (bottom).

To further explore the effect of microchamber culture of rat NSCs, we seeded the cells at high density under mildly hypoxic conditions (3% O_2). After four days, the cells formed neurospheres. Automated, in-plate immunostaining for nestin and Sox2 revealed that the core of this tightly packed mass contained only bright spots of Sox2 while the outer ring of the neurospheres exhibited both nestin and Sox2 expression.



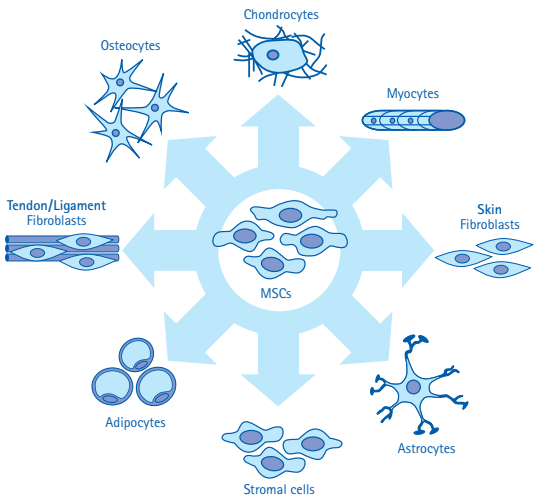
High density seeding of NSCs in a microfluidic chamber under conditions of mild hypoxia causes formation of neurospheres (top row, inset box at 72 hours). Neurospheres were also imaged at higher magnification (20X, bottom left). When stained for nestin and Sox2 (bottom right), only the outer layers of the neurospheres showed both nestin (green) and Sox2 (red) expression; the core displayed bright spots of Sox2 expression.

In summary, we have demonstrated the combinatorial effect of microenvironment parameters on rat NSCs and the capability of imaging them with fluorescent microscopy for further analysis. The platform promises to facilitate assay development for NSCs and provides a better-controlled *in vitro* model system for neurogenesis and neural development research.

4

Mesenchymal Stem Cells

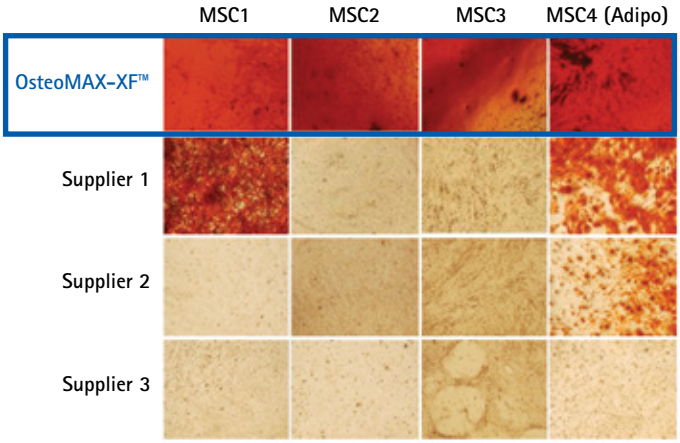
Human mesenchymal stem cells are adult stem cells, which have the capacity for multi-lineage differentiation, giving rise to a variety of mesenchymal phenotypes such as osteoblasts (bone), adipocytes (fat), and chondrocytes (cartilage). Human mesenchymal stem cells, or hMSCs, usually reside in the bone marrow stroma, but have also been found in liver, spleen, peripheral blood, umbilical cord blood, and other mesenchymal tissues. Due to their capacity for self-renewal over long periods of time and the ability to differentiate into specialized cells, interest in understanding the biology of MSC cultures has increased, especially for their therapeutic potential for a variety of diseases.



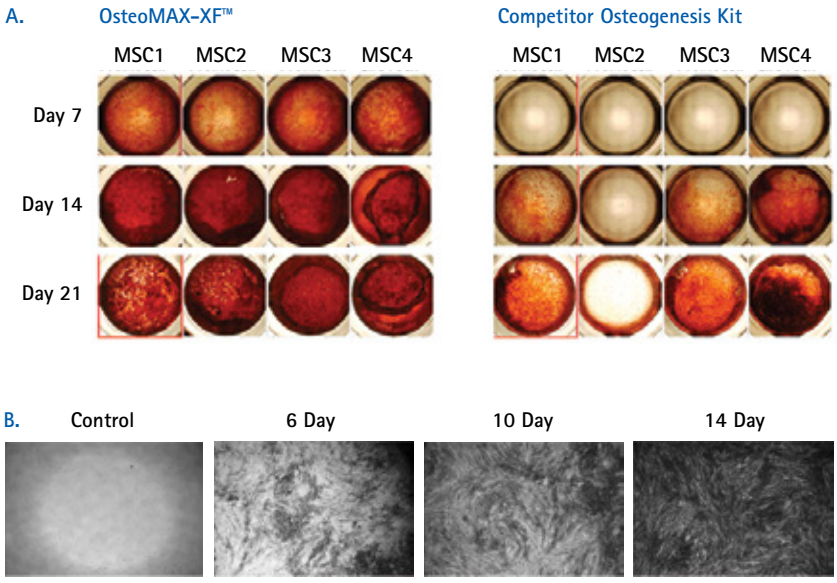
The multi-lineage differentiation potential of mesenchymal stem cells MSC cultures have the multi-lineage capacity to differentiate towards a variety of cell types. Based on the stimulus presented, MSCs can differentiate towards either osteoblasts (bone), adipocytes (fat), or chondrocytes (cartilage), to name a few. Given the ability of MSCs to give rise to a number of cell types, these cells are highly attractive models for investigation, especially in regenerative medicine applications.

OsteoMAX-XF™ Differentiation Medium

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that have the capacity to differentiate into bone, cartilage, and fat cells. The generation of osteoblasts from hMSCs has enormous potential for developing and evaluating drugs for alleviation of orthopedic conditions.



Alizarin red staining of MSC cultures differentiated for 28 days using OsteoMAX-XF™ vs. other commercial suppliers; cell lines 1-3: bone marrow-derived MSC; cell line 4: adipose-derived MSC.



Mineralization kinetics of human bone-marrow derived MSC (Cat. No. SCC034) differentiated in OsteoMAX-XF™ Medium.

Difficulties in directing MSCs and other stem cells to generate fully functional, specific cell types hamper the realization of their full potential. Generating specific cell types in large scale, in a reproducible and cost effective manner, is even more challenging. To overcome these difficulties, Merck Millipore has developed a novel, serum-free, xeno-free, differentiation medium, termed OsteoMAX-XF™, for the generation of osteoblasts from hMSCs that may be suitable for drug discovery and clinical development. Screening of roughly 3,500 combinations of serum and xeno-free medium using Combicult™ technology from Plasticell, we discovered a remarkably efficient, fully defined, reproducible medium to promote the differentiation of hMSCs into osteoblasts, which offers an excellent, cost effective method to produce large amounts of human bone cells for multiple applications.

OsteoMAX-XF™ Advantages

- **Faster differentiation**: Mineralization and bone nodule formation in as little as 7 days.
- **Efficient**: Generate greater than 80% positive osteocytes (measured by alkaline phosphatase and alizarin-red staining) in as little as 7 days compared to 21 days with standard osteogenesis media and protocols.
- **Xeno-free**: Maintain a completely humanized cell culture system by using our xeno-free and serum-free media formulation.

Description	Cat. No.
OsteoMAX-XF™ Differentiation Medium	SCM121
Mesenchymal Stem Cell Osteogenesis Kit	SCR028
In Vitro Osteogenesis Assay Kit	ECM810
Osteogenesis Quantitation Kit	ECM815
Alkaline Phosphatase Detection Kit	SCR004
Alizarin Red Stain Solution	TMS-008-C
Quantitative Alkaline Phosphatase ES Characterization Kit	SCR066

Human Mesenchymal Stem Cell Lines

Human Bone Marrow Mesenchymal Stem Cells

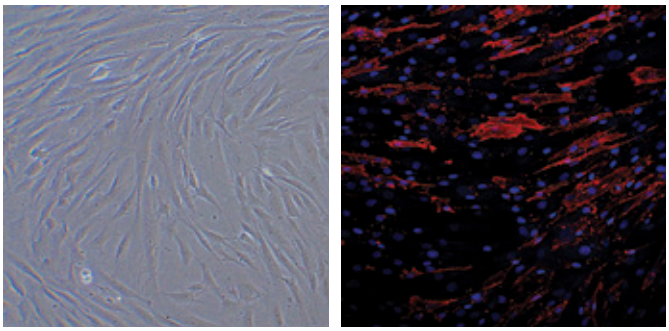
Bone marrow mesenchymal stem cells are the most widely studied type of mesenchymal stem cell and are considered the "gold standard" for mesenchymal stem cell research. To aid researchers in the accurate identification and characterization of mesenchymal stem cells, Merck Millipore provides cryopreserved human MSCs and optimized media that have been isolated from the iliac crest of a normal human bone marrow donor.

Human Adipose Mesenchymal Stem Cells

Adipose tissue represents an abundant, accessible source of multipotent stromal cells. These human adipose-derived MSCs are isolated from human adipose tissue collected during liposuction procedures and are cryopreserved as secondary cells to ensure optimal phenotype and the highest viability and plating efficiency. Cells are provided at passage 2 and display rapid cell proliferation rates.

Human Embryonic Stem Cell–derived Mesenchymal Stem Cell

These MSCs are derived from MEL-1 human embryonic stem cells (hESC). The cells proliferate as an adherent cell monolayer and can be expanded for up to 10 passages.



(Left) Phase contrast images of Human Bone Marrow Mesenchymal Stem Cells one day after thawing. (Right) Immunocytochemical staining of Merck Millipore's cultured human bone marrow-derived mesenchymal stem cells with STRO-1 (red) (Cat. No. MAB4315: 1/500 dilution). Nuclei of the cells were visualized with DAPI (blue).

Description	Cat. No.
Human Mesenchymal Stem Cell Kit (Derived from Bone Marrow)	SCR108
Human Mesenchymal Stem Cells (derived from hES cells)	SCC036
Human Adipose Mesenchymal Stem Cells	SCC038
Human Adipose Mesenchymal Stem Cell Kit	SCR038
Rat Mesenchymal Stem Cells	SCR027

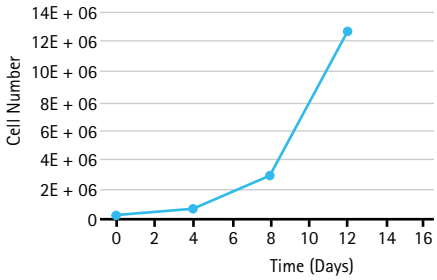
Featured Products for Mesenchymal Stem Cells

Human Mesenchymal-LS Expansion Medium

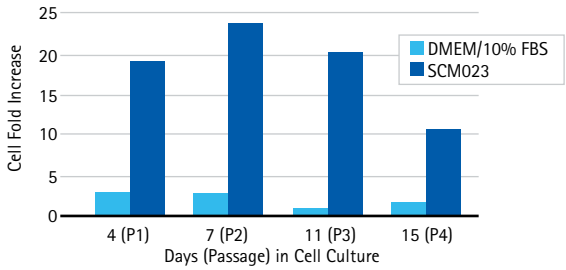
(Cat. No. SCM023)

This medium provides a low-serum (2%) cell culture environment for human mesenchymal stem cells, including those derived from human adipose, bone marrow tissues and ES/iPS cells. This low-serum formulation has been shown to promote cell proliferation at rates that meet or exceed rates exhibited in commercially available serum-containing media, while maintaining excellent cell morphology.

A. Bone Marrow MSC



B. Adipose MSC



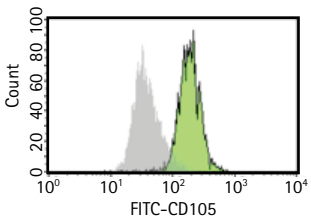
Improved proliferation of Human Bone Marrow-derived (Cat. No. SCR108) and Adipose-derived (Cat. No. SCC038) Mesenchymal Stem Cells cultured in Human Mesenchymal-LS Expansion Medium. Adipose MSCs cultured in the Human Mesenchymal-LS Expansion Medium (Cat. No. SCM023) for four passages yielded a 4-fold greater increase in cell number compared to DMEM medium containing 10% FBS.

FlowCellect® Human Mesenchymal Stem Cell Characterization Kit

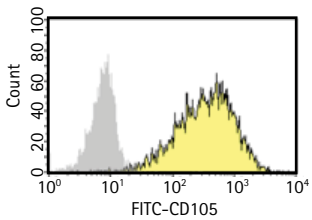
(Cat. No. FCSC100184)

This flow cytometry kit provides a quantitative solution for clearly identifying human MSC cultures with single cell resolution. The kit contains four directly conjugated antibodies to phenotypically characterize hMSCs. Three of the antibodies recognize positive expressing markers (CD105, CD90, and CD44), and one recognizes the negative marker, CD19. These antibodies have been validated on a variety of different mesenchymal stem cell culture types such as adipocyte-derived stem cells (ADSCs), bone marrow-derived hMSCs and ES-derived hMSCs..

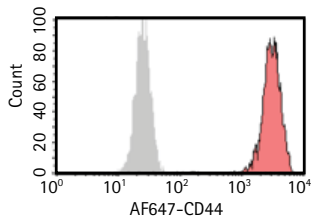
A. Anti-human CD105/FITC



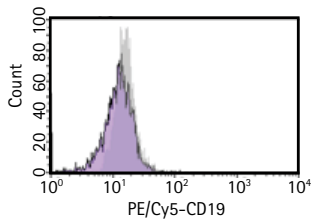
B. Anti-human CD90/PE



C. Anti-human CD44/Alexa Fluor®



D. Anti-human CD19/PE Cy5



Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) were phenotypically identified by CD105, CD90, and CD44 conjugated antibodies. Along with isotype controls, hBM-MSC cultures were individually stained with positive markers, CD105, CD90, and CD44. As illustrated, positive expression is evident for all three cultures compared to the isotype control stained cells (gray histograms). As expected, when CD19 was examined in a similar fashion there was no expression detected, further validating that these four antibodies used in multiplex are a legitimate way to characterize MSCs.

Description	Cat. No.
Human Mesenchymal Stem Cell Characterization Kit	SCR067

Mobius® CellReady Single-use Bioreactor

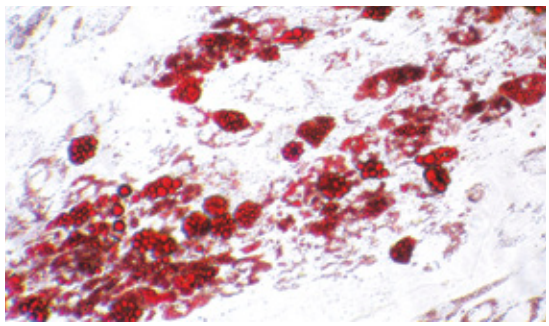
The increased knowledge of the differentiation capability as well as of the immunologic properties of mesenchymal stem cells (hMSCs) has stimulated the interest in their use as therapeutic agents. However, a key hurdle in the clinical application of hMSCs is the high cost of manufacturing and successful scale up of cell culture production. The Mobius® CellReady 3 L single-use bioreactor has successfully been used to grow hMSCs on microcarriers. After 2 weeks of culture, cells reach densities greater than 2×10^5 cells/mL while maintaining their identity as shown by the surface expression of CD105, CD90 and CD73 and the absences of CD14, CD34 and CD45. This system provides a cost-effective approach for the production of clinical grade hMSCs.

Description	Cat. No.
Single-use 3L stirred tank bioreactor (green)	CR0003L200
Applikon style 3L motor adaptor	CR0003L102
Sartorius BIOSTAT® A+ (1L, 2L, 5L) style 3L motor adapter	CR0003L103
Sartorius BIOSTAT® B-DCU style 3L motor adapter	CR0003L104
New Brunswick Celligen® Plus Style 3L motor adapter	CR0003L107
New Brunswick Celligen® 115 Style motor adapter	CR0003L108

Mesenchymal Adipogenesis Kit

(Cat. No. SCR020)

Excessive weight gain and obesity pose significant health challenges in many industrialized nations. Understanding molecular mechanisms that underlie adipogenesis, the process by which adipose or fat tissue is formed, is thus of critical importance. Merck Millipore's Mesenchymal Stem Cell Adipogenesis Kit contains reagents that readily differentiate mesenchymal stem cells to an adipogenic lineage as assessed with Oil-Red-O staining of lipid vacuoles in mature adipocytes. These factors include dexamethasone, IBMX, insulin, and indomethacin. Along with Oil Red O staining solution, a hematoxylin solution is provided to counterstain the cell nucleus.

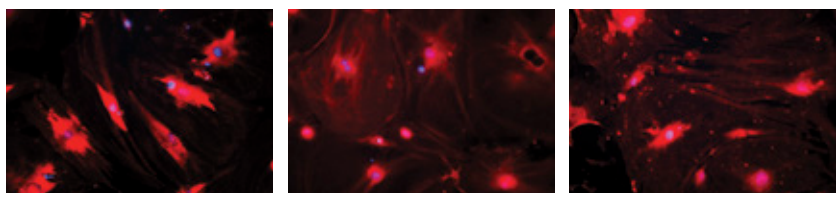


Using the mesenchymal stem cell adipogenesis kit, human bone marrow mesenchymal stem cells differentiated after 21 days to mature adipocytes as indicated by the accumulation of lipid vacuoles that stain with Oil Red O (20x magnification).

Cardiomyocyte Characterization Kit

(Cat. No. SCR059)

Cardiomyocytes are striated heart muscle cells that possess the unique ability to "self-contract" without central nervous system innervation. The inherent contractile activity of cardiomyocytes serves a critical function to pump blood throughout the circulatory system. This kit enables researchers to characterize cardiomyocytes in culture using basic immunocytochemistry techniques. Included in the kit are antibodies that are critical to the structure (actinin and desmin), contractile function (tropomyosin and troponin) and homeostatic control (ANP) of cardiac myocytes to a variety of signals that increase blood pressure.



Troponin I **Desmin** **Actinin**

Cultured CSCs retain their stem cell characteristics and efficiently differentiate into cardiomyocytes expressing mature markers for cardiomyocytes (Troponin I, Desmin, and Actinin).

Key Products for Mesenchymal Stem Cells

Cells, Media and Kits

Description	Cat. No.
Cryopreserved Rat Mesenchymal Stem Cells	SCR027
Rat Mesenchymal Stem Cell Characterization Kit	SCR018
Human Mesenchymal Stem Cell Characterization Kit	SCR067
Cardiac Stem Cell Maintenance Medium	SCM101
Cardiomyocyte Differentiation Medium	SCM102
Cardiac Stem Cell Isolation Kit	SCR061
Cardiomyocyte Characterization Kit	SCR059
Bone Marrow Harvesting and Hematopoietic Stem Cell Isolation Kit	SCR051
Pancreatic Cell DTZ Detection Assay	SCR047
Mesenchymal Stem Cell Expansion Medium (1x)	SCM015
Mesenchymal Stem Cell Osteogenesis Kit	SCR028
LT2 Immortalized Pancreatic Mesenchymal Cell Line	SCR013
Alizarin Red Staining Solution	TMS-008-C
Safranin-O Staining Solution	TMS-009-C
Alcian-Blue Staining Solution	TMS-010-C

Antibodies

Description	Cat. No.
Anti-CD73, clone AA60-E3-3	MABD122
Anti-H-CAM, clone F10-44-2	CBL154
Anti-Thy-1, clone F15-42-1	CBL415
Anti-STRO-1, clone STRO-1	MAB4315
Anti-MCAM, clone P1H12	MAB16985
Anti-Nuclei, clone 235-1	MAB1281
Anti-Bone Morphogenetic Protein 4, clone 3H2	MAB1049
Anti-BMP-7, clone 2A10	MAB4350
Anti-c-Kit, clone YB5.B8	MAB1162
Anti-Stem Cell Factor	AB1498P
Anti-CD349/Frizzled 9, clone W3C4	MABD86

Growth Factors and Cytokines

Description	Cat. No.
BMP-2, Human Recombinant	GF166
BMP-4, Human Recombinant Animal Free	GF302
BMP-6, Human Recombinant	GF168
Hepatocyte Growth Factor, recombinant human	GF116
Macrophage-Colony Stimulating Factor, recombinant human	GF053
Oncostatin M, recombinant human	GF016
SCF, Human Recombinant Animal Free	GF312
Stromal Cell-Derived Factor-1α, recombinant human	GF073
Tumor Necrosis Factor-α, recombinant human	GF023
VEGF165, Human Recombinant Animal Free	GF315

Small Molecule Modulators

Description	Cat. No.
Hepatic Differentiation Inducer, SJA710-6	375110
Tankyrase1/2 Inhibitor, XAV939	575545
Trichostatin A	647925

5

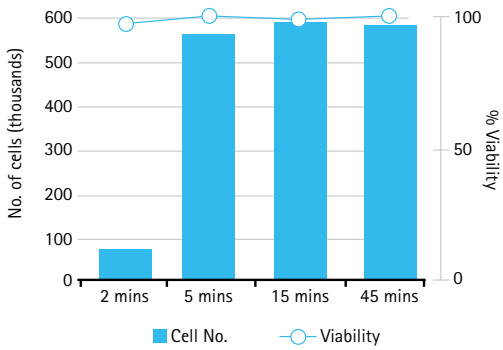
General Cell Culture

Ensure that your discoveries have the highest impact and biological relevance by relying on quality cell and tissue preparations. Count on Merck Millipore's wide variety of reagents, devices, surfaces and cell counting platforms to provide cell growth, structure, and function that more closely mimic what occurs *in vivo*. Merck Millipore's trusted line of sterile filtration tools have been specifically designed to eliminate contaminants and ensure the reproducibility of your downstream analyses.

For a complete listing of our cell culture solutions, visit: **www.merckmillipore.com/cellculture**

Enzymatic Cell Dissociation Solutions

Description	Cat. No.
Accutase® Reagent	SCR005
Accumax® Reagent	SCR006
Trypsin 0.25%, In Hank's Balanced Salt Solution, without Ca^{2+} and Mg^{2+}	SM-2001-C
Low Trypsin-High EDTA, PBS-Based, 0.025% Trypsin and 0.75 mM EDTA (1X), without Ca^{2+} and Mg^{2+}	SM-2004-C
Enzyme-Free Cell Dissociation Solution Hank's Based (1X), liquid	S-004-B
Enzyme-Free Cell Dissociation Solution PBS-Based (1X), liquid	S-014-B



Various constructs of genetically engineered CHO cells, BHK cells and a hybridoma were grown in suspension in serum-free or protein-free medium. Representative cell aliquots were treated with an equal volume of PBS or Accumax™ cell detachment solution and incubated for 5 minutes at 37 °C. Cell number was then determined with a Coulter Counter® Device.

Featured Products for General Cell Culture

Sterile Filtration

Description	Cat. No.
Stericup®-GP Filter Units	SCGPUORE
Steriflip®-GP Filter Unit	SCGP00525

Cultureware

Millicell® EZ SLIDE

Description	Cat. No.
Millicell® EZ SLIDE (4-well glass)	PFHYS0616, PFHYS1008
Millicell® EZ SLIDE (8-well glass)	PEZGS0816, PEZGS0896
Millicell® EZ SLIDE Slide Holder	PEZXMSH01

Millicell® Membrane-Based Cell Culture

Description	Cat. No.
Millicell® 96- Cell Culture Insert Plates	PSHT004R5, PSHT004S5

Millicell® HY (High-Yield) Cell Culture Flasks

Description	Cat. No.
Millicell® HY Flask	PFHYS0616, PFHYS1008

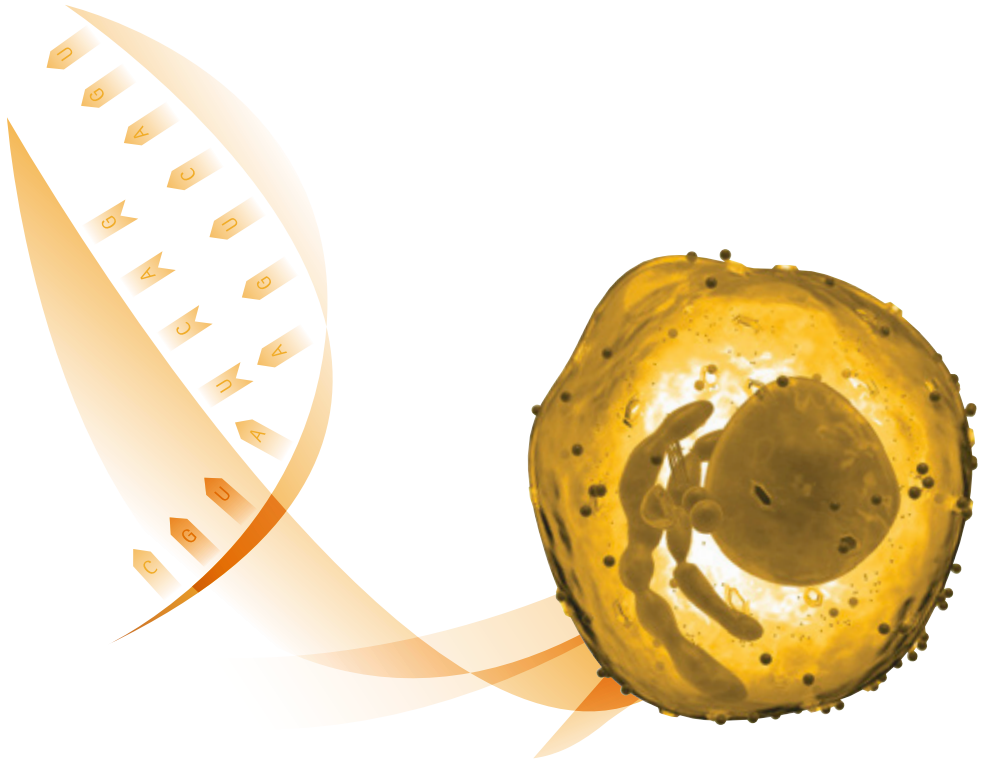
Cell Counting

Description	Cat. No.
Scepter™ 2.0 Handheld Automated Cell Counter, w/50pk of 40 µM Sensors	PHCC20040
Scepter™ 2.0 Handheld Automated Cell Counter, w/50pk of 60 µM Sensors	PHCC20060

Live Cell RNA Detection
SmartFlare™ RNA Detection Probes

Instead of lysed cells, switch to live cells. And while you're at it, eliminate sample preparation and transfection steps altogether. Live cell RNA detection is now possible, in a single incubation step using inert nanoparticle technology to specifically detect native RNA. And when you're done, the probes exit the cells, allowing you to perform downstream analyses with the same, unperturbed cells.

- Monitor reprogramming status of cells based on RNA content.
- Characterize differentiated or pluripotent stem cells by examining RNA expression in real time.
- Obtain relative quantification of RNA content at single cell resolution when combined with flow cytometry.
- Target RNA sequences for detecting markers that were previously difficult to study.



Make the smart change!

To learn more, please visit:
www.merckmillipore.com/SmartFlare

To Place an Order or Receive Technical Assistance

In Europe, please call Customer Service:

France: 0825 045 645
Germany: 01805 045 645
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: **www.merckmillipore.com/offices**

For Technical Service visit: **www.merckmillipore.com/techservice**

Get Connected!

Join Merck Millipore Bioscience on your favorite social media outlet for the latest updates, news, products, innovations, and contests!

facebook.com/MerckMilliporeBioscience

twitter.com/Merck4Bio

MERCK MILLIPORE

www.merckmillipore.com/offices

Lit No. PB6112ENEU BS-GEN-13-08089 Printed in the USA 05/2013

Discovered by CombiCult ®