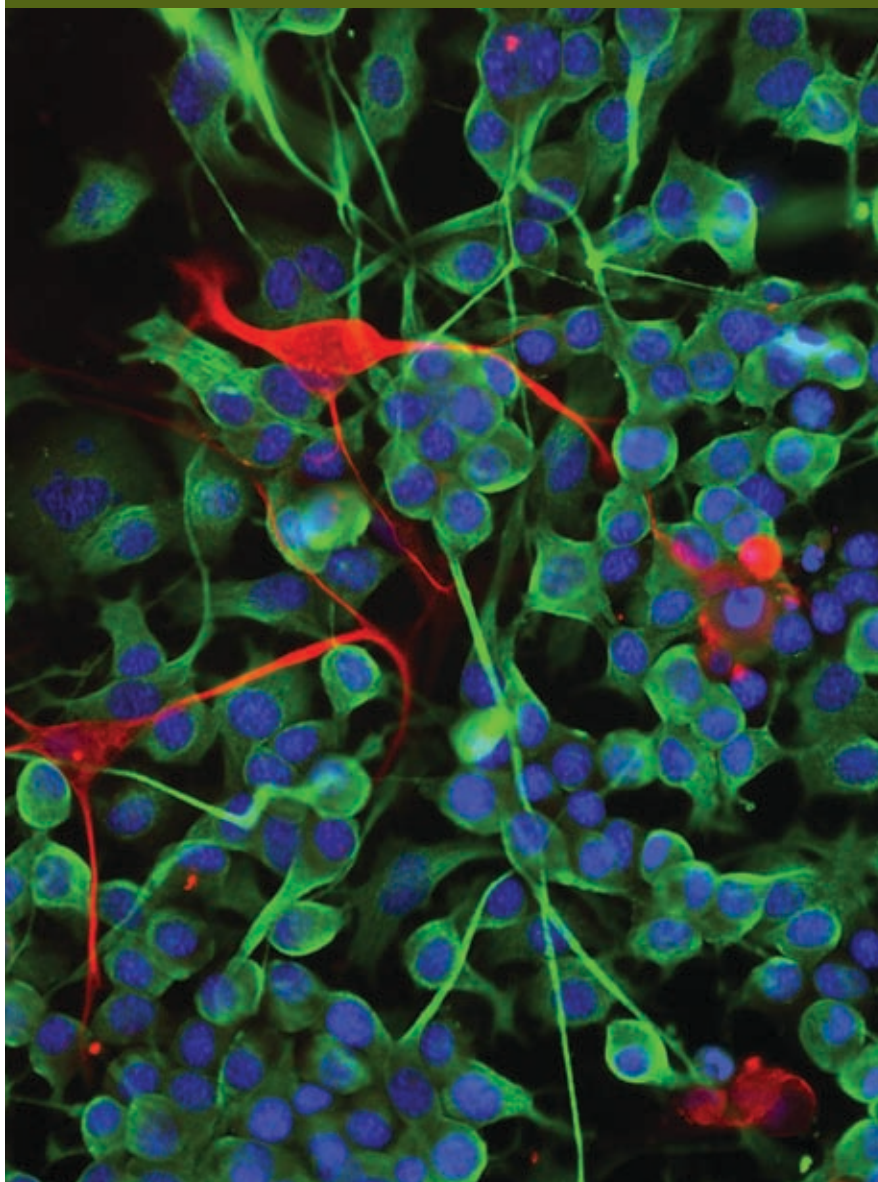




Cellutions | 2009 VOLUME 3

THE NEWSLETTER FOR CELL BIOLOGY RESEARCHERS



INSIDE:

- 3 Characterization of Human Induced Pluripotent Stem Cells Using the FlowCelect™ hESC Kit
- 6 Comparison of Three Methods of Purifying Lentiviral Vectors for use in Embryonic Stem Cell Transduction
- 8 A Human Neural Stem Cell Toxicity Assay that Combines Live Cell Monitoring with Quantitative Assessment of Neuronal Differentiation
- 11 Detection of Cancer Proliferation Marker Ki-67 by Flow Cytometry Provides Insight into Biological Correlation for PI3K/MAPK Activation and Cross Talk
- 14 Exciting New Products for Cell Biology Research

Cover photo: Neuron astrocyte co-culture with β III-tubulin (green) GFAP (red) and Hoechst nuclear dye (blue).



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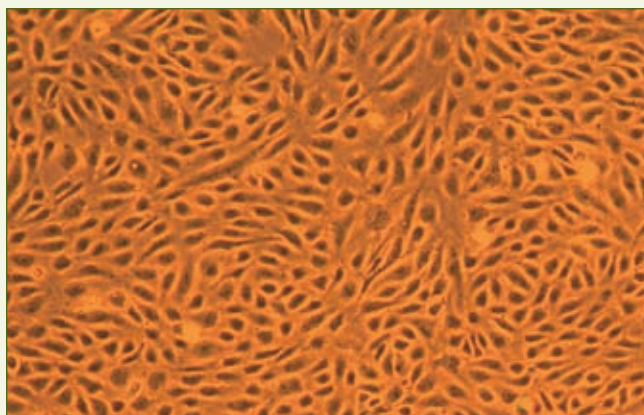
The new product line includes four uniquely optimized media formulations for large vessel and microvascular endothelial cells, as well as low passage human umbilical vein endothelial cells (HUVECs). Used together, these tools provide an ideal model for vascular biology research.

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- Lower serum concentrations than standard endothelial cell culture media
- Dramatically improves proliferation rates while maintaining excellent cell morphology
- No phenol red or antimicrobials, which can cause cell stress and masking effects
- Extensive QC and exacting standards to ensure lot-to-lot consistency
- Special UV packaging helps prevent light damage
- Built-in temperature gauges assist in contamination-free media warming

EndoGRO HUVECs

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- Ideal model system when used with EndoGRO media



EndoGRO HUVECs, P2, 6 days after innoculation at 100x.

Media Type	Description
EndoGRO-LS	Low serum culture media for human endothelial cells, HUVECs, aortic endothelial cells, and other large vessel endothelial cells
EndoGRO-VEGF	Low serum, VEGF-supplemented media for the rapid proliferation of human endothelial cells, HUVECs, aortic endothelial cells, and other large vessel endothelial cells
EndoGRO-MV	Low serum culture media for human microvascular endothelial cells
EndoGRO-MV-VEGF	Low serum, VEGF-supplemented media for the rapid proliferation of human microvascular endothelial cells

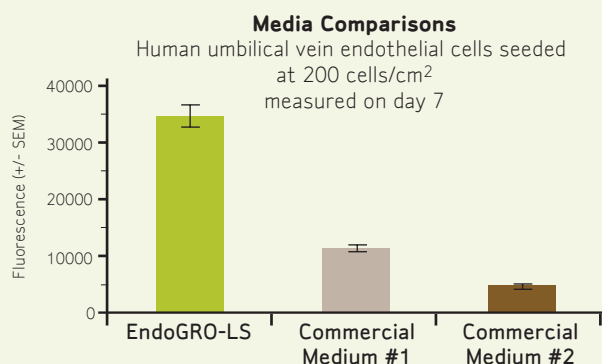
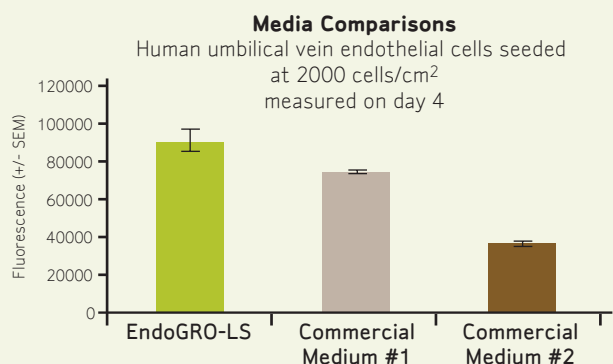


Figure 1. A comparison of proliferation rates of HUVEC cultured in EndoGRO media and other commercially available media at two different seeding densities. EndoGRO media shows superior proliferation at both seeding densities.

Description	Qty/Pk	Catalogue No.
EndoGRO-LS Complete Media Kit*	500 mL	SCME001
EndoGRO-VEGF Complete Media Kit	500 mL	SCME002
EndoGRO-MV-VEGF Complete Media Kit	500 mL	SCME003
EndoGRO-MV Complete Media Kit*	500 mL	SCME004
EndoGRO HUVECs	5 x 10 ⁵ cells	SCCE001

*Not currently available in Europe.

Characterization of Human Induced Pluripotent Stem Cells Using the FlowCollect hESC Kit

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Abstract

Cytometric analysis is commonly used to evaluate cells by quantifying and separating sub-populations of cells within heterogenous cell mixtures; it is widely used for the characterization of both human embryonic stem cells (hESCs) and induced pluripotent stem (iPS) cells. Flow cytometry allows researchers to evaluate percentages of cells expressing specific markers, to determine culture quality, and to track gene expression changes during a differentiation protocol. While there are many flow cytometry protocols available, it is often necessary to troubleshoot and optimize a protocol and validate the antibody. Here, we report the successful use of a flow cytometry kit to characterize pluripotent iPS cells, enabling rapid and efficient assessment of iPS cell lines.

Introduction

The derivation of induced pluripotent stem (iPS) cells from adult human somatic cells^{1,2} holds enormous potential applications for regenerative medicine, *in vitro* disease modeling, and drug screening. Further, examination of iPS cells will likely shed light on basic developmental questions regarding the transition from one cell type to another.

iPS cells are generated using virus and non-virus methods that insert transcription factor genes or manipulate their expression in early development^{1,2,3}. Novel iPS cell production techniques are beginning to address challenges associated with teratoma production, disease models, and therapeutic options, while reducing time and expense. Rapid, efficient, and reliable methods must be established to determine similarities and differences among various iPS cell lines, particularly those generated via different techniques and with different transcription factor gene cocktails, relative to hESCs.

hESCs and iPS cells are evaluated by their expression of markers associated with the undifferentiated and early embryogenesis states. Specifically, Oct-4, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81 are important markers for determining pluripotency of human stem cells^{4,5}. Additionally, SSEA-1 is a useful marker for monitoring the transition of undifferentiated hESCs to a differentiated state. Gleaning

information about a population of cells is key to screening and evaluating a specific cell line's degree of pluripotency and examining the conversion to a differentiated cell fate.

Here, we employ the new FlowCollect human ESC nuclear marker characterization kit to characterize and compare three iPS cell lines (derived by expression of Oct-4, KLF4, Nanog, and Lin28¹) and two parent fibroblast cell lines (cell lines used for reprogramming into iPS cells). This kit eliminates time-consuming antibody optimization by including validated fluorescent antibodies that have been optimized for cytometric analysis on the Guava® easyCyte™ Plus benchtop flow cytometer. The kit also includes important reagents including buffers, fixative, and cell strainers that have been validated for stem cell use.

Materials & Methods

Cells: The three iPS cell lines (iPS[IMR90]-3, iPS[IMR90]-4, and iPS[foreskin]-2) were a generous gift from J. Thomson (University of Wisconsin/UC Santa Barbara). iPS cells were maintained on mitomycin-C-inactivated mouse embryonic fibroblasts (MEFs) in DMEM/F12 culture medium supplemented with 20% KnockOut™ serum replacement, 2 mM GlutaMAX™-I, 0.1 mM β-mercaptoethanol, 0.1 mM non-essential amino acids (all from Invitrogen) and 100 ng/mL zebrafish bFGF (gift of J. Thomson). Cells were passaged by manual dissection every 5–7 days and media was replaced every 2 days. Parent fibroblast cell lines IMR90 (fetal lung fibroblast, ATCC) and CCD-1079sk (newborn foreskin fibroblast, ATCC) were included as controls and were cultured in Eagle's minimum essential medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate (Invitrogen). Cells were passaged with 0.05% trypsin and 0.5 mM EDTA (Invitrogen) every 3–4 days and were replated at a dilution of 1:3–1:6. All cells were grown in a humidified incubator at 37 °C in 5% CO₂.

Flow Cytometry: For flow cytometric analysis, Millipore's FlowCollect human ESC nuclear marker characterization kit was used. Adherent cells were washed

once with PBS, then dissociated with 0.05% trypsin and 0.5 mM EDTA. hESC media (without bFGF) supplemented with 10% FBS was added to inactivate the trypsin-EDTA. Cells were strained through a 40 μ m mesh filter and then counted on a haemocytometer. Viability was assessed via trypan blue exclusion. After a PBS wash, cell pellets were resuspended in wash buffer (included in kit). Fixation and antibody labeling were performed according to kit instructions. Antibodies were targeted against Oct-4 (Alexa Fluor® 488, green), SSEA-4 (PE, yellow), SSEA-1 (PE/Cy5, red), and isotype controls. Results were acquired on a Guava EasyCyte Plus flow cytometer using Guava Express® Pro software. Final graphs were prepared using FCS Express 3 (De Novo Software).

Results & Discussion

Our investigation reveals that the iPS cell line, iPS(foreskin)-2, maintains a distinct Oct-4 and SSEA-4 positive population that shows significant separation from the parent foreskin fibroblast cell line, CCD-1079sk. There is a clear shift in fluorescence intensity indicating a gain in Oct-4 and SSEA-4 expression within the iPS(foreskin)-2 cell line, indicating acquired pluripotency (Figure 1). Likewise, the population of iPS(foreskin)-2 cells expressing SSEA-1, a marker that coincides with hESC differentiation, is reduced in the iPS(foreskin)-2 cell line (Figure 1). It is of interest that the parent foreskin fibroblast cell line, CCD-1079sk, was SSEA-1-positive, as previous work shows that a different foreskin fibroblast cell line, HFF-1, was SSEA-1-negative⁶. Detecting three fluorophore channels per cell allows for the

identification of Oct-4/SSEA-4-positive and SSEA-1-negative cells. Indeed, the iPS(foreskin)-2 cell sample comprised a high proportion of Oct-4- and SSEA-4-positive cells (75.4%) with few expressing SSEA-1 (Figure 2). These data correspond with published studies of hESCs that show a high proportion of Oct-4/SSEA-4-positive and SSEA-1-negative cells⁵.

We also used flow cytometry to compare different clones of the same iPS cell line. iPS(IMR90)-3 displayed an Oct-4, SSEA-4, and SSEA-1 cell population signature similar to the iPS(foreskin)-2 cell line (Figure 3). iPS(IMR90)-4 exhibited a related trend, however, fewer Oct-4- and SSEA-4-positive cells, along with a decrease in fluorescence intensity, suggests that these cells possess reduced pluripotency and may be in the process of differentiation. Specifically, only 40% of iPS(IMR90)-4 cells showed signals for both Oct-4 and SSEA-4, whereas iPS(IMR90)-3 demonstrated an 84% coincidence (Figure 4). Both iPS(IMR90)-3 and iPS(IMR90)-4 were negative for SSEA-1.

Conclusion

The ability to quickly and effectively assess cell populations is a powerful tool for the field of stem cell biology. Flow cytometry is widely used for the characterization of hESCs and iPS cells, and the FlowCollect™ human ESC nuclear marker characterization kit provides a rapid, reliable and sensitive screen that can be utilized for evaluation of iPS cell lines, establishing a feasible pluripotency screen for both routine and investigational purposes.

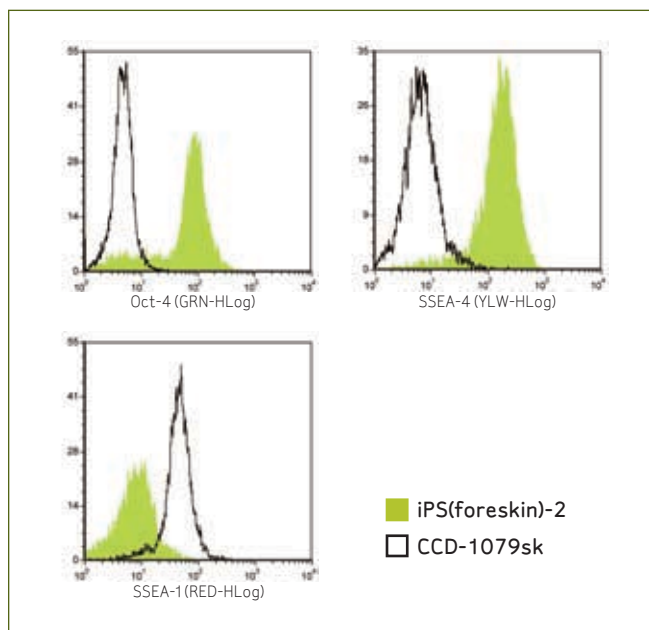


Figure 1. Flow cytometric analysis of iPS(foreskin)-2 and the parent foreskin fibroblast line CCD-1079sk for Oct-4, SSEA-4, and SSEA-1. Arrows indicate change in expression of Oct-4, SSEA-4, and SSEA-1 as CCD-1079sk cells were reprogrammed to iPS(foreskin)-2 cells.

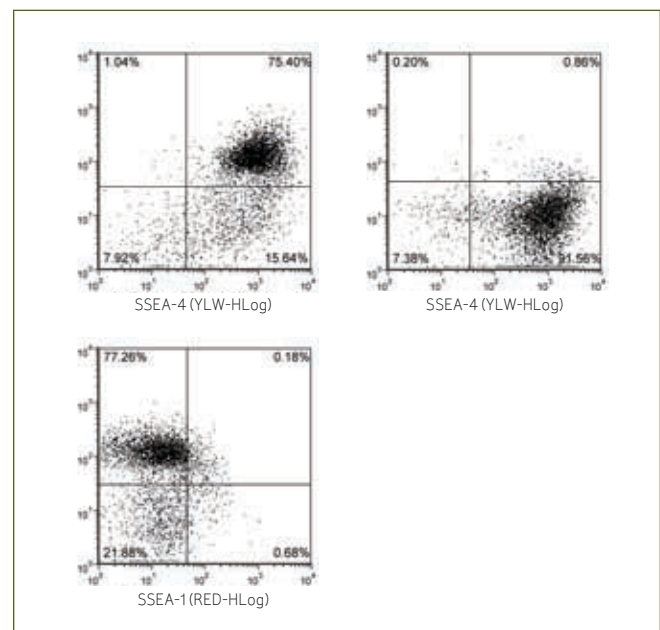


Figure 2. Flow cytometric analysis of iPS(foreskin)-2 for Oct-4, SSEA-4, and SSEA-1.

References

1. Yu J, Vodyanik MA, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007 Dec 21;318(5858):1917-20.
2. Takahashi K, Tanabe K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007 Nov 30;131(5):861-72.
3. Yu J, Hu K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009 May 8;324(5928):797-801.
4. International Stem Cell Initiative, Adewumi O, Aflatoonian B, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol*. 2007 Jul;25(7):803-16.
5. Henderson JK, Draper JS, et al. Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. *Stem Cells*. 2002;20(4):329-37.
6. Tateishi K, He J, et al. Generation of Insulin-secreting Islet-like Clusters from Human Skin Fibroblasts. *J Biol Chem*. 2008 Nov 14;283(46):31601-7

Related Products

Description	Catalogue No.
FlowCelect Mouse ESC Nuclear Marker Characterization Kit	FCMEC25110
FlowCelect Human ESC Nuclear Marker Characterization Kit (Oct-4, SSEA-1, and SSEA-4)	FCHEC25102
FlowCelect Human ESC Surface Marker Characterization Kit (HESCA-1, SSEA-1, and SSEA-4)	FCHEC25104
FlowCelect Human ESC Surface Marker Characterization Kit (TRA-1-60, SSEA-1, and SSEA-4)	FCHEC25106
FlowCelect Rodent NSC Characterization Kit (Neural)	FCRNC25112
FlowCelect Rodent NSC Characterization Kit (Astrocyte)	FCRNC25114

Figure 3. Flow cytometric analysis of iPS(IMR90)-3, iPS(IMR90)-4, and the parent lung fibroblast line IMR90 for Oct-4, SSEA-4, and SSEA-1 expression. Arrows indicate the change in expression of Oct-4, SSEA-4, and SSEA-1 as IMR90 cells were reprogrammed into iPS(IMR90) cells.

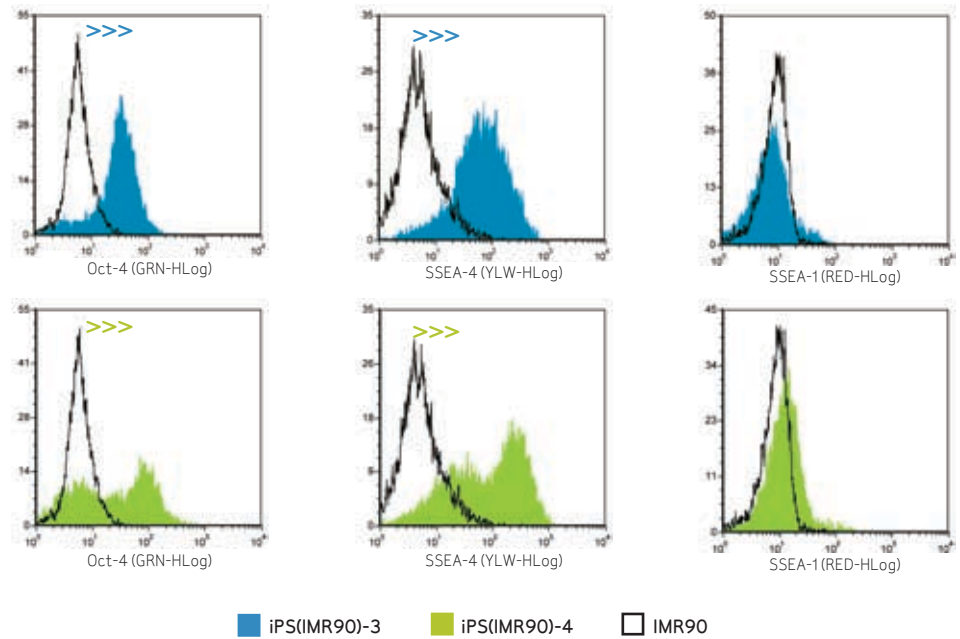
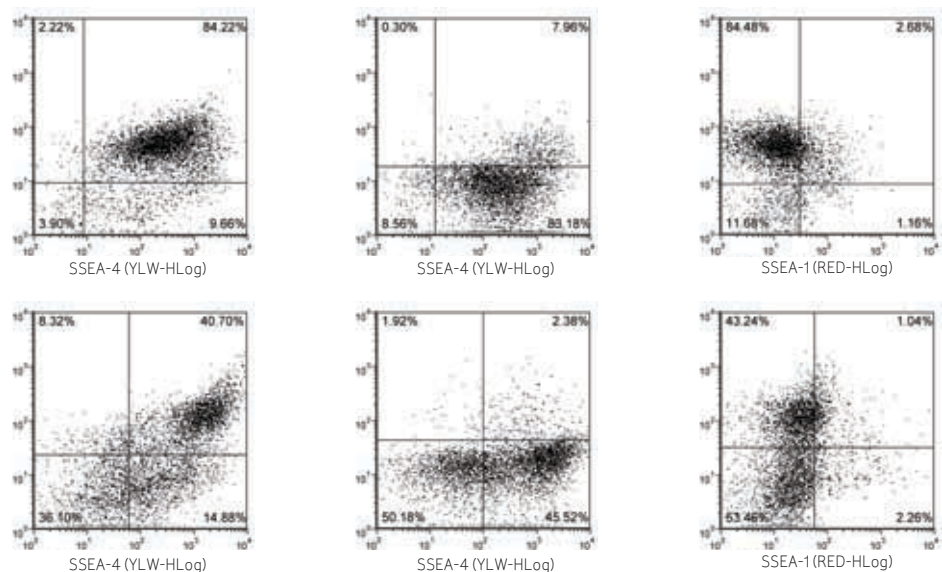


Figure 4. Flow cytometric analysis of iPS(IMR90)-3 and iPS(IMR90)-4 cell lines for Oct-4, SSEA-4, and SSEA-1.



Comparison of Three Methods of Purifying Lentiviral Vectors for use in Embryonic Stem Cell Transduction

Patrick W. Heiser, Ph.D., Harvard University

Abstract

Lentiviral vectors offer many advantages as vehicles for gene delivery and are useful for modifying embryonic stem cells. However, conventional virus purification methods such as ultracentrifugation and column concentration often deliver low viral concentrations. Here we present data comparing three viral purification methods and their infection efficiencies in murine embryonic stem cells. In our study, the Fast-Trap[®] lentivirus purification and concentration kit gave higher recoveries than the other two methods and allowed for robust, reproducible infection at up to 80% efficiency.

Introduction

Lentiviral vectors have been used successfully to mediate gene transfer in embryonic stem (ES) cells^{1,2}. They offer a fast and efficient alternative to more conventional electroporation techniques used to modify ES cells. Moreover, the development of replication-incompetent and self-inactivating lentiviral vectors allows for their safe use in a laboratory setting.

The efficiency of lentiviral infection of mouse ES cells is known to be low when compared to other cell types (20-80%)². This limitation can be partially circumvented through the use of antibiotic resistance to select for cells that have successfully incorporated and expressed the viral DNA.

In the present study we asked whether purification regimes which produce very concentrated lentivirus could help boost the infection efficiency. Such methodology would prove useful when greater throughput is required, such as during screening of shRNA or cDNA libraries. We directly compared three different methods for the production of concentrated lentivirus: ultracentrifugation, column concentration, and the Millipore Fast-Trap purification kit.

Materials & Methods

Lentivirus preparation (2nd generation construct):

Five 10 cm dishes of HEK293T cells (passage <10) were transfected using the Lipofectamine[™] 2000 reagent with 3 µg each of the following plasmids: pTRIPZ (empty vector), pMD2.G (VSV-G containing plasmid), and psPAX2 (packaging plasmid containing gag, pol, and rev genes). 48 hours after transfection, the tissue culture supernatant from each of the

plates was collected and centrifuged at 800 rpm for 10 minutes at 4 °C to pellet debris. Next, the supernatant was decanted into a 0.45 µm Steriflip[®] column and filtered for further clarification. Lentivirus was then purified and concentrated by ultracentrifugation, column concentration, or purification with the Fast-Trap kit.

For ultracentrifugation, 50 mL of viral tissue culture supernatant was spun at 3×10^4 rpm for 120 minutes at 4 °C to pellet the viral particles. The supernatant was carefully decanted to avoid disturbing the viral pellet. Tubes were then inverted in order to allow the remainder of the media to drain from the tube and allowed to air dry for 30 minutes. Next, 100 µL of PBS was added to the pellet, and placed at 4 °C for 12 hours. The pellet was then gently solubilized via slight agitation; the resultant viral solution was snap frozen in liquid nitrogen and stored at -80 °C.

For column concentration, 50 mL of viral tissue culture supernatant was concentrated via the use of an Amicon[®] Ultra-15 filter unit with a molecular weight cut-off of 100,000 kDa. The supernatant was divided into 5 columns and spun at 3×10^3 rpm for 45 minutes at 4 °C. The concentrated viral solution was then placed into a new Amicon Ultra-15 column and spun for an additional 10 minutes. The viral solution was snap frozen in liquid nitrogen and stored at -80 °C.

For purification with the Fast-Trap kit, 50 mL of viral tissue culture supernatant was concentrated according to the kit instructions. Briefly, viral supernatant was clarified by vacuum filtration through a Steriflip-HV 0.45 µm filter. The virus solution was then filtered through a lentivirus-specific membrane to which the viral particles adsorbed. The membrane was washed to remove contaminants, and purified virus was eluted via a quick spin. The buffer exchange/concentration column included in the kit was used to resuspend the purified virus in PBS.

All prepared virus stocks were titered using a QuickTiter[™] ELISA kit.

Mouse ES-cell culture and lentiviral infection

All cells used in these experiments were cultured in the following media: EmbryoMax[®] DMEM, 1X non-essential amino acids, and 1X nucleosides.

96-well plates were coated with 100 μL /well gelatin solution for 30 minutes at room temperature. The gelatin was aspirated and 3×10^4 mouse embryonic fibroblasts were plated in each well. 24 hours later, 10^4 wild type mouse embryonic stem cells (C57/B6 genetic background) were plated in each well. After 4 hours, the indicated amount of viral particles was added to each well. Infections were allowed to proceed for 24 hours before the viral media was removed and replaced with fresh ES-cell media.

Quantification of lentiviral infection efficiency

The pTRIPZ encoding lentivirus contains a tetracycline-inducible promoter which drives the expression of turbo RFP (tRFP). 48 hours after exposure of the mouse embryonic stem cells to 1 $\mu\text{g}/\text{mL}$ doxycycline, cells which have successfully been infected with virus and incorporated the transgene can be readily visualized via fluorescence microscopy. Four fields were chosen at random for each of the viral concentrations and imaged. Viral infection efficiencies were determined by dividing the number of tRFP+ ES cell colonies by the total number of colonies in the field.

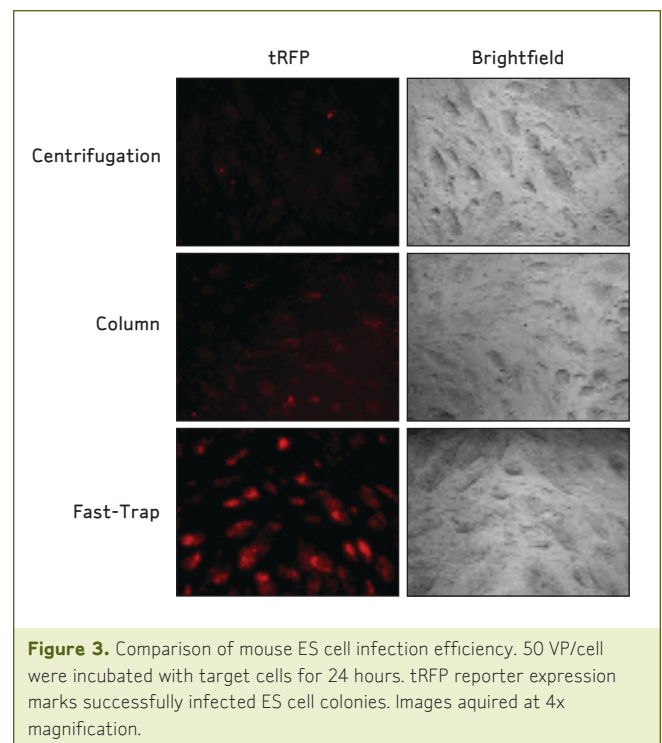
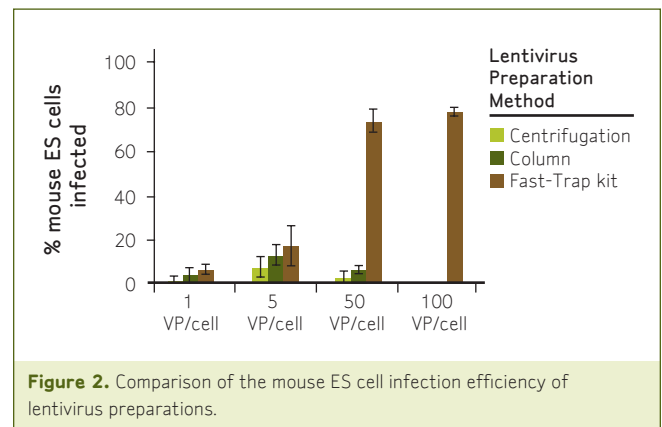
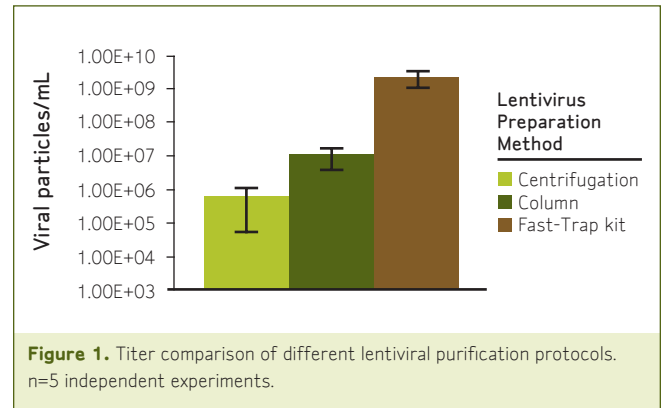
Results

All three of the lentiviral purification methodologies evaluated in this study provided reproducible virus concentrations. However, the Fast-Trap kit yielded 10^9 viral particles (VP)/mL on average, a concentration that was at least two orders of magnitude greater than virus prepared via the other two methods (Figure 1). As a result, the use of the Fast-Trap kit allows significantly more virus to be added to target cells without undesirable dilution of the growth medium.

Next, viral activity was assayed via infection of mouse embryonic stem cells. Successfully infected ES cells can be easily visualized via expression of tRFP driven by the lentiviral construct. Based upon our analysis, infection efficiency was low but equivalent between the three purification protocols at low concentrations (1-5 VP/cell, Figure 2). However, at 50 VP/cell, the infection efficiency of the Fast-Trap lentivirus was 75% or greater. In contrast, the virus prepared by centrifugation or column concentration actually infected fewer cells at this concentration (Figure 3). Further increasing the concentration of the Fast-Trap-prepared virus to 100 VP/cell did not result in any further increase in the number of ES cells infected. This suggests that 50 VP/cell may represent a maximum infectious threshold for this viral pseudotype and ES cell strain. Centrifugation or column viral preparations could not be evaluated at 100 VP/cell because they are too dilute.

Discussion & Conclusion

The Fast-Trap lentiviral purification and concentration kit offers many advantages over conventional preparation techniques. Highly concentrated and active virus can be prepared in less than two hours with reproducible titers.



Mouse ES cells can be infected with efficiencies reaching 80%. Moreover, the kit's design results in a very clean preparation, unlike the other two purification schemes evaluated in this study which also concentrated undesirable proteins and metabolites from the HEK293 growth media. Treatment of mouse ES-cells with concentrated, virus-free HEK293 conditioned media results in a decrease in cell viability and increased differentiation; this effect is not seen with Fast-Trap processed virus-free HEK293 media (data not shown). This accounts for the decrease in infection efficiency when

greater volumes of virus solution from ultracentrifugation and column preparations were used (Figures 2,3). In conclusion, Fast-Trap purified lentivirus provides an excellent tool for the genetic modification of mouse ES cells.

References

1. Suter DM, Cartier L, Bettiol E, *et al.* Rapid generation of stable transgenic embryonic stem cell lines using modular lentivectors. *Stem Cells*. 2006 Mar;**24**(3):615-23.
2. Ma Y, Ramezani A, Lewis R, Hawley RG, Thomson JA. High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. *Stem Cells*. 2003;**21**(1):111-7.

Related Products

Description	Catalogue No.
Fast-Trap Lentivirus Purification and Concentration Kit	FTLV00003
Fast-Trap Adenovirus Purification and Concentration Kit	FTAV00003
Fast-Trap Adeno-Associated Virus (AAV) Purification and Concentration Kit	FTAA00003
Amicon Ultra-15 Centrifugal Filter Unit, with Ultracel™-100 membrane	UFC910008
Steriflip-HV Filter Unit, 0.45 µm, PVDF, radio-sterilized	SE1M003M00
EmbryoMax ES Cell Qualified DMEM (1X), liquid, with 4500 mg/L glucose, 2.25 g/L sodium bicarbonate and L-glutamine, without sodium pyruvate	SLM-120-B
EmbryoMax ES Cell Qualified MEM (100X), non-essential amino acids	TMS-001-C
EmbryoMax ES Cell Qualified Nucleosides (100X)	ES-008-D
EmbryoMax Primary Mouse Embryo Fibroblasts, mitomycin-C-treated, neo-resistant	PMEF-N

A Human Neural Stem Cell Toxicity Assay that Combines Live Cell Monitoring with Quantitative Assessment of Neuronal Differentiation

Christine Chen and Vi Chu, Millipore Corporation

Abstract

Neural stem cells (NSCs) are generating considerable attention as relevant *in vitro* model systems to assess the potential neurotoxicity of compounds. To further this research, a human neural stem cell line was generated with a GFP reporter under the control of the nestin promoter. Here we show that this cell line can be used to assess neuronal differentiation via live cell tracking and quantitative methods.

Introduction

Neurotoxins may adversely affect cell proliferation, cell

viability, and differentiation. Effects on cell proliferation and survival are easily and rapidly quantifiable by existing assays; however, the effects of potential neurotoxins on differentiation are much more complicated and difficult to examine.

A previous group has used ReNcell® CX cells, a human neural progenitor cell line developed from the cortical region, to screen for chemical effects on cell proliferation and viability in an automated, high-throughput assay with known anti-proliferative compounds¹. To facilitate even more rapid screening of potential neurotoxins, we have created a new reporter cell line in which fluorescence intensity is linked to expression of a neural stem cell marker.

The new MilliTrace™ CX nestin GFP reporter NSC line was developed by transfecting ReNcell CX cells with a proprietary bicistronic plasmid containing humanized mulleri green fluorescent protein (hmGFP) under the control of the mouse nestin promoter. Nestin expression is commonly associated with undifferentiated neural stem and early progenitor cells that are actively proliferating. With the onset of differentiation, nestin is down-regulated. In the transfected cell line, this causes a corresponding decrease in the expression of hmGFP which is easily detected as a drop in the fluorescence intensity of the live cells. Here we show that the MilliTrace CX nestin GFP reporter human NSC line can be used to assess neuronal differentiation, both through live cell monitoring and quantitative ELISA evaluation.

Methods

For immunofluorescent staining, MilliTrace CX nestin GFP reporter cells were cultured under proliferating conditions containing FGF-2 and EGF or differentiated for two weeks via growth factor withdrawal. Cells were then stained with stem cell markers (anti-nestin, 1:500 dilution; and anti-SOX-2, 1:1000 dilution), differentiation markers (anti- β III-tubulin, 1:1000 dilution; and anti-gial fibrillary acidic protein [GFAP], 1:250 dilution), and a nuclear stain (DAPI). GFP expression was also noted.

For Western blot analysis, MilliTrace CX nestin GFP reporter cells were differentiated by growth factor withdrawal and harvested at approximately 3-day intervals. 10 μ g lysates from each time point were loaded on a 4-12% gradient gel and prepared for Western blot detection. Antibodies against nestin (stem cell marker) and GAPDH (loading control) were used to analyze the blot.

For quantitative ELISA, approximately $1-2 \times 10^4$ cells were plated in 100 μ L of ReNcell NSC maintenance medium containing 20 ng/mL FGF-2 and 20 ng/mL EGF per well in a 96-well plate and cultured for 24-48 h prior to differentiation. Differentiation was initiated by growth factor withdrawal. Cells were fixed with 4% paraformaldehyde after 0, 1, and 2 weeks of differentiation. GFP intensity was acquired using a fluorometric reader (Molecular probe) with a FITC setting (Ex/Em 435/485nm) and normalized to the total cell numbers as quantified on plate reader using a colorimetric cell staining kit. GFP intensity at each time point was presented as the percentage of undifferentiated cells. In the ELISA, antibodies against GAPDH, MAP2, and β III-tubulin were used to monitor the level of neuron differentiation; again, signal intensity was normalized with cell numbers.

Results

Relative levels of GFP expression were reduced 10-fold (Figure 1, A vs. F) as estimated by the fluorescence intensity in the FITC channel using the same exposure time. Under proliferating conditions containing FGF-2 and EGF, GFP-positive cells expressed high levels of the NSC markers, nestin (B) and SOX-2 (C) with minimal to low expression of lineage-specific markers, β III-tubulin and GFAP (data not shown). Nestin and SOX-2 antibodies mark cells that are in the undifferentiated neural stem cell stage. After two weeks of differentiation via growth factor withdrawal, GFP expression was down regulated (F) and lineage-specific markers, β III-tubulin (G) and GFAP (H) was up-regulated indicating the presence of neurons and astrocytes, respectively. Approximately 30% of cells were strongly β III-tubulin-positive after 14 days of differentiation.

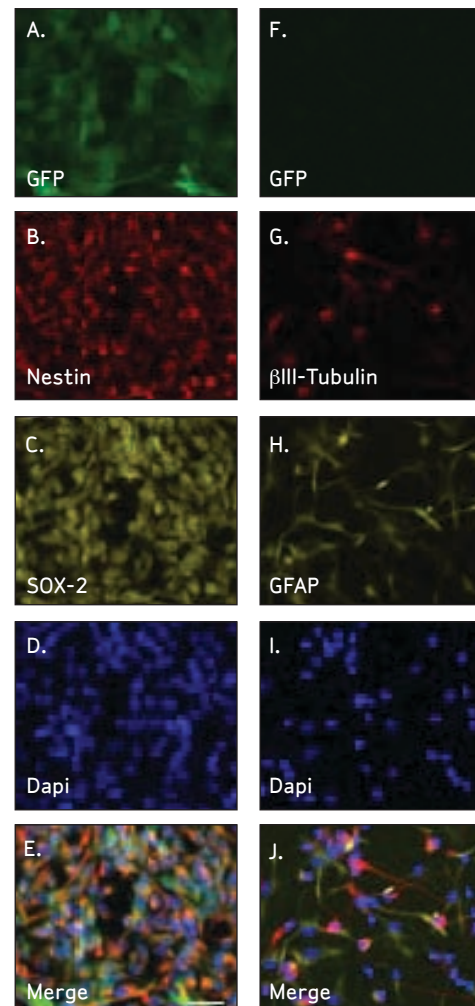


Figure 1. Immunofluorescent staining of undifferentiated and differentiated MilliTrace CX nestin GFP reporter cells. Indirect immunofluorescent staining shows stem cell markers in undifferentiated (A-E) cells and lineage markers in differentiated (F-J) cells. Scale bar = 50 μ m.

Qualitative Western blot analysis (Figure 2A) showed that nestin was highly expressed in undifferentiated cells and gradually decreased as differentiation progressed. By normalizing the nestin intensity for each time point to the corresponding GAPDH band, quantitative analysis showed that nestin expression levels had declined by 50% at day 5 of differentiation (B).

A quantitative ELISA demonstrated the expected up-regulation of neural lineage markers as cells differentiated (Figure 3B). Likewise, the decline of GFP expression in the reporter cell line (Figure 3A) corresponded to the rise of MAP2 and β III-tubulin. The increase of these lineage markers was similar in both the parental ReNcell CX cells (data not shown) and the reporter line.

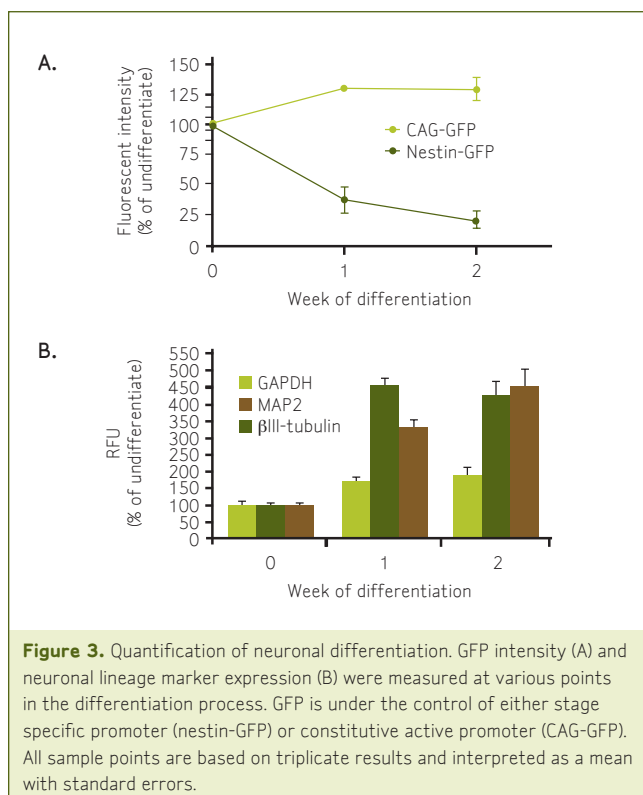
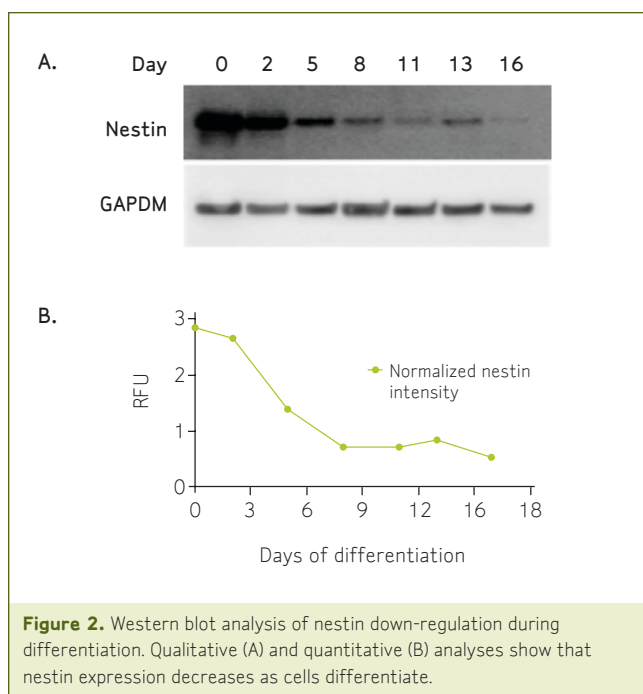
Discussion and Conclusion

A quantitative ELISA assay that measures the capacity and extent of differentiation would shed further light into the specificity of the neurotoxic effect, allowing scientists to explore questions like whether neurotoxins affect neuronal rather than glial differentiation.

The MilliTrace CX nestin GFP reporter cell line is ideal for use in neurotoxicity screens. The robust cell line offers rapid, simple, and reproducible analysis via both live cell fluorescent tracking and quantitative ELISA assays. As demonstrated, the cells are multipotent and their GFP levels are closely tied to the expression of nestin, enabling sensitive differentiation tracking in live cells. Also, human-derived reporter cells like the MilliTrace line naturally offer better *in vitro* models of the developing and adult central nervous system than animal models do. The cells are suited for high-throughput and high-content neurotoxicity screens due to their sensitivity, specificity, and adaptability.

References

- Breier J, Radio NM, Mundy WR, Shafer TJ. Development of a high-throughput screening assay for chemical effects on proliferation and viability of immortalized human neural progenitor cells. *Toxicol. Sci.* 2008;105(1):119-133.



Related Products

Description	Catalogue No.
MilliTrace CX Nestin GFP Reporter Human Neural Stem Cells and Media Kit	SCRO96
Anti-Nestin, clone 10C2	MAB5326
Anti-SOX-2	AB5603
Anti- β III-Tubulin, C-terminus, clone TU-20 (similar to TuJ1)	MAB1637
Anti-Glial Fibrillary Acidic Protein (GFAP)	AB5804
Anti-GAPDH	MAB374

Detection of Cancer Proliferation Marker Ki-67 by Flow Cytometry Provides Insight into Biological Correlation for PI3K/MAPK Activation and Cross-Talk

Mark Santos, Wenying Zhang, Kevin Su, Melanie Chan, Roberto Renteria, Jason Whalley, Kerry Paradis, Patrick Schneider, and Matthew Hsu, Millipore Corporation.

Abstract

Much excitement in the field of signal transduction has centered on the discovery of increasing cross-talk among multiple signaling pathways ¹. Complicated cellular events, including many downstream effectors which can result in cancer cell proliferation, can be elucidated through simultaneous, multiparameter analysis of signaling cross talk and proliferation markers.

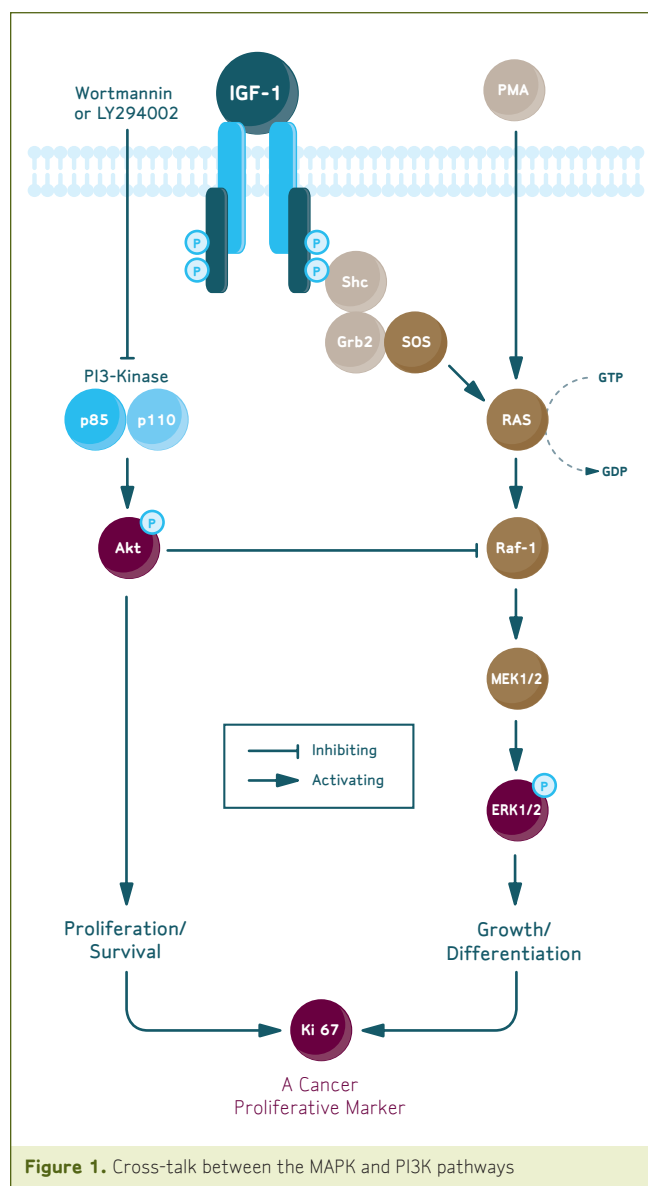
Recent evidence suggests that there is cross talk between the PI3K and MAPK signaling pathways. To assist scientists in evaluating the biological significance of this cross-talk, Millipore developed a new flow-cytometry-based kit. Here we demonstrate that the kit, with a novel cell cycle arrest agent and antibodies against phospho-ERK1/2, phospho-Akt, and Ki-67, enables scientists to evaluate cross talk between the PI3K and MAPK pathways and develop a more complete picture of its biological effects.

Introduction

Recent evidence suggests that cross-talk exists between the PI3K and MAPK signaling pathways downstream of the cell surface ^{2,3}. Antibodies against phosphorylated Akt (pAkt) and phosphorylated ERK (pERK) can be used to examine key interactions between the PI3K/MAPK signaling pathways (Figure 1).

It has been suggested that the phosphorylation of Akt can result in the inhibition, or dephosphorylation, of phospho-Raf on Ser 259. Inactivating Raf will essentially block the MAPK signaling pathway, preventing ERK phosphorylation. In HEK293 cells, insulin-like growth factor 1 (IGF-1) stimulation will activate both the PI3K and MAPK pathways, leading to the phosphorylation of both Akt and ERK. But, since there is cross talk between the two pathways, it is critical to investigate their interactions in terms of both pathway activation and timing.

In addition, the use of phospho-specific antibody labeling as a biomarker may give some measure of target activation, but it may not necessarily correlate with the



desired biological effect (e.g. growth inhibition or apoptosis). Researchers will benefit from the inclusion of both phospho-specific signal transduction markers and a proliferation marker to allow a true measure of the biological effect ⁴.

In this study, we used the new FlowCollect PI3K/MAPK dual pathway activation and cancer marker detection kit to examine the effects of IGF-1 stimulation on both the PI3K and MAPK signaling pathways in HEK293 cells. Optimized for the Guava® line of flow cytometers, this new kit uses a trio of directly conjugated antibodies for simultaneous detection. Cross-talk is analyzed using phospho-specific antibodies and Ki-67 is used to evaluate the influence of this cross-talk on cell proliferation.

Ki-67 is present in all phases of the cell cycle except for G₀ and is a reliable tumor proliferative marker⁵. However, Ki-67 is difficult to detect via flow cytometry because its staining patterns are punctate in most phases, thus producing weaker signals. We have developed a novel detection method that uses the Cell Cycle Stop™ reagent to arrest the cell cycle at M phase for stronger Ki-67 signals.

Methods

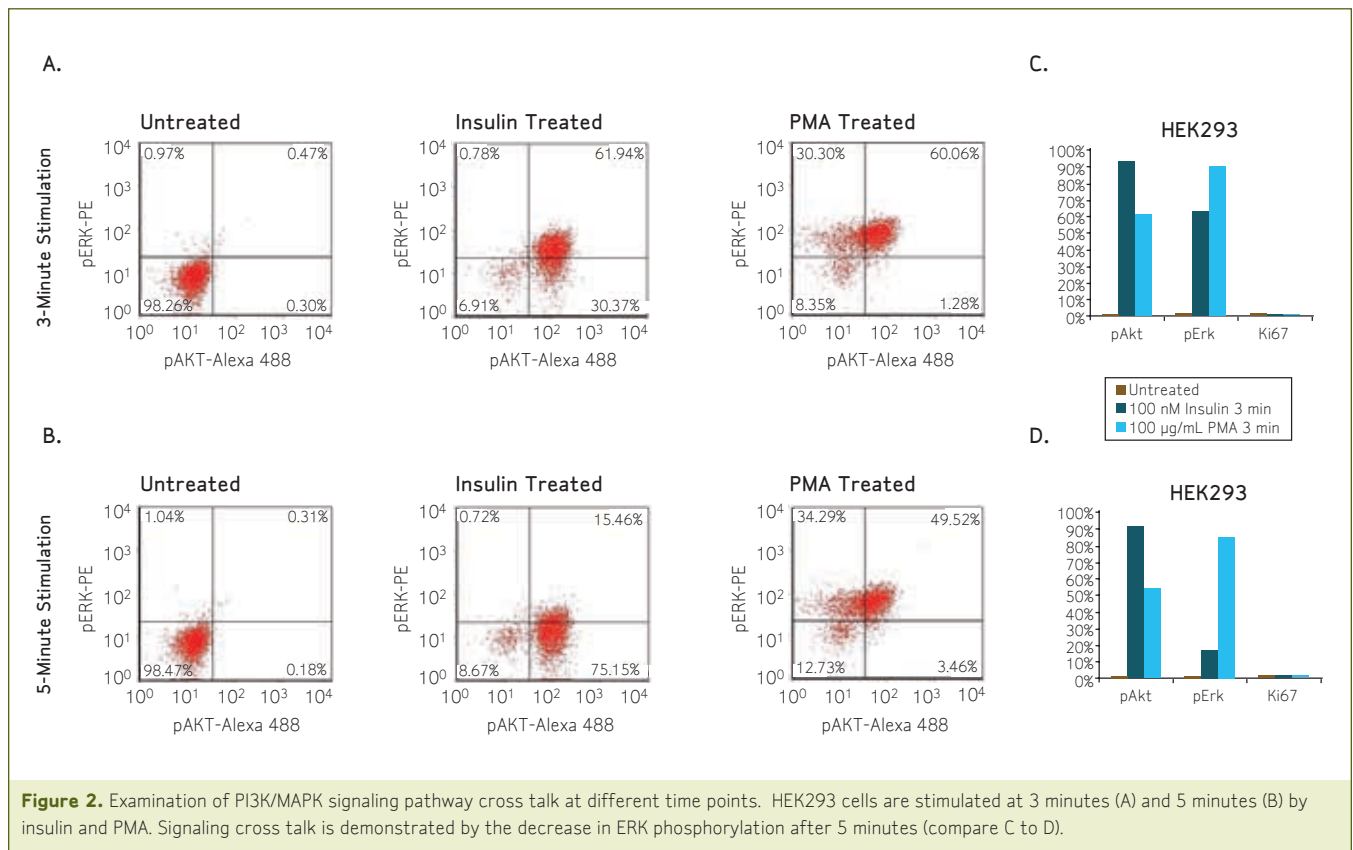
To determine the cross-talk between the PI3K and MAPK pathways, HEK293 cells were stimulated with IGF-I or PMA and evaluated via flow cytometry. First, cells were serum starved for 24 hours. Samples of 2.5 x 10⁵ cells each were then stimulated for 3 or 5 minutes with 100 nM insulin or 100 ng/mL PMA and immediately fixed in the kit's fixation

buffer for 20 minutes on ice. Samples were then permeabilized, stained with directly labeled antibodies against phospho-ERK1/2 (Thr202/Tyr204, Thr185/Tyr187) and phospho-Akt1/PKBα (Ser473), and analyzed using the guava easyCyte™ Plus and 8HT flow cytometers.

To confirm the biological effect of signaling cross-talk on cell proliferation, HEK293 cells were stimulated with insulin and evaluated via flow cytometry. Cells were first starved for 24 hours to reset the cell cycle back to the G₀ phase⁶. Samples of 2.5 x 10⁵ cells each were then incubated overnight (approximately 16 hours) at 37 °C with the Cell Cycle Stop reagent only or the Cell Cycle Stop reagent and 100 nM insulin. The next day, samples were fixed, permeabilized, stained with a directly labeled antibody against Ki-67, and analyzed using the guava easyCyte 8HT flow cytometer.

Results

ERK and Akt phosphorylation were examined in HEK293 cells following 3- or 5-minute stimulation with insulin or PMA. The dot plots (Figure 2, A and B) and resulting bar graphs (C, D) demonstrate cross-talk between the PI3K and MAPK signaling pathways.



As previously shown (Figure 1), although insulin initially activates both pathways independently, the activation of the PI3K pathway (indicated by the phosphorylation of Akt) will inhibit the downstream portion of the MAPK pathway. When Akt is phosphorylated, it dephosphorylates Raf, which in turn affects ERK signaling activity. This is demonstrated by the sharp decrease in ERK phosphorylation between 3 minutes and 5 minutes (C, D).

Having established the presence of cross-talk between the PI3K and MAPK pathways, we measured the expression of the Ki-67 proliferation marker to evaluate the biological significance of the cross-talk. The results indicate that cells have proliferated in response to insulin treatment when compared to the negative control, as demonstrated by the increase in Ki-67 expression in the insulin treated sample. Also, as indicated by the dot plots (Figure 3, A) and the bar graph (B), the use of Cell Cycle Stop greatly enhances Ki-67 measurements.

Discussion and Conclusion

Using flow cytometry analysis and Millipore's FlowCelect PI3K/MAPK dual activation and cancer marker detection kit, we have shown that cross-talk does exist between the PI3K and MAPK signaling pathways. This is demonstrated in HEK293 cells, in which we have confirmed that the phosphorylation of Akt will cause inhibition, or desphosphorylation, of phospho-Raf on Ser 259, essentially blocking the MAPK signaling pathway and preventing the activation of ERK. We have also validated the biological effect of pAkt and pERK1/2 signaling by confirming the correlation between phosphorylation and Ki-67 expression after stimulation with insulin.

These results demonstrate the power of multiparameter flow cytometry in studying signaling cross-talk and its effects in mixed cell populations. The FlowCelect PI3K/MAPK kit and others like it will help scientists gain greater insight into the biological effects of signaling cross-talk in cell proliferation and cancer.

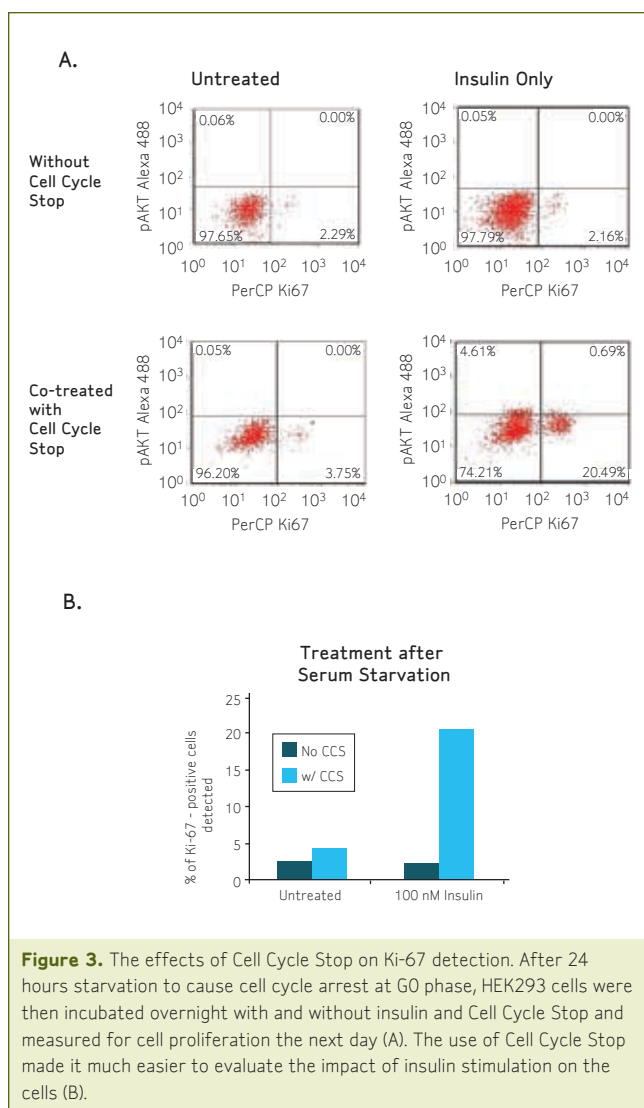


Figure 3. The effects of Cell Cycle Stop on Ki-67 detection. After 24 hours starvation to cause cell cycle arrest at G0 phase, HEK293 cells were then incubated overnight with and without insulin and Cell Cycle Stop and measured for cell proliferation the next day (A). The use of Cell Cycle Stop made it much easier to evaluate the impact of insulin stimulation on the cells (B).

References

- Jun T, et al. Tangled webs: evidence of cross-talk between c-Raf-1 and Akt. *Sci STKE*. 1999 Dec 21;1999(13):PE1.
- Moelling K, et al. Regulation of raf-Akt cross-talk. *J Biol Chem*. 2002 Aug 23;277(34):31099-106. Epub 2002 Jun 4.
- Zimmermann S, Moelling K. Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science*. 1999 Nov 26;286(5445):1741-4.
- Smalley KS, et al. Ki67 expression levels are a better marker of reduced melanoma growth following MEK inhibitor treatment than phospho-ERK levels. *Br J Cancer*. 2007 Feb 12;96(3):445-9. Epub 2007 Jan 23.
- Ishikuro A, et al. Ki-67 labeling indices in non-small cell lung cancer: comparison between image cytometry and flow cytometry. *Cytometry*. 1997 Aug 15;30(4):186-91.
- Littleton RJ, et al. Kinetic aspects of Ki-67 antigen expression in a normal cell line. *Virchows Arch B Cell Pathol Incl Mol Pathol*. 1991;60(1):15-9.

Related Products

Description	Catalogue No.
FlowCelect PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection Kit	FC05025100
FlowCelect PI3K-mTOR Signaling Cascade Mapping Kit	FC05025210
FlowCelect Multi-STAT Activation Profiling Kit	FC05025550
Guava easyCyte 8HT Base Flow Cytometry System	0500-4008

Exciting New Products for Cell Biology Research

STEM CELL RESEARCH

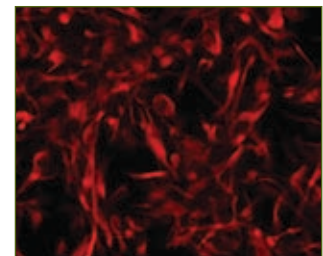
Arctic Ground Squirrel Neural Stem Cells

Arctic ground squirrel neural stem cells (AGS-NSCs) are isolated from the hippocampus of adult arctic ground squirrels following hibernation. Both the isolated cells and the whole animals are tolerant of ischemic insult and reperfusion. Using these cells, researchers can identify potentially novel proteins and genes key to neuronal tolerance, neuroprotection, and neurogenesis. Millipore's AGS-NSCs can be used to screen drugs, genes, and proteins for protective changes caused by oxygen and/or glucose deprivation. These cells can potentially identify future targets for novel stroke therapeutics. As stem cells, these cells can be used in transplantation studies using rodent stroke models to investigate tolerance to ischemic injury. AGS-NSCs can be expanded with the AGS-NSC Expansion Media Kit, and then differentiated into neurons using the AGS-NSC differentiation media kit.

Description	Qty/Pk	Cat. No.
Adult Hippocampal Arctic Ground Squirrel Neural Stem Cells	4 x 10 ⁵ cells	SCCE002
AGS-NSC Expansion Media Kit	500 mL	SCMA002
AGS-NSC Differentiation Media Kit	500mL	SCMA003

MilliTrace CX Nestin GFP Reporter Human Neural Stem Cell Kit

This kit provides a quick, convenient method to study the role of nestin and other factors in neural stem cell differentiation, maintenance and self-renewal. MilliTrace CX nestin GFP reporter human neural stem cells are labeled with the humanized mulleri green fluorescent protein (hmGFP) under the regulation of the nestin promoter. Upon differentiation, nestin is down-regulated and GFP expression is switched off. In addition to the cells, the kit contains expansion medium to help maintain expression of the transgene. Also available from Millipore are human neural stem cells that constitutively express hmGFP.



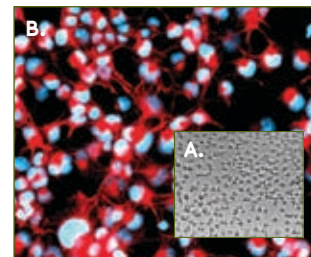
Description	Qty/Pk	Cat. No.
MilliTrace CX Nestin GFP Reporter Human Neural Stem Cell Kit	10 ⁶ viable cells, plus 500 mL expansion medium	SCR096
MilliTrace VM Constitutive GFP Reporter Human Neural Stem Cell Kit	10 ⁶ viable cells, plus 500 mL expansion medium	SCR092
MilliTrace CX Constitutive GFP Reporter Human Neural Stem Cell Kit	10 ⁶ viable cells, plus 500 mL expansion medium	SCR095

STEM CELL RESEARCH

Synthetic Extracellular Matrix (ECM) Proteins

Millipore's synthetic laminin peptide is a defined ECM substrate that has been specifically optimized to support the cell adhesion, proliferation, and multi-lineage differentiation of rat neural stem cells (NSCs) *in vitro*. Rat NSCs grown on tissue culture plates coated with this synthetic laminin peptide (A) display the characteristic neural stem cell markers, nestin (B) and SOX-2, and furthermore possess the capacity to preferentially differentiate down both glial and neural lineages.

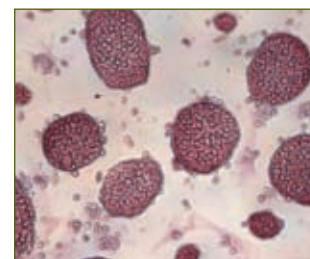
Description	Qty/Pk	Cat. No.
Synthetic Laminin Peptide for Rat Neural Stem Cells	5 x 3 mg	SCR127



ESGRO Complete™ PLUS Medium for Mouse ES Cell Culture

For improved serum-free and feeder-free mouse embryonic stem (ES) cell culture, Millipore has developed ESGRO Complete PLUS, a two-component system containing the original ESGRO Complete clonal grade medium, plus an optimized GSK3 β inhibitor supplement to help maintain pluripotency. The new formulation eliminates the drawbacks associated with the use of FBS and feeder layers, while enhancing the growth and maintenance of undifferentiated mouse ES cells in controlled conditions.

Description	Qty/Pk	Cat. No.
ESGRO Complete PLUS Clonal Grade Medium	100 mL	SF001-100P
ESGRO Complete PLUS Clonal Grade Medium	500 mL	SF001-500P



PluriStem® Mouse Embryonic Stem Cell Lines

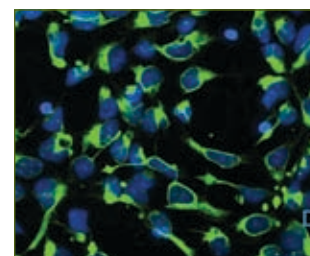
The PluriStem range of murine ES cells is derived from well characterized, common inbred mouse strains. These new mouse ES cell lines are provided at passage 9, have an apparent normal male karyotype (40, XY), and have been genotyped by SNP analysis.

Description	Qty/Pk	Cat. No.
PluriStem C57BL/6N Mouse ES Cell Line	2 vials, 2.5 x 10 ⁶ cells ea.	SCC050
PluriStem BALB/c Mouse ES Cell Line	2 vials, 2.5 x 10 ⁶ cells ea.	SCC052
PluriStem FVB/N Mouse ES Cell Line	2 vials, 2.5 x 10 ⁶ cells ea.	SCC053
PluriStem DBA/2 Mouse ES Cell Line	2 vials, 2.5 x 10 ⁶ cells ea.	SCC054
PluriStem C3H Mouse ES Cell Line	2 vials, 2.5 x 10 ⁶ cells ea.	SCC055

Animal-Free Recombinant Human Basic Fibroblast Growth Factor

Millipore's recombinant human basic fibroblast growth factor (FGF) has been developed without animal-derived ingredients and supports human ES cell and induced pluripotent stem (iPS) cell maintenance and expansion. With our newly introduced 100 μ g pack size, we have several options for you to choose from. Don't see what you need here? Custom packaging is available; please inquire for details.

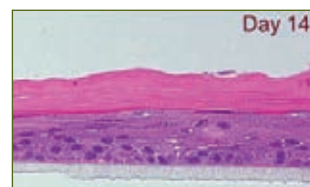
Description	Qty/Pk	Cat. No.
Basic FGF, animal-free, recombinant human	50 μ g	GF003
Basic FGF, animal-free, recombinant human	100 μ g NEW SIZE!	GF003AF-100UG
Basic FGF, animal-free, recombinant human	1 mg	GF003AF-1MG



PRIMARY CELLS

Epidermal Keratinocyte 3D Prime Medium for Generating 3D Skin Models

This novel 3D cell culture medium enables the easy and reliable generation of 3D epidermal models in your own laboratory, on your own schedule, when used with CELLnTEC™ human epidermal keratinocytes and Millipore's Millicell® membrane inserts. The media can also be used with human keratinocytes you have isolated and grown in CELLnTEC's CnT-07 or CnT-57 media. Human epidermal keratinocytes cultured in this medium can generate a 3D model of human epidermis, with all layers (stratum corneum, granulosum, spinosum, basale), within 14-18 days. The 3D medium is fully defined, serum-free, BPE-free, and optimized for 3D epidermal growth. Full, detailed protocols for establishment of 3D keratinocyte models and histological sectioning and staining are available.



Description	Qty/Pk	Cat. No.
Epidermal Keratinocyte 3D Prime Medium, defined	100 mL	CnT-02-3DP1
Epidermal Keratinocyte 3D Prime Medium, defined	500 mL	CnT-02-3DP5

Related Products for 3D culture

Description	Qty/Pk	Cat. No.
PCT Epidermal Keratinocyte Medium, Low BPE (for 2D isolation and growth)	500 mL	CnT-57
PCT Epidermal Keratinocyte Medium, defined (for 2D isolation and growth)	500 mL	CnT-07
Human Epidermal Keratinocyte Progenitors, pooled donor	5 x 10 ⁵ cells	HPEKP.05
Human Epidermal Keratinocyte Progenitors, single donor	5 x 10 ⁵ cells	HPEKS.05
Millicell Single Well Inserts, PCF, 0.4 µm pore size, 30 mm	50	PIHP03050
Millicell Single Well Inserts, PCF, 0.4 µm pore size, 12 mm	50	PIHP01250

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PRIMARY CELLS

EndoGRO Advanced Media Formulations for Endothelial Cell Culture

EndoGRO media provide an optimal cell culture environment for many types of endothelial cells, including HUVEC, aortic endothelial cells, and other human large vessel and microvascular endothelial cells. These low-serum media formulations have been shown to grow endothelial cells at rates that match or exceed commercially available serum-containing media, while maintaining excellent cell morphology. Four media formulations are available:

- **EndoGRO-LS:** Low-serum culture media for human endothelial cells, HUVEC, aortic endothelial cells, and other large vessel endothelial cells
- **EndoGRO-VEGF:** Low-serum, VEGF-supplemented media for rapid proliferation of human endothelial cells, HUVECs, aortic endothelial cells and other large vessel endothelial cells
- **EndoGRO-MV:** Low-serum culture media for human microvascular endothelial cells
- **EndoGRO-MV-VEGF:** Low-serum, VEGF-supplemented media for rapid proliferation of human microvascular endothelial cells

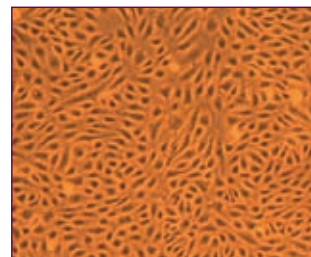


Description	Qty/Pk	Cat. No.
EndoGRO-LS Complete Media Kit	500 mL	SCME001
EndoGRO-VEGF Complete Media Kit	500 mL	SCME002
EndoGRO-MV-VEGF Complete Media Kit	500 mL	SCME003
EndoGRO-MV Complete Media Kit	500 mL	SCME004

EndoGRO Human Umbilical Vein Endothelial Cells (HUVECs)

EndoGRO HUVECs are cultured for only one passage before cryopreservation to ensure the highest viability and plating efficiency. When cultured in EndoGRO low serum media formulations, EndoGRO HUVECs proliferate for at least 15 population doublings at rates equal or greater than standard serum-supplemented media. EndoGRO HUVECs have not been exposed to antimicrobials or phenol red, providing a model system with a more physiological environment.

Description	Qty/Pk	Cat. No.
EndoGRO HUVECs	500,000 cells	SCCE001



CELL CULTUREWARE

Millicell ERS-2 Volt-Ohm Meter

An updated Electrical Resistance System with New Accessories

The Millicell ERS-2 volt-ohm meter is a great QC tool for measuring cell membrane potential, resistance of epithelial cells in culture, cell health, and confluence (TEER). The improved Millicell ERS-2 has improved ergonomic handling by allowing for single-handed data collection. The system now comes with an internal rechargeable battery and accessories such as replacement electrodes, adjustable electrodes, and a specialized electrode for use with the Millicell-96 plate.



Description	Cat. No.
Millicell ERS-2 Volt-Ohm Meter	MERS00002
Replacement Fixed Electrode Set	MERSSTX01
Replacement Test Electrode Set	MERSSTX04
Adjustable Electrode Set (Optional)	MERSSTX03
Specialized Electrode Set for Millicell-96 Plate (Optional)	MERSSTX00
Replacement Battery	MERSBAT01

Related Products

Description	Qty/Pk	Cat. No.
Millicell Hanging Inserts:		
Millicell Hanging Cell Culture 24 well PET 8um	48	PIEP12R48
Millicell Hanging Cell Culture 24 well PET 0.4um	48	PIHT12R48
Millicell Hanging Cell Culture 12 well PET 1um	48	PIRP15R48
Millicell Hanging Cell Culture 12 well PET 0.4um	48	PIHT15R48
Millicell Hanging Cell Culture 6 well PET 1um	48	PIRP30R48
Millicell Hanging Cell Culture 6 well PET 0.4um	48	PIHP30R48
Millicell Standing Inserts:		
Millicell Standing Cell Culture 24 well PCF 0.4um	48	PIHP01250
Millicell Standing Cell Culture 6 well PCF 0.4um	48	PIHP03050
Millicell-24 well plates:		
24-well culture plate with receiver tray, PCF 3.0um	5	PSST010R5
24-well culture plate with single well tray, PCF 0.4um	5	PSHT010R5
24-well culture plate with single well tray, PET 1.0um	5	PSRP010R5
Millicell-96 well plates:		
96-well culture plate with receiver tray, PCF 0.4um	5	PSHT004R5
96-well culture plate with single well tray, PCF 0.4um	5	PSHT004S5
96-well culture plate with receiver tray, PET 1.0um	5	PSRP004R5
Product Description Receiver Plates:		
24-Well Cell Culture Multiwell Plate, TC, Sterile	50	PIMWS2450
12-Well Cell Culture Multiwell Plate, TC, Sterile	50	PIMWS1250
6-Well Cell Culture Multiwell Plate, TC, Sterile	50	PIMWS0650

Introducing ESGRO Complete PLUS Medium

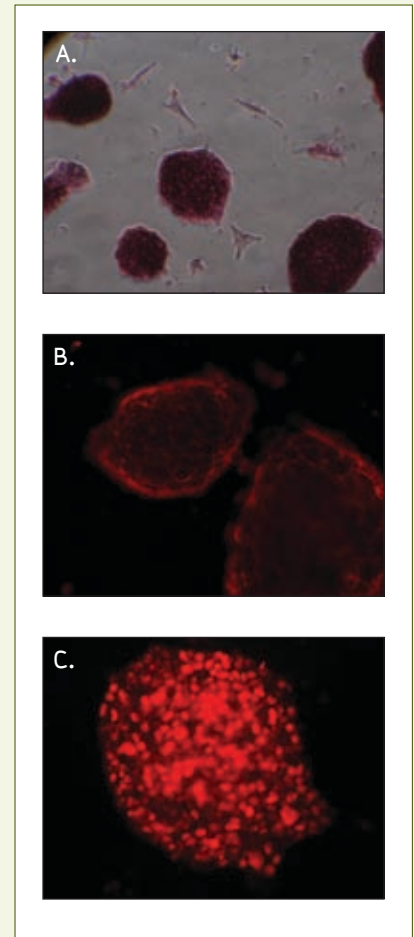
Innovative medium for serum-free, feeder-free culture of mouse ES and iPS cells

ESGRO Complete PLUS is a new and improved medium for the feeder- and serum-free culture of mouse embryonic stem (ES) and induced pluripotent (iPS) cells. This new medium comes as a two part pack featuring the original ESGRO Complete clonal grade medium and an optimized GSK3 β inhibitor to help maintain pluripotency. Cells cultured in ESGRO Complete PLUS medium supplemented with the GSK3 β inhibitor consistently display better growth characteristics, cell morphology, viability, and proliferation rates when compared with cells cultured in the original ESGRO Complete medium alone.

Advantages:

- Eliminates the need for feeder cells or serum
- Complete formulation contains mLIF, BMP4, and GSK3 β inhibitor
- Enables *in vitro* differentiation studies in controlled conditions
- ES cells propagate at clonal density while maintaining pluripotency
- Germline transmission of ES cells confirmed

Figure 1 (right). To confirm pluripotency of iPS cells after ten passages in ESGRO Complete PLUS medium supplemented with GSK3 β inhibitor, cells were stained for alkaline phosphatase (A) and immunostained for SSEA-1 (B) and Oct-4 (C).



Related Products

Description	Qty/Pk	Catalogue No.
ESGRO Complete PLUS Medium	100 mL	SF001-100P
ESGRO Complete PLUS Medium	500 mL	SF001-500P
ESGRO Complete Basal Medium	100 mL	SF002-100
ESGRO Complete Basal Medium	500 mL	SF002-500
ESGRO Complete Derivation Kit	1 kit	SF003
ESGRO Complete Switch Kit	1 kit	SF004
ESGRO Complete Accutase Dissociation Solution	100 mL	SF006
ESGRO Complete Enzyme-Free Dissociation Solution	100 mL	SF009
ESGRO Complete Trypsin	100 mL	SF007
ESGRO Complete Freezing Medium	50 mL	SF005
ESGRO Complete Gelatin	500 mL	SF008
Alkaline Phosphatase Detection Kit	1 kit	SCR004
Anti-Oct-4 (POU5F1), clone 7F9.2	100 μ g	MAB4419
Anti-SSEA-1, clone MC-480	100 μ g	MAB4301

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