Reprogramming Cell Fate and Function

Novel Strategies for iPSC Generation, Characterization, and Differentiation

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Merck Millipore is a division of MERCK

Platforms, Technologies, and Services

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 stem cells, human embryonic stem cells, and a complete line of mouse embryonic stem cells. Endothelial and epithelial cells are also available.

CELL CULTURE MEDIA AND REAGENTS

Merck Millipore provides media designed for virtually all types of stem cells, including embryonic, mesenchymal, and neural, and for both human and rodent 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.

ANTIBODIES AND IMMUNOASSAYS

Merck Millipore offers an extensive, focused portfolio of antibodies and immunoassays. 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.

CELL-BASED ASSAYS AND QUANTITATIVE CELL IMAGING

Merck Millipore offers a significant portfolio of live cell, whole-cell and cell-based activity assays and reporter systems for direct and indirect detection. These technologies facilitate protein target validation, identify cellular pathways and determine mechanism of action for lead optimization environments. Merck Millipore also offers an array of assays for high-content, multiparametric cell imaging, enabling identification of cellular responses and events under user-defined conditions.

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

FLOW CYTOMETRY ASSAYS AND SYSTEMS

Flow cytometry is an essential tool for in-depth cell analysis, with the capacity to simultaneously measure multiple parameters on individual cells. Guava® flow cytometers provide direct, precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. Merck Millipore also offers FlowCellect™ reagents, kits and Milli-Mark™ conjugated antibodies that are optimized for guava systems and compatible with traditional core lab environments, along with application-specific analysis software modules, to provide a complete solution for flow cytometry.

MILLIPLEX® MAP MULTIPLEX ASSAYS

MILLIPLEX MAP assays offer the broadest selection of multiplex kits and reagents in a wide variety of therapeutic areas, measuring multiple biomarkers using a small sample size. Compared to conventional methods, such as ELISAs and Western blots, MILLIPLEX MAP enables the simultaneous detection of multiple soluble or intracellular biomarkers. Using the Luminex® xMAP® bead-based technology, Merck Millipore's flexible and customizable assays are exhaustively tested and qualified for sensitivity, specificity, reproducibility and wide dynamic range.

CALBIOCHEM® COMPOUNDS

Merck Millipore's Calbiochem line of high quality inhibitors, biochemicals, antibodies, proteins, and kits have been cited in thousands of peer-reviewed publications. 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.

CELL CULTUREWARE AND STERILE FILTRATION DEVICES

Merck Millipore's innovative cell culture workflow solutions help optimize cell growth and maintenance. Designed for fast flow and maximum flexibility, our sterile filtration devices have many membrane options. Also available are the Millicell® membrane-based cell culture inserts and multiwell plates that provide a more *in vivo*-like environment and coculture options.

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Introduction

Novel iPS Cell Strategies for Reprogramming Cell Fate and Function

In C.H. Waddington's classic epigenetic landscape metaphor for biological development, cell fates are established much like marbles rolling down hill in valleys, split into different lineages by various ridges (Waddington, 1953). Terminal cell differentiation, like a marble at its lowest local elevation, was thought to be stable and immutable.

Modern advances in the molecular mechanisms underlying epigenetics has led to the realization that, through treatment with small molecules or induced gene expression, cells can be dedifferentiated and refocused to different lineages like a marble pulled back uphill and redirected down a different path.



The current science of inducing pluripotency in cells has yielded practical technologies and protocols for a new generation of applied stem cell research. These innovations have advanced all steps of the reprogramming workflow: iPS cell generation; iPS cell culture; iPS cell characterization; and iPS cell differentation. iPS cell clones must be carefully characterized before any application in diagnosis or therapy. Following characterization of ES-like state, the iPS cells can be guided down distinct differentiation pathways using various growth factors, small molecules, or other extracellular microenvironment manipulations.

Merck Millipore is dedicated to developing and refining these induced pluripotent stem (iPS) cell technologies. With Merck Millipore's comprehensive portfolio of reagents and antibodies, including the expertise of Upstate®, Chemicon®, and Calbiochem® brands, researchers now have reliable, high-quality solutions for cellular reprogramming available to them.

REPRODUCTION INTERDITE SAUF AUTORISATION EXPRESSE DE MILLIPORE SAS

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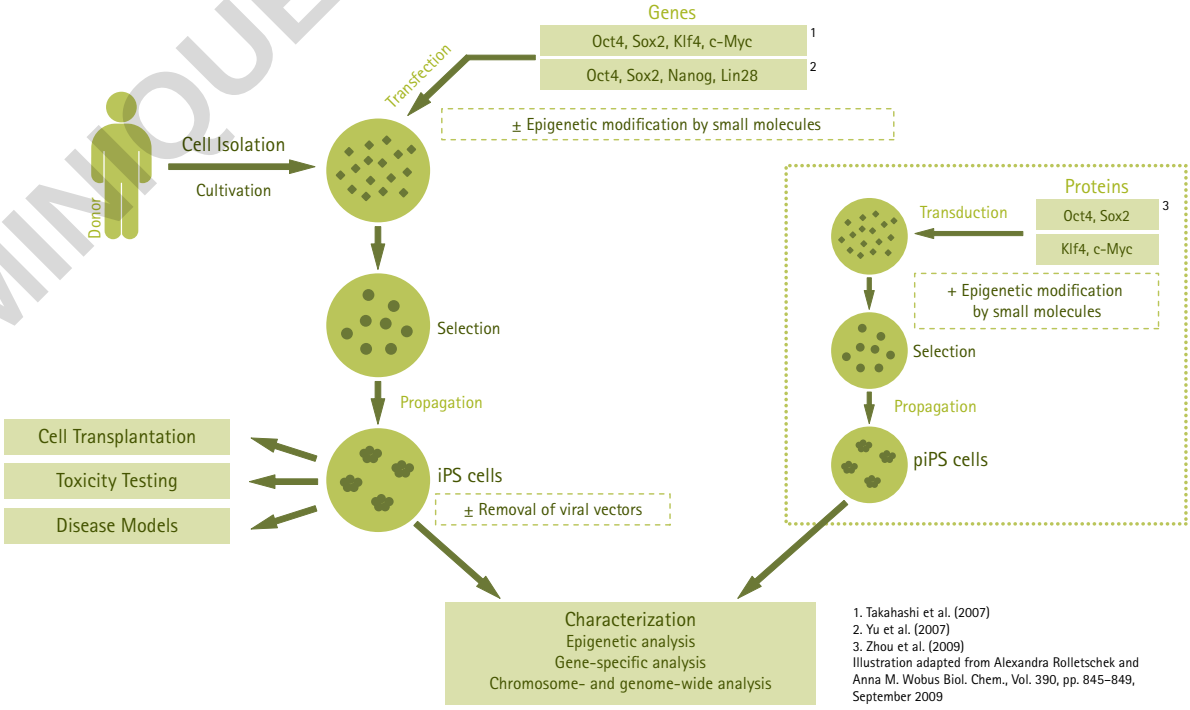
24 Measuring Differentiated Cell Function

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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 vectors. Mouse cells have been reprogrammed by directly transducing protein products of reprogramming genes, generating protein-induced pluripotent stem (piPS) cells. Because the iPS cell generation process is sensitive to multiple variables, Merck Millipore supports the entire workflow with resources including fibroblasts validated for efficient reprogramming, optimized media and reagents, and virus purification kits for improved transduction.

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1. Takahashi et al. (2007)
2. Yu et al. (2007)
3. Zhou et al. (2009)
Illustration adapted from Alexandra Rolletschek and Anna M. Wobus Biol. Chem., Vol. 390, pp. 845–849, September 2009

Principle strategies to obtain human iPS cells. Dashed line boxes: Methods that have been established for generating mouse and only partially established for human iPS cells. These methods include strategies to reduce the number of reprogramming viral vectors and/or to enhance the reprogramming efficiency by small molecules, and techniques to remove viral vectors after successful reprogramming. Dotted line box: Generation of protein-induced pluripotent stem (piPS) cells, reported in mouse cells[3].

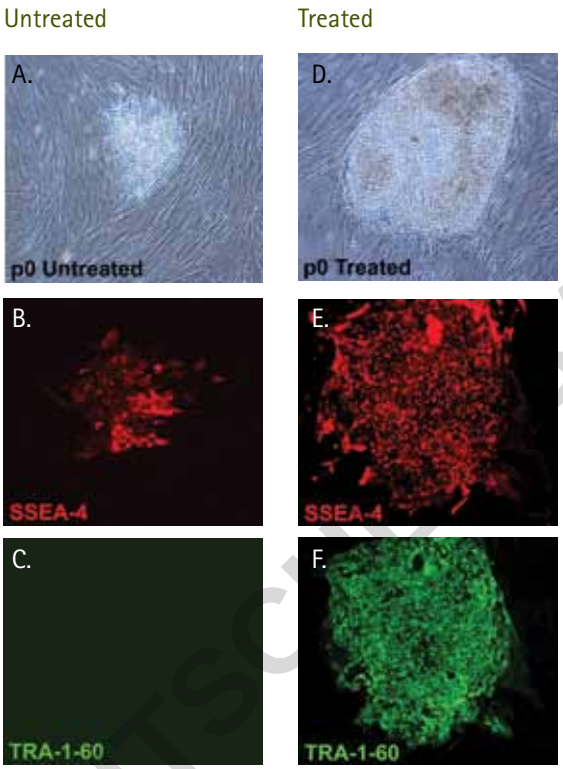
Human iPS Cell Boost Supplement

Increase the quality and number of your human iPS cells with Merck Millipore's new small molecule Human iPS Cell Boost Supplement. No need to optimize your small molecule concentrations, each supplement is provided in a ready to use format for supplementing 300 mL of medium.

When used in conjunction with the Human STEMCCA™ lentivirus reprogramming kits, the Human iPS Cell Boost Supplement conveys the following advantages:

- Decreases time required to establish fully reprogrammed colonies by 50%
- Enhances generation of fully reprogrammed colonies (SSEA-4+ TRA-1-60+ Hoechst Dim)
- Improves colony morphology — colonies possess distinctive flat 2D morphology and can be easily passaged
- More than doubles reprogramming efficiency

In addition, Merck Millipore also offers a Mouse iPS Cell Boost Supplement containing three proprietary small molecules provided at optimized concentrations for supplementing 300 mL of medium. You can enhance the efficiency of mouse iPS colony formation up to three-fold by adding this supplement to your iPS cell culture medium.



Addition of Human iPS Cell Boost Supplement to a polycistronic lentivirus-based reprogramming regime (STEMCCA) decreases the time required to establish fully reprogrammed human iPS clones (D,E,F). p0 human iPS colonies exhibited larger colony sizes, a flat 2D morphology (D), and are SSEA-4-positive (E), and TRA-1-60-positive (F). This is in contrast to untreated control where the colonies are smaller, 3D in morphology (A) and are SSEA-4 positive (B) but TRA-1-60 negative (C) at p0.

Description	Qty	Catalogue No.
Human iPS Cell Boost Supplement	Kit	SCM088
Mouse iPS Cell Boost Supplement	Kit	SCM087

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FibroGRO® Xeno-Free Human Foreskin Fibroblasts FibroGRO LS Complete Medium

(Catalogue No. SCC058)

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 FibroGRO LS (low serum) Complete Medium (Cat. No. SCMF002). 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 Kit (Cat. No. SCR530).

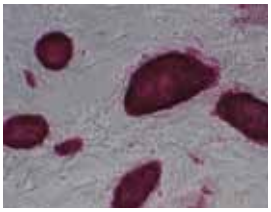


Human iPS cell colonies generated from FibroGRO HFFs and plated on mouse embryonic fibroblasts (MEFs, Cat. No. PMEF-CF).

Mouse Embryonic Fibroblasts

(Catalogue No. PMEF-CFL)

Feeder cells, including primary mouse embryonic fibroblast (PMEF) cells, support ES cell growth by secreting important growth factors that help maintain pluripotency and by providing a cellular matrix. EmbryoMax® PMEFs are ideal for ES and iPS cell culture and conveniently eliminate the need for time-consuming feeder cell isolation and preparation. Several varieties are available, including actively dividing, growth-arrested (mitomycin-C treated), and drug-resistant feeder cells. The actively dividing, non-mitomycin-C treated PMEFs also provide good starting material for the generation of mouse iPS cells.

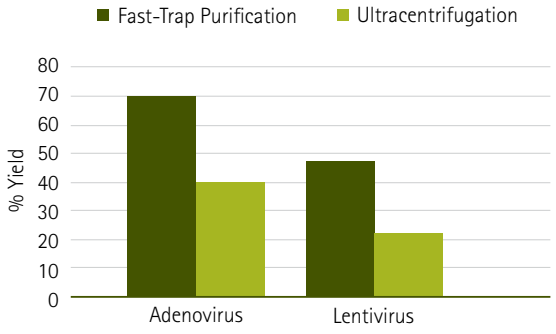


Mouse embryonic fibroblasts infected with the STEMCCA lentivirus display characteristic ES cell morphology and marker expression. Passage 3 mouse iPS cells express high levels of Oct-4 (Cat. No. MAB4419).

Fast-Trap® Virus Purification Kits

(Catalogue No. FTAV00003, FTLV00003)

When generating virus for reprogramming experiments, use Merck Millipore's Fast-Trap kits for a fast, safe, and easy alternative to traditional viral purification. The kits contain the necessary components to accommodate the entire virus purification workflow. The purification results in high recovery (up to 70%) of viable viral particles from cellular contaminants and the expressed recombinant transgene. It yields concentrated virus in the exchange buffer of choice, suitable for *in vitro* and animal studies.



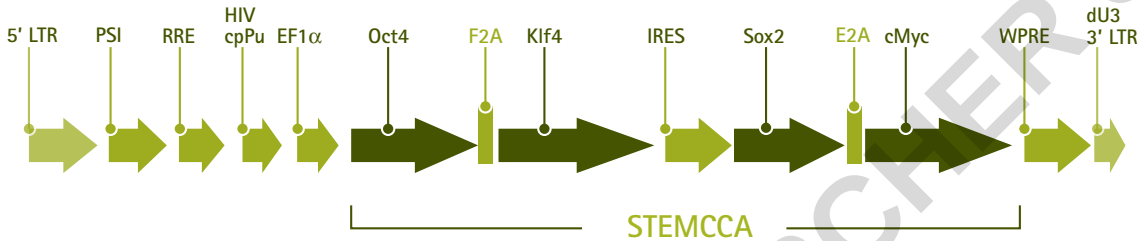
Virus purification using Fast-Trap kits resulted in higher yields than those obtained by purification by ultracentrifugation.

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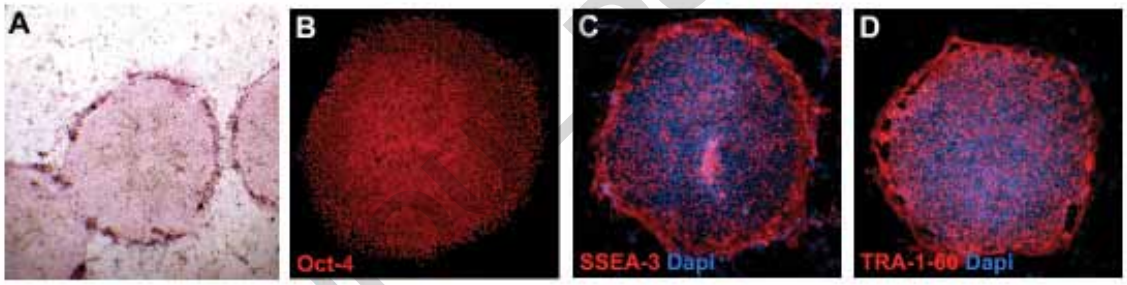
STEMCCA™ Reprogramming Kits

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.



The STEMCCA vector is comprised of the transcription factors Oct-4, Klf4, SOX-2, 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	Qty/pack	Catalogue No.
Human STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	30 µL lentivirus + Polybrene® transfection reagent	SCR544
Human STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	30 µL lentivirus + Polybrene transfection reagent	SCR545
Mouse STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	15 µL lentivirus + Polybrene transfection reagent	SCR510
Mouse STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	45 µL lentivirus + Polybrene transfection reagent	SCR530
Mouse STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	15 µL lentivirus + Polybrene transfection reagent	SCR511
Mouse STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	45 µL lentivirus + Polybrene transfection reagent	SCR531

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Key Products

Cells

Available from www.millipore.com

Description	Catalogue No.
EmbryoMax Primary Mouse Embryo Fibroblasts, neo resistant, not mitomycin-C treated, strain FVB, passage 3	PMEF-NL
EmbryoMax Primary Mouse Embryo Fibroblasts, hygro resistant, not mitomycin-C treated, strain C57BL/6, passage 3	PMEF-HL

Inhibitors

Available from www.merck4biosciences.com

Description	Catalogue No.
Valproic Acid, Sodium Salt	676380
TGF-βRI Kinase Inhibitor II	616452

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iPS Cell Culture

The success of iPS cell generation and subsequent redifferentiation protocols is highly dependent upon the media, growth factors, and ECM environments of the developing colonies. Complete reprogramming is complex, involving changes in gene expression, chromatin structure, and protein and DNA modification state. 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. Fortunately, high quality media and supplements are now commercially available, reducing variation and increasing success of target terminal differentiation.

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ESGRO®-2i Medium

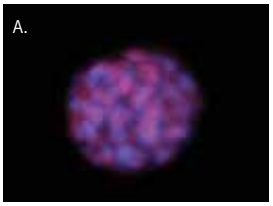
(Catalogue No. SF016-100, SF016-200)

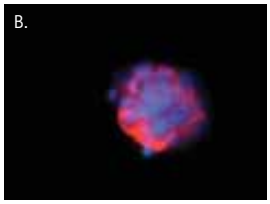
This serum-free medium is designed to maintain pluripotency and promote growth at clonal density of iPS and mouse ES cells. ESGRO-2i medium is a defined medium formulated with Merck Millipore's gold standard ESGRO medium supplement, a highly potent formulation of mouse leukemia inhibitory factor (LIF), and provided with GSK3β and Mek1/2 inhibitor supplements. In addition, 2i/LIF-based media have been shown to promote partially reprogrammed cells to full pluripotent status.

ESGRO Complete™ Plus Medium

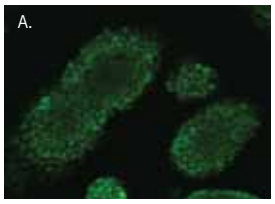
(Catalogue No. SF001-500P, SF001-100P)

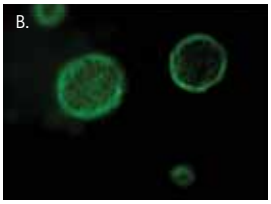
The first complete medium for the serum-free and feeder-free culture of mouse ES cells, the ESGRO Complete system enables more reproducible studies under controlled conditions. The defined formulation of ESGRO Complete Plus includes mouse LIF, BMP4, and a GSK3β inhibitor at optimized concentrations. This formulation eliminates the inconsistency and expense associated with the use of FBS and feeder layers, while providing a complete medium that enhances the growth and maintenance of undifferentiated mouse ES and iPS cells.





ESGRO-2i adapted 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.

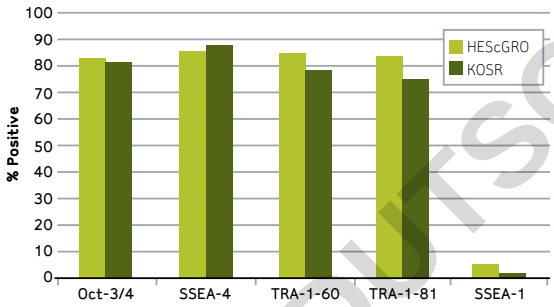




To confirm pluripotency of ES cells after culturing in ESGRO Complete PLUS medium, cells were immunostained for Oct-4 (A) and SSEA-1 (B).

HEScGRO® hES Cell Medium
(Catalogue No. SCM020, SCM021)

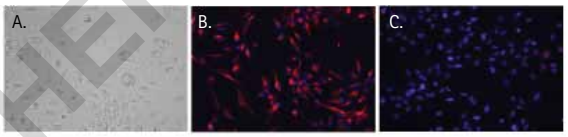
HEScGRO hES cell medium is the first animal-component-free medium that is specially formulated to meet the unique requirements of human embryonic stem cell culture, enabling more reproducible hES cell research under controlled conditions. HEScGRO has been extensively tested and proven to maintain the pluripotent nature of several hES cell lines, including MEL-1, MEL-2, H1, H7, and H9. This medium is fully defined and does not require additional supplementation to maintain cells in their pluripotent state. Human feeders are required to maintain hES cells in an undifferentiated state.

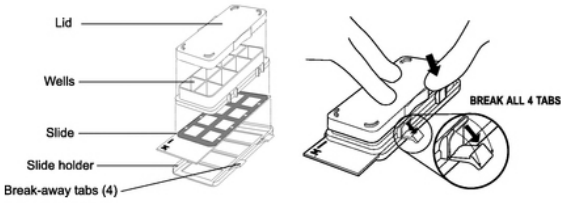


hES cells grown in HEScGRO Medium express pluripotency markers. Flow cytometry analysis of cells cultured with both HEScGRO and KnockOut™ Serum Replacement (KOSR) medium show that marker expression patterns are stable for pluripotency under both conditions after 5 passages. hES cells grown in HEScGRO medium continue to express the same levels of pluripotency markers even after 20 passages (data not shown).

Millicell EZ Slides
(Catalogue No. PEZGS0416, PEZGS0816)

Simplify your cell analysis by using the Millicell EZ Slide to culture, fix, stain, and view your sample, all in one device. There's no need to remove the media chamber from the slide prior to fixing or staining. Easy well removal eliminates the worry of breaking slides or harming cells during analysis - acquire data simply and quickly with Millicell EZ Slides. Finally, these slides are STEM CELL TESTED using ReNcell® Human Neural Stem Cells; after five passages of culture, cells still expressed Sox-2, a marker of multipotency.





ReNcell CX cells cultured on Millicell 8-well glass EZ Slides show staining for nestin (red, B) and Sox-2 (red, C). Nuclei (blue) are stained with DAPI. Lower panel illustrates the integrated, stackable design of the Millicell EZ Slide and a demonstration of easy tab removal.

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Key Products

Growth Factors

Available from www.millipore.com

Description	Catalogue No.
Fibroblast Growth Factor basic, recombinant human	GF003, GF003AF-MG
Leukemia Inhibitory Factor, recombinant mouse	LIF2010, LIF2005, LIF2050
ESGRO mLIF medium supplement	ESG1106, ESG1107

ECM Proteins

Available from www.millipore.com

Description	Catalogue No.
Human Collagen Type I	CC050
Human Collagen Type IV	CC076
Human Vitronectin	CC080, 08-126
Human Laminin	AG56P
Human Fibronectin, cellular	08-102
Human Plasma Fibronectin, purified protein	FC010, FC010-5MG, FC010-10MG, FC010-100MG
ECL Cell Attachment Matrix (EHS Mouse Tumor)	08-110

Inhibitors

Available from www.merck4biosciences.com

Description	Catalogue No.
Cyclopamine-KAAD	239804
JAK Inhibitor I	420099
LY 294002	440202
PD 98059	513000
PP2	529573
Rapamycin	553210
SB 203580	559389
γ-Secretase Inhibitor IX	565770
U0126	662005
MEK1/2 Inhibitor III, PD0325901	444966
GSK-3 Inhibitor XVI, CHIR99021	361559
ROCK inhibitor Y27632	688000

Cells and Media

Available from www.millipore.com

Description	Catalogue No.
FibroGRO Inactivated Xeno-free Human Foreskin Fibroblasts	SCC057
DMEM w/ 4.5 g/L Glucose, 2.25 g/L Sodium Bicarb w/o Sodium Pyruvate, 500 mL	SLM-220-B
DMEM w/ 4.5 g/L Glucose, 2.25 g/L Sodium Bicarb w/o Sodium Pyruvate, 400 mL	SLM-220-M
DMEM/F12, w/ HEPES, w/ GLUT, 500 mL	DF-041-B
DMEM/F12, w/o HEPES, w/ GLUT, 500 mL	DF-042-B
EmbryoMax cell culture freezing media (1X), DMEM, 10% DMSO, calf & fetal bovine serum	S-002-D, S-002-5F, S-002-10F
EmbryoMax cell culture freezing medium (2X), 20% DMSO & fetal bovine serum	ES-002-D, ES-002-5F, ES-002-10F

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Key Products

Reagents

Available from www.millipore.com

Description	Catalogue No.
EmbryoMax 0.1% Gelatin Solution	ES-006-B
Penicillin-Streptomycin solution	TMS-AB2-C
Accutase® Cell Dissociation Solution	SCR005
Accumax™ Cell Detachment Solution	SCR006
Enzyme-free cell dissociation solution (1X), Hank's based	S-004-B, S-004-C
Enzyme-free cell dissociation solution (1X), PBS based	S-014-B, S-014-C

Cultureware and Sterile Filtration Devices

Available from www.millipore.com

Description	Catalogue No.
Millicell HY Culture Flask, 3 layer, T600, sterile	PFHYS0616
Millicell HY Culture Flask, 5 layer, T1000, sterile	PFHYS1008
Millicell-24 Cell Culture Plate, single well feeder tray, 24-well receiver tray and lid, PET membrane, 1.0 µm pore size STEM CELL TESTED	PSRP010R1
Millicell-24 Cell Culture Plate, single well feeder tray and lid, PET membrane, 1.0 µm pore size STEM CELL TESTED	PSRP010R5

Description	Membrane/Application	Pore Size (µm)	Funnel Capacity (mL)	Receiver Bottle	Catalogue No.
Stericup®-GP Filter Units STEM CELL TESTED	Millipore Express® PLUS (PES) /fast filtration of tissue culture media and buffers	0.22	500	500 mL	SCGPU05RE
Steritop®-GP Filter Units STEM CELL TESTED	Millipore Express PLUS (PES) / filtration of high value biomolecules, lowest protein binding	0.22	500	33 mm thread	SCGPS05RE
				45 mm thread	SCGPT05RE

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iPS Cell Characterization

The unique advantage of using iPS cells for studying differentiation, compared to isolating naturally occurring stem cells from adults or embryos, is that the iPS cell approach uses common, easily accessible cell types such as fibroblasts. The dedifferentiation of a single cell type can generate extremely versatile ES cell-like cells capable of broad pluripotency.

However, after manipulating the extracellular and/or intracellular environment of cells to achieve reprogramming, the resulting cells must be thoroughly characterized for ES cell-like pluripotency, marked by expression of key undifferentiated state markers. This step is an important verification that the cells resulting from these molecular manipulations have pluripotent phenotypes in common with naturally occurring stem cells and have no residual compounds or viral constructs that could cause abnormalities in gene expression, or cellular dysfunction.

During the reprogramming process, cells undergo dynamic, 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 gaining confidence in the dedifferentiated stage. Comparing ES and iPS cells continues to be an active area of investigation. Specifically, recent studies have shown that iPS cells display more gene copy number variation as well as areas of aberrant methylation compared to ES cells[1,2].

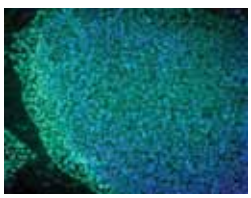
1. Laurent LC et al. Dynamic Changes in the Copy Number of Pluripotency and Cell Proliferation Genes in Human ESCs and iPSCs during Reprogramming and Time in Culture. Cell Stem Cell 2011 Jan 7; Vol 8(1):106-118.
2. Lister R et al. Hotspots of Aberrant Epigenomic Reprogramming in Human Induced Pluripotent Stem Cells. Nature. 2011 Mar 3;471(7336): 68-73.

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Anti-HESCA-2, clone 060818-7A6

(Catalogue No. MAB4406)

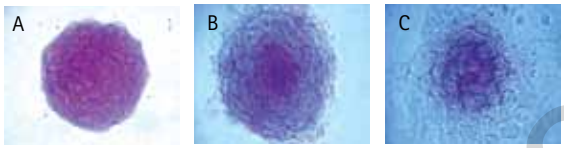
Anti-HESCA-2, developed in collaboration with Abeome Corporation, recognizes a newly discovered 200 kDa cell surface marker that is expressed on pluripotent human embryonic stem cells (hESC), and may serve as a useful tool in the identification, characterization, and isolation of undifferentiated hES and hiPS cells from differentiating hESC and feeder cell types. Merck Millipore offers several other novel monoclonal antibodies against surface antigens of undifferentiated hESCs, showing strong reactivity against undifferentiated, but not differentiated hESCs.



H9 (WA09) human ES cells labeled with the HESCA-2 antibody (green) and DAPI. Only pluripotent human ES cells are labeled by anti-HESCA-2; the antibody does not recognize the cells differentiating from the human ES cell colony.

ESC Characterization Kit
(Catalogue No. SCR001)

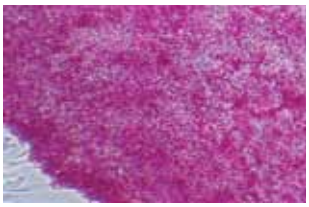
Merck Millipore's ES Cell Characterization Kits are specific and sensitive tools for the phenotypic assessment of the differentiation status of human and mouse ES and iPS cells. This kit measures alkaline phosphatase activity, as well as expression of SSEA-1, SSEA-4, TRA-1-60, and TRA-1-81 antigens. A combinatorial analysis of marker expression using these kits enables a more accurate assessment of stem cell phenotype, compared to assessment based solely on single stem cell markers.



Alkaline phosphatase staining of ES cells using the ES Cell Characterization Kit (Cat. No. SCR001). High magnification of undifferentiated (A) and differentiated murine ES cells (B & C) following alkaline phosphatase (AP) staining using the ES cell characterization kit. Results show diminished AP expression by differentiated cells, as indicated by a decrease in staining intensity, following ES cell differentiation.

Alkaline Phosphatase Detection Kit
(Catalogue No. SCR004)

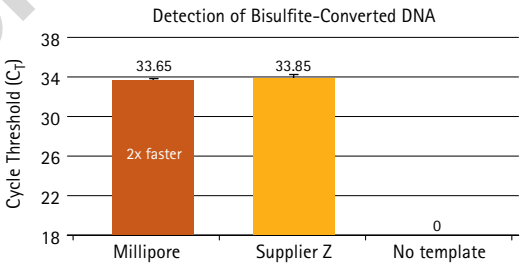
Merck Millipore's Alkaline Phosphatase Detection Kit is a specific and sensitive tool for the phenotypic assessment of ES cell differentiation by the determination of AP activity. Endogenous AP expression in undifferentiated ES cells can be readily detected by intense staining following the recommended staining procedure. A quantitative version of the kit is also available.
(Catalogue No. SCR066)



Alkaline phosphatase staining of H9 cells cultured in HEScGRO medium, using the Allkaline Phosphatase Detection Kit (Catalogue No. SCR004).

CpGenome™ Turbo Bisulfite Modification Kit
(Catalogue No. S7847)

Given the reported epigenomic differences between ES cells and iPS cells, identifying differential patterns of DNA methylation can help characterize iPS cells and may help elucidate the mechanisms by which reprogramming confers pluripotency. Accelerate your studies of gene methylation with the fastest bisulfite modification kit available. We have optimized our proprietary conversion reagent and protocol for short incubation times and >99.5% efficiency of conversion of unmethylated cytosines to uracil. Starting with as little as 500 pg of unmodified input DNA, obtain pure, ready to use, bisulfite-modified DNA in under 90 minutes.



Equivalent bisulfite conversion, twice as fast. DNA (S7821) was bisulfite-converted using either the CpGenome Turbo Kit or a kit from Supplier Z. 1 ng of bisulfite-converted DNA was amplified using the CpG Wiz® MGMT methylated primer set (S7803).

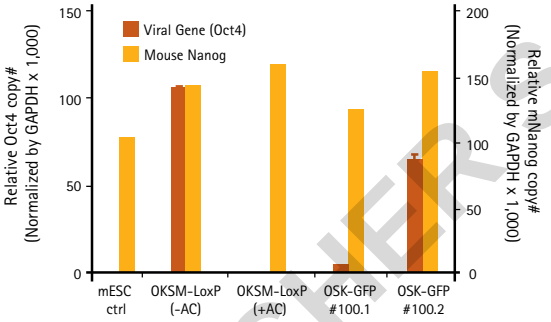
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STEMCCA Viral Gene Detection Multiplex qPCR Kits

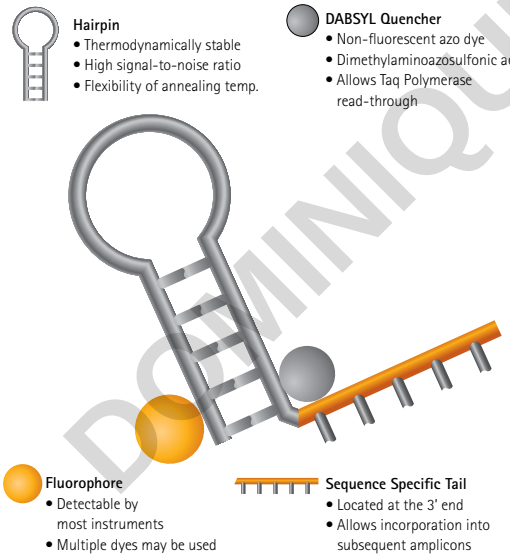
For iPS cell clones generated using STEMCCA lentiviral vectors, our new multiplex RT-PCR kits facilitate the identification of partially and fully reprogrammed colonies. These kits use the Amplifluor® real-time quantitative PCR technology, which enables the detection of multiple targets in a simple, one-step closed-tube system. Included in the kit are three Amplifluor primer sets conjugated with distinct fluorophores that detect the expression of:

- STEMCCA viral transgenes
- Endogenous levels of the pluripotency gene Nanog
- Housekeeping GAPDH gene for normalization of gene expression levels

The kits have been pre-optimized using the included positive control standard. Also available are several individual Amplifluor primer sets that may be used to characterize the pluripotency of ES or iPS cells in single gene detection assays or together with other fluorophore-conjugated primer sets in multiplex detection assays.



Viral Oct-4 and mouse endogenous Nanog mRNA levels from mouse ES cells and iPS cells were quantified using STEMCCA viral gene detection qPCR multiplex kit (mouse). Copy numbers of three target genes were extrapolated from the standard curve. Relative mRNA copy numbers for viral Oct-4 and endogenous Nanog were normalized using GAPDH expression levels. Viral transgenes were continuously expressed in most iPS clones. Analysis of a mouse iPS cell clone with successful Cre-mediated excision of the integrated viral transgenes [OKSM-LoxP (+AC)] indicated a complete absence of viral transgene expression and comparable levels of endogenous Nanog expression to mESC control.



The Amplifluor system consists of the fluorescent Amplifluor hairpin primer and an unconjugated reverse primer. In combination, these two primers produce a fluorescently labeled amplicon, which can be measured by real-time PCR. The carefully designed Amplifluor molecule consists of four parts: a fluorophore, a hairpin structure, a DABSYL quencher, and a target-sequence specific tail.

Description	Catalogue No.
STEMCCA Viral Gene Detection qPCR Multiplex Kit (Human)	SCR580
STEMCCA Viral Gene Detection qPCR Multiplex Kit (Mouse)	SCR581
Amplifluor Human/Mouse Oct-4 FAM Primer Set	SCR584
Amplifluor Human/Mouse Oct-4 JOE Primer Set	SCR585
Amplifluor Mouse Nanog FAM Primer Set	SCR586
Amplifluor Human Nanog FAM Primer Set	SCR587
Amplifluor Mouse Nanog JOE Primer Set	SCR588
Amplifluor Human Nanog JOE Primer Set	SCR589
Amplifluor Mouse Viral OCT-4 FAM Primer Set	SCR583
Amplifluor Human/Mouse GAPDH JOE Primer Set	SCR590
Amplifluor Human/Mouse 18S rRNA JOE Primer Set	SCR591
Amplifluor Human/Mouse GAPDH FAM Primer Set	SCR592
Amplifluor Human/Mouse 18S rRNA FAM Primer Set	SCR593
Amplifluor Human/Mouse GAPDH Texas Red Primer Set	SCR594
Amplifluor Human/Mouse 18S rRNA Texas Red Primer Set	SCR595

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Key Products



Download the Histone Modifications App for ready access to the biological significance and epigenetic implications of core histone amino acid modifications in your iPS, ES, and differentiated cells.

Through this interactive nucleosome experience, you can explore published histone modifications to obtain relevant bioinformatic data, supporting references, and available research reagents (such as antibodies, ChIP kits, and modifying enzymes) for histone modification and epigenetics research.

Download at www.itunes.com or visit www.millipore.com/histonemodapp to learn more

Antibodies

Available from www.millipore.com

Description	Catalogue No.
Anti-Undifferentiated hESC Surface Marker, clone mAb14	MAB4439
Anti-HESCA-1, , clone 051007-4A5 (human)	MAB4407
Anti-Oct-4 (human)	MAB4401, MAB4419
Anti-SSEA-3, clone MC-631 (human)	MAB4303
Anti-SSEA-4, clone MC-813-70 (human)	MAB4304
Anti-ShSCP-5, clone 8H9.3 (human)	MAB4408
Anti-TRA-1-60, clone TRA-1-60 (human)	MAB4360
Anti-TRA-1-81, clone TRA-1-81 (human)	MAB4381
Anti-Dppa1, clone 4D10.2 (mouse)	MAB4355
Anti-Genesis [FoxD3] (mouse)	AB5687
Anti-Nanog (mouse)	AB5731
Anti-Podocalyxin-like protein I (Cytotoxic), clone mAb 84 (mouse)	MAB4414
Anti-Pramel-4 (mouse)	AB4304
Anti-Rex-1 (mouse)	MAB4316
Anti-Sox-2, clone 6F1.2 (mouse)	MAB4343
Anti-SSEA-1 (mouse)	AB4304
Milli-Mark Anti-monomethyl-Histone H3 (Lys27)-Alexa Fluor488	FCABS304A4
Milli-Mark Anti-monomethyl-Histone H3 (Lys29)-Alexa Fluor488	FACBS301A4
ChIPAb+™ Bmi-1	17-664
ChiPAb+REST	17-641
ChIPAb+HDAC1	17-608
ChIPAb+Sox-2, clone 6F1.2	17-656
ChIPAb+Acetyl-Histone H3	17-615
ChIPAb+Acetyl Histone H4	17-630

Assays

Available from www.millipore.com

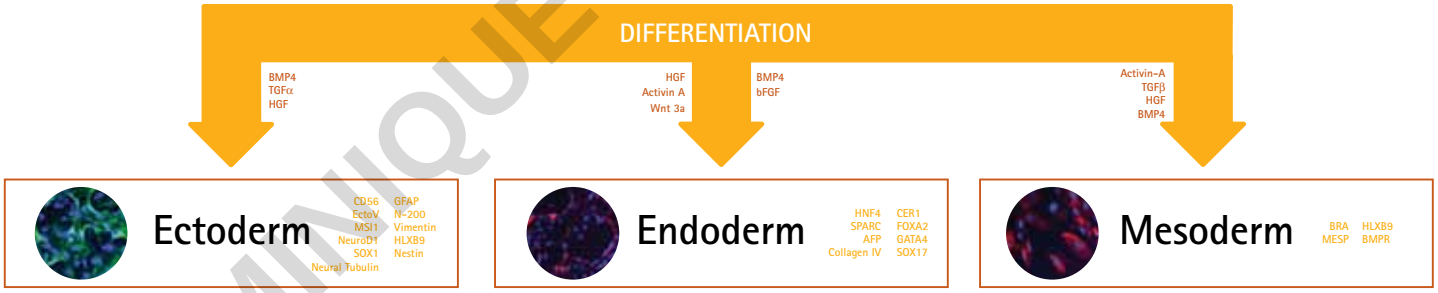
Description	Catalogue No.
FlowCellect Embryonic Stem Cell Surface Marker Characterization Kits	FCHEC25106, FCHEC25104, FCHEC25102, FCMEC25110
Germ Layer PCR Kit	SCR063
Human Embryonic Germ Layer Characterization Kit	SCR030
Quantitative Alkaline Phosphatase ES Cell Characterization Kit	SCR066
Magna ChIP™ Universal ChIP DNA Microarray Quad Kit (12 assays)	17-1004
Magna ChIP Universal ChIP DNA Microarray Kit (3 assays)	17-1000
Magna ChIP^2 ™ Mouse Promoter 244K Microarray Kit	17-1002
Magna ChIP^2 Human Promoter 244K Microarray Kit	17-1001
ChIP Assay Kit	17-295
EZ-ChIP™	17-371
EZ Magna ChIP™ Kits	17-408 and 17-409
Magna ChIP™ Kits	17-610 and 17-610
Magna GrIP Rack	20-400
Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Library Preparation Kit	17-1010

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iPS Cell Differentiation

Pluripotent stem cells can be differentiated into cells of all three embryonic germ layers. The characterization of differentiated progeny is important in studies relating to the use of pluripotent stem cells to treat degenerative diseases, such as diabetes, chronic heart disease, and Parkinson's disease. *In vivo*, the paths taken by ES cells towards particular developmental fates are determined by signaling within the tissue microenvironment. *In vitro*, ES cells and iPS cells may be guided towards particular cell fates by mimicking lineage-specific microenvironments in culture. Current stem cell research has focused on defining the factors, media, small molecules, and early-expressed lineage markers needed to create particular transforming microenvironments and in subsequent differentiation analysis. Merck Millipore offers a wide range of quality media, supplements, small molecules, and antibodies against differentiation markers. These reagents improve the reproducibility of differentiation conditions while also elucidating the molecular mechanisms underlying tissue-specific differentiation.

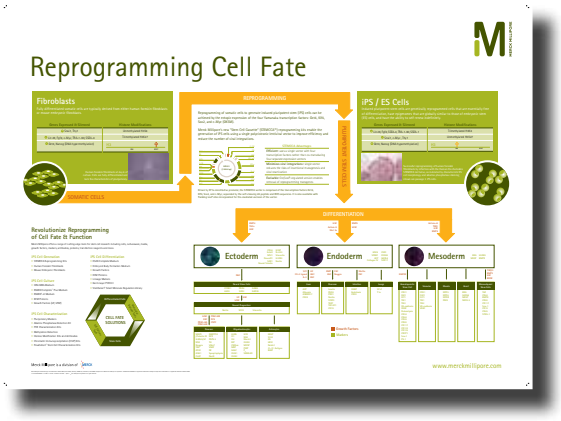
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Pluripotent stem cells can differentiate into the three germ layers in response to signals (red). Markers expressed in each germ layer are listed in orange.

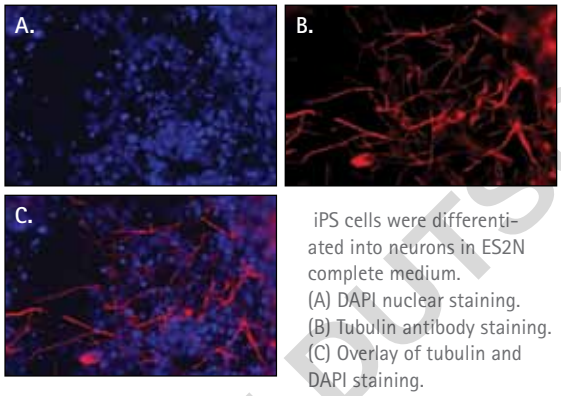
The new "Reprogramming Cell Fate" poster depicts reprogramming and differentiation pathways while highlighting key characteristics of each step along the way.

To request a copy of the new Reprogramming Cell Fate Poster, use your smart phone to scan the 2D barcode to the right of the poster, or visit www.millipore.com/cellfateposter.



ES2N Complete Medium
(Catalogue 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. This medium provides a means for testing the differentiation potential of iPS and ES cells without undergoing EB formation. With the ES2N Complete Medium, cells readily differentiate into neuron monolayers within 9-12 days on gelatin-coated culture dishes.



iPS cells were differentiated into neurons in ES2N complete medium.
(A) DAPI nuclear staining.
(B) Tubulin antibody staining.
(C) Overlay of tubulin and DAPI staining.

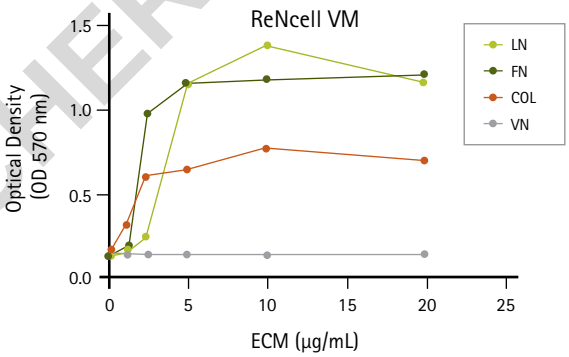
Wnt-5a, Recombinant Mouse
(Catalogue No. GF146)

Wnt signaling has been implicated in the control of differentiation of stem cells. The Wnts have also been shown to have putative roles in the regulation of adult stem cells. Wnt-5a, a member of the class of non-canonical Wnt family, signals independently of β-catenin and is secreted by stem cells into the microenvironment to affect differentiation.

In addition to Wnt-5a, Merck Millipore offers a comprehensive range of cytokines and growth factors for cell culture and stem cell differentiation. Every lot produced is thoroughly tested for bioactivity, purity, and endotoxin levels. Whether your project is big or small, we offer high quality recombinant proteins to meet your needs. Visit our website at www.millipore.com/cellbiology for a complete list of Human, Mouse, Rat, or Other Cytokines & Growth Factors.

ECM Cell Culture Optimization Arrays
(Catalogue No. ECM541, ECM542, ECM546)

Adding ECM proteins to *in vitro* cell cultures can promote cellular adhesion, viability, proliferation, and can affect cell differentiation. The ECM Cell Culture Optimization Array provides a multiparametric assay that quickly identifies ECM protein(s) that promote desired phenotypes and pinpoints optimal concentrations for a differentiation towards a particular lineage.



ReNcell VM adhesion profiling against ECMs. ReNcell VM human neural stem cells were seeded on the ECM Cell Culture Optimization Array at 10^5 cells per well for 2 hours at 37°C. Cell adhesion levels were measured by crystal violet staining and analyzed by spectrometer. Each data point represents three replicates.

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StemSelect® 384-Well Small Molecule Regulators Library

(Catalogue No. 569744, available from www.merck4biosciences.com)

The StemSelect Small Molecule Regulators 384-Well Library I consists of 303 pharmacologically active, structurally diverse small molecules, including extracellular domain-targeting reagents as well as cell-permeable compounds that effectively regulate intracellular targets. The reagents in this library 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. The library is supplied with a compact disk containing comprehensive documentation for each compound.

Format

- Eppendorf® 384-well, Polypropylene, nonpyrogenic, deep well plate
- Corning® 384-well robolid microplate seal
- Individual silicone seal for each well that exhibits DMSO resiliency and protects from moisture
- Minimizes cross-contamination

Small Molecule Regulators Characteristics

- Cell permeable*
- Potent and selective*
- Reversible*
- Structurally diverse
- Known pharmacological activity
- Stable in DMSO or H_2O as supplied
- Less toxic

Comprehensive Documentation

- SD Files
- CAS numbers (where available)
- Concentration
- Target
- Categorical index
- PubChem Substance ID (where available)
- Lot specific purity
- Molecular formula
- Molecular weight
- Structure
- Web links to Calbiochem and PubChem for individual small molecule regulators

*Pertains to a majority of the regulators



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Key Products

Antibodies

Available from www.millipore.com

Description	Catalogue No.
Anti-Achaete Scute homolog 2, clone 7E2	MAB4418
Anti-PDX-1, clone 6F6.1	MAB4425
Anti-LEF1, all isoforms, clone 1C3.1D10	MAB3750
Anti-LEF1, β-catenin binding domain, clone REMB1	MAB3751
Anti-BCRP, clone 5D3	MAB4155
Anti-c-Kit (CD117) (pTyr703)	07-803
Anti-BMP7, clone 2A10	MAB4350
Anti-Plet1 (Placenta-expressed transcript 1 protein), clone 1D4	MAB4416
Anti-SNAI2, clone 2B6	MAB4371
Anti-SRF [Serum Response Factor], clone 1E1	MAB4369
Anti-Ago2, clone 9E8.2	04-642
Anti-EVX1	MAB11030
Anti-ISL1	AB4326
Anti-Nestin, clone 10C2	MAB5326

Inhibitors

Available from www.merck4biosciences.com

Description	Catalogue No.
ROCK Inhibitor Y-27632	688000
TGF-B RI KInase Inhibitor II	616452
Valproic Acid	676380
JAK Inhibitor I	420099
Rapamycin	553210
Cyclopamine-KAAD	239804
LY 294002	440202
SB 203580	559389

Growth Factors

Available from www.millipore.com

Description	Catalogue No.
Human Basic FGF (FGF-2)	GF003, GF003AF-MG, 01-106
Human Epidermal Growth Factor (EGF)	GF144, 01-107
Human Hepatocyte Growth Factor (HGF)	GF116
Human IGF-I	GF138
Human IGF-II	01-142
Human PDGF-AA	01-309, GF142
Human PDGF-BB	01-305, GF149
Human Stem Cell Factor	GF021
Human TGF-β2	GF113
Human TGF-β1	GF111
Human VEGF	GF094, 01-185
Mouse EGF	01-101, GF155
Mouse Stem Cell Factor (SCF)	GF141
Mouse VEGF	GF140

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Characterization Kits

Available from www.millipore.com

Description	Catalogue No.
Human Embryonic Stem Cell Neurogenesis Characterization Kit	SCR065
Human Embryonic Germ Layer Characterization Kit	SCR030
Cardiomyocyte Characterization Kit	SCR059
Pancreatic Islet Cell Characterization Kit	SCR045
Human Mesenchymal Stem Cell Characterization Kit	SCR067
MSC Characterization Kit	SCR018
Oligodendrocyte Characterization Kit	SCR601
Human NSC Characterization Kit	SCR060
Human Neurogenesis CELISA Assay (Colorimetric)	SCR109
Human ESC Neurogenesis Characterization Kit	SCR065
NSC Characterization Kit	SCR019

Differentiation Kits

Available from www.millipore.com

Description	Catalogue No.
Human Oligodendrocyte Differentiation Kit	SCR600
MSC Adipogenesis Kit	SCR020
MSC Osteogenesis Kit	SCR028
Mouse ESC Neurogenesis kit	SCR101
Mouse ESC Adipogenesis Kit	SCR100

Media and Supplements

Available from www.millipore.com

Description	Catalogue No.
N21 Medium Supplement	SCM081
NDiff Neuro2 Medium Supplement	SCM012
NDiff Neuro27 Medium Supplement	SCM013
N-Base Neural Basal Medium	N014-B
EB Formation Medium	SCM018
MSC Expansion Medium	SCM015
FibroGRO Expansion Medium (Low serum formulation for fibroblasts and human mesenchymal stem cells)	SCMF002

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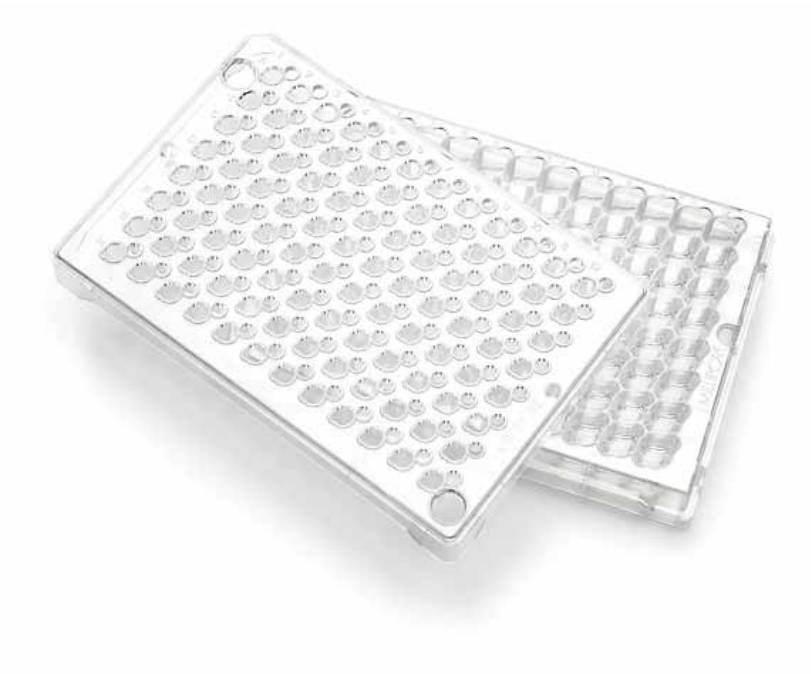
Measuring Differentiated Cell Function

The ultimate goal of stem cell research is to replace specific lost or damaged cells in order to restore or augment important tissue function. As with ES cell differentiation, characterizing downstream biological performance of iPS-redirected cells requires analysis beyond detection of early cell-type markers. For example, cardiac myocytes should be analyzed for proper, characteristic gap junction formation, oligodendrocytes are expected to generate myelin wrapping extensions, while neurons should be tested for presence of mature processes and functional synapses. In considering the final disposition of ES/iPS cell-derived mature cells, several important studies should be undertaken to demonstrate correct graft or xenograft localization, expression of mature state markers; characteristic performance in functional cellular assays, and, ideally, restored function in the modified tissue *in vivo*.

MultiScreen®-MIC 96-well Plates

(Catalogue No. MAMIC3S10, MAMIC5S10, MAMIC8S10)

Cell migration is a critical component of tissue development and remodeling by immature cells. MultiScreen-MIC filter plates are ideal for assays of differentiated cell phenotypes, including migration, invasion, chemotaxis, co-culture, angiogenesis, and transmigration. These 96-well plates incorporate polycarbonate membranes in a variety of pore sizes to support a range of cell types. Results show that the plates demonstrate high assay consistency with little inter-assay variability. The sterile plates are also shown to support longer incubation times and enable assay set-up and analysis in the same device.

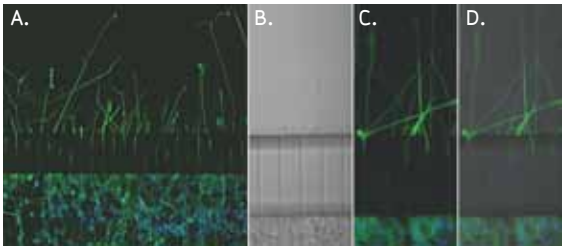


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AXIS® Axon Investigation System

(Catalogue No. AX15010, AX45010, AX50010)

The Axon Investigation System (AXIS) is Merck Millipore's 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 AX150 device. 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.

InhibitorSelect™ Wnt Signaling Pathway Panel

(Catalogue No. 681666, available from www.Merckbiosciences.com)

The Wnt signaling pathway is an evolutionarily-conserved pathway involved in fate specification, development, cell proliferation, cell 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. Merck Millipore's Wnt Signaling Pathway Panel consists of 15 highly potent, selective, and cell-permeable inhibitors useful for the investigation of the Wnt signaling pathway.

MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel

(Catalogue No. HMHMAG-34K)

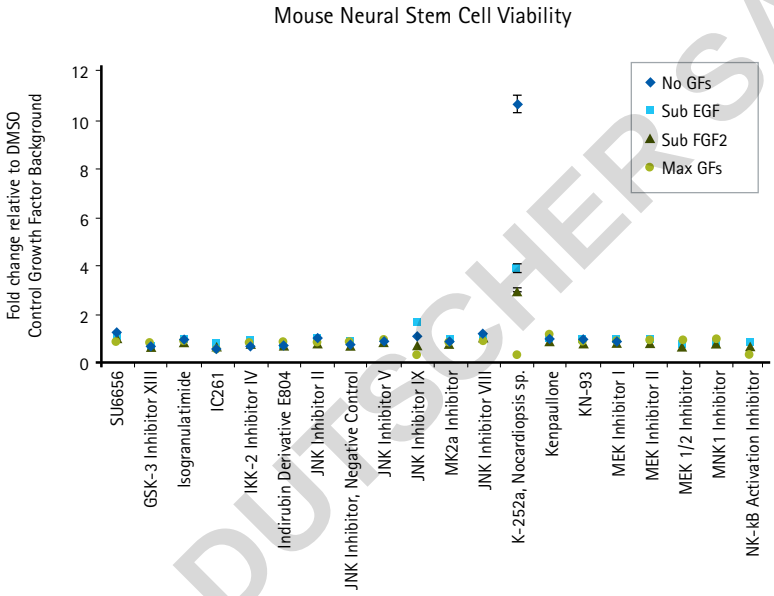
MILLIPLEX MAP Human Metabolic Panels enable you to measure simultaneously either total or active amylin, C-peptide, active ghrelin, total GIP, active GLP-1, glucagon, IL-6, insulin, leptin, MCP-1, pancreatic polypeptide, PYY and TNF. MILLIPLEX MAP enables you to investigate the modulation and expression of multiple analytes simultaneously, giving you the advantage of speed and sensitivity, and dramatically improving productivity. MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel is the most versatile system available for metabolic hormone research.

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Calbiochem InhibitorSelect 96-Well Protein Kinase Inhibitor Library I

(Merck Chemicals Catalogue No. 539744*)

This panel of compounds consists of 80, well-characterized protein kinase inhibitors, the majority of which are cell-permeable and ATP-competitive. The library is useful for cancer signaling pathway analysis, cell-based assays, target identification in drug discovery, screening new protein kinases, and other related applications. It is supplied with a CD containing comprehensive documentation for each inhibitor.



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 – Sub-optimal 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

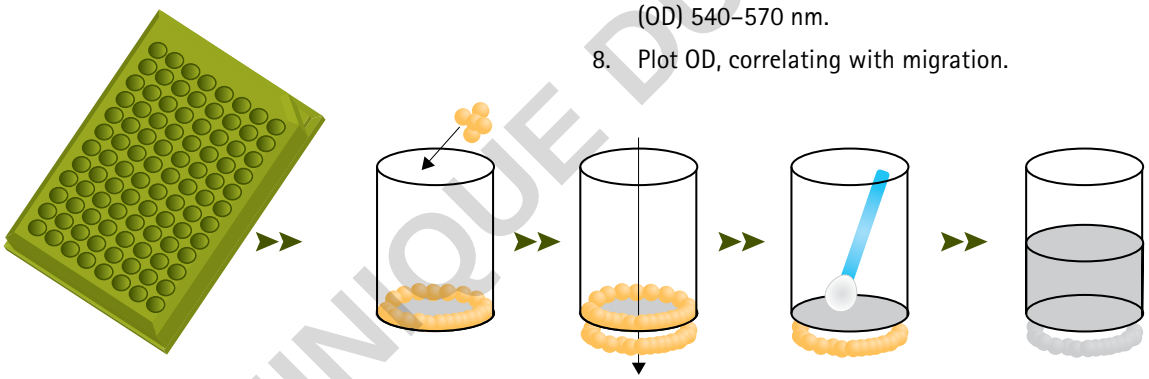
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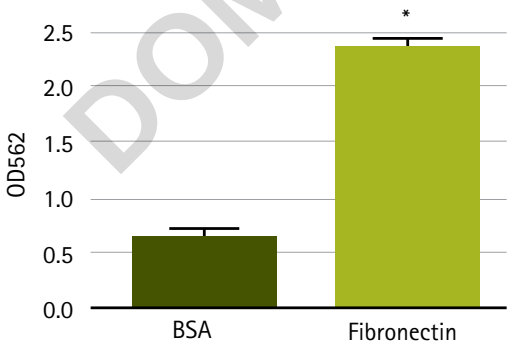
QCM Haptotaxis Cell Migration Assays

QCM Haptotaxis Cell Migration Assays measure cell movement toward an immobilized ECM protein gradient. The Boyden chambers have been outwardly pre-coated with an appropriate ECM or BSA (negative control) to allow optimal cell migration, and include built-in migration and adhesion controls for each sample. An 8 µm pore size supports the optimal haptotactic migration of epithelial and fibroblast cells. These assays enable quantitative analysis using a standard microplate reader. Precoated ECM options include fibronectin, vitronectin, and collagen.

Overview of Cell Migration Colorimetric Assay

1. Harvest subject cells, pellet, and resuspend to 0.75–2.5 x 10^6 cells per mL.
2. Place 125–500 µL of cells per well in ECM-coated test chambers, BSA control chambers, and ECM-coated control wells.
3. Incubate for 2–24 hours in a CO_2 incubator.
4. Stain ECM-coated control wells and visualize with a microscope to confirm attachment morphology. Remove non-migrating cells from coated chambers with a swab.
5. Stain migration chambers, and rinse away excess stain.
6. Solubilize stained migratory cells with extraction buffer.
7. Transfer 50–150 µL of extraction buffer from wells to microplate, and read optical density (OD) 540–570 nm.
8. Plot OD, correlating with migration.





Migration of HT-1080 cells towards a fibronectin matrix was assayed using the QCM Haptotaxis Cell Migration Assay. Cells were incubated and then stained according to the assay instructions. Cell migration was measured by plotting the optical density at a wavelength of 562 nm. Values represent mean of three separate experiments ± standard error of the mean. Asterisk indicates *P*<0.001 versus migration in BSA-coated control chambers.

Description	Catalogue No.
QCM Haptotaxis Cell Migration Assay, Fibronectin, 24-well, colorimetric	ECM580
QCM Haptotaxis Cell Migration Assay, Vitronectin, 24-well, colorimetric	ECM581
QCM Haptotaxis Cell Migration Assay, Collagen I, 24-well, colorimetric	ECM582

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Key Products

Antibodies

Available from www.millipore.com

Description	Catalogue No.
Milli-Mark Pan Neuronal Marker	MAB2300
Milli-Mark FluoroPan Neuronal Marker, Alexa Fluor 488 conjugated	MAB2300X
Milli-Mark ChromaPan Neuronal Marker	NS420
Milli-Mark ChromaPan Neuronal Marker-OMC	NS330
Milli-Mark ChromaPan Neuronal Marker-ORC	NS340
Actin Cytoskeleton and Focal Adhesion Staining Kit	FAK100
Anti-Vimentin, clone V9	MAB3400
Anti-Synaptophysin, clone SP15	MAB329
Anti-MBP	AB980

Engraftment Markers

Available from www.millipore.com

Description	Catalogue No.
Anti-BrdU, clone IIB5	MAB3222
Anti-Golgi Zon , clone 371-4	MAB1271
Anti-GFP	MAB3580
Anti-Ki-6 , clone Ki-S5	MAB4190
Anti-Mitochondria, clone 113-1	MAB1273
Anti-NCAM, extracellular, clone ERIC-1, Azide free	MAB2120Z
Anti-Nuclear Ribonucleoprotein, clone 58-15	MAB1287
Anti-Human Nuclei, clone 235-1	MAB1281

Compound Libraries Available from Merck Millipore

Available from www.merck4biosciences.com

Description	Catalogue No.
InhibitorSelect 96-Well Protein Kinase Inhibitor Library II	539745
InhibitorSelect 96-Well Protein Kinase Inhibitor Library III	539746
InhibitorSelect 384-Well Protein Kinase Inhibitor Library I	539743
StemSelect Small Molecule Regulators 384-Well Library I	569744

Assays

Available from www.millipore.com

Description	Catalogue No.
QCM Chemotaxis Cell Migration Assay, 24-well (8µ), Colorimetric	ECM508
QCM Chemotaxis Cell Migration Assay, 24-well (8µ), Fluorimetric	ECM509
QCM ECMatrix™ Cell Invasion Assay, 24-well (8µ), Colorimetric	ECM550
QCM ECMatrix Cell Invasion Assay, 24-well (8µ), Fluorimetric	ECM554
Calpain Activity Assay Kit, Fluorogenic	QIA120 (available from www.Merckbiosciences.com)
In Vitro Osteogenesis Assay	ECM810
Osteogenesis Quantitation Kit	ECM815
MAP Kinase/Erk Assay	17-191

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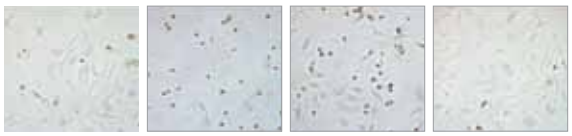
(Catalogue No. 71902, available from www.merck4biosciences.com)

NanoJuice Transfection Kit is specially designed to deliver the highest transfection efficiency for difficult cell types. The kit is comprised of a Core Reagent and a Booster Reagent, developed to work synergistically. By combining these two reagents at different ratios, transfection can be fine-tuned for each cell type, enabling maximum transfection efficiency while minimizing cytotoxicity.



Cell lines were plated in 24-well plates 18-24 h prior to transfection, such that cells were 80% confluent at time of transfection. Transfections were performed according to the manufacturers' optimized protocols. For transfection, 0.25 µg of low endotoxin-purified pTriEx-6 Rluc plasmid DNA was complexed with the reagents in the NanoJuice Transfection Kit or with competitor's reagents (LX or L2), and introduced into each well. After 24-48 h, the cells were extracted with Reportasol Extraction Buffer and Rluc activity was assayed. Data are represented as relative light units per well (RLU/well). All values reflect an average of four replicate cultures with standard errors.

NanoJuice Transfection Kit is less cytotoxic than competitor reagents. Three replicate Saos-2 cultures were either left untreated, transfected with commonly used competitor's reagents (L2 or LX0, or transfected with NanoJuice Transfection Kit according to recommended protocols. Photographs were taken 48 h post-transfection.



NanoJuice Transfection Kit | Reagent L2 | Reagent LX | No transfection

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Related Research Support Tools

Ensure that your discoveries have the highest impact and biological relevance by starting with quality cell and tissue samples. Merck Millipore's sterile filtration tools, cultureware, and automated cell counting system enable robust, uniform, contamination-free cell and tissue culture.

Sterile Filtration



Eliminating contaminants from your cell growth media and additives is absolutely crucial to preserving the integrity and accuracy of your cell cultures. Merck Millipore's comprehensive line of sterile filtration tools have been specifically designed to address these needs and to ensure the reproducibility of your cancer research.

Vacuum-Driven Sterile Filters

Stericup® filter devices combine a filter unit with a receiver flask and cap for processing and storage.

Description	Membrane/Application	Pore Size (µm)	Funnel Capacity (mL)	Receiver Bottle	Catalogue No.
Stericup-GP Filter Units	Millipore Express PLUS (PES) / fast filtration of tissue culture media and buffers	0.22	500	500 mL	SCGPU05RE

Cultureware



Millicell Membrane-Based Cell Culture

For more relevant cell-based assays, try growing your cells on Merck Millipore's membrane-based cell culture products. The optimized membranes result in cells with structure and function that more closely mimic what occurs *in vivo*. Obtain high quality results for screening, cell signaling assays, proliferation studies, and more.

Description	System Components	Membrane/Pore Size	Qty/Pk	Catalogue No.
Millicell-96 cell culture insert plates	96-well cell culture plate, single-well feeder tray and lid	Isopore (Polycarbonate)	5	PSHT004R5
	96-well cell culture plate, 96-well receiver tray and lid	Isopore (Polycarbonate)	5	PSHT004S5

Millicell HY (High-Yield) Cell Culture Flasks



Simplify your cell culture. Obtain robust cell growth for your next big experiment by using Millicell HY multilayer flasks to save time, incubator space, reagents, and money.

Description	No. of Layers	Total Surface Area (cm^2)	Qty/Pk	Catalogue No.
Millicell HY Flask STEM CELL TESTED	3	600	16	PFHYS0616
	5	1000	8	PFHYS1008

Millicell EZ SLIDES

Use the Millicell EZ SLIDE to culture, fix, stain and view your cells all in one device. There's no need to tediously move cover slips from your culture dish to a slide. Leave the wells intact to fix and stain and acquire data simply and quickly with Millicell EZ SLIDES.

Description	Qty/Pk	Catalogue No.
Millicell EZ SLIDE (4-well glass)	16	PFHYS0616
	8	PFHYS1008
Millicell EZ SLIDE (8-well glass)	16	PEZGS0816
	96	PEZGS0896
Millicell EZ SLIDE Microscope Slide Holder	1	PEZXMSH01



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