

# Differentiation of pluripotent stem cells cultured in StemFlex Medium

## Introduction

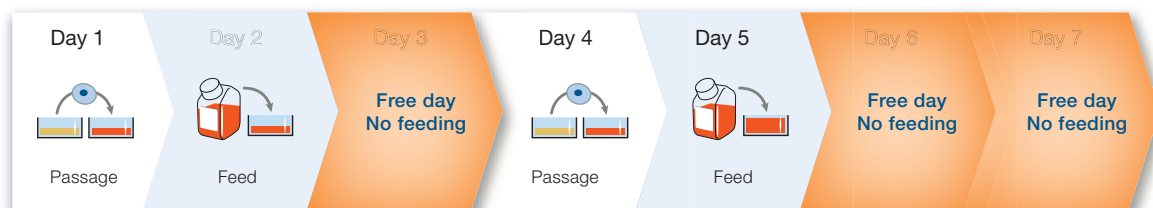
Pluripotent stem cell (PSC) culture systems have evolved over time from feeder-dependent culture systems in which PSCs are cultured on a layer of inactivated mouse embryonic fibroblasts, to simpler, more defined, feeder-free culture systems in which PSCs are cultured on a variety of extracellular matrices (e.g., Gibco™ rhLaminin-521, Geltrex™ matrix, or Vitronectin Recombinant Human Protein, Truncated). The movement to these feeder-free systems has led to simpler, more efficient methods for routine culture and expansion of PSCs, while maintaining the following hallmarks of PSCs: high nuclear-to-cytoplasmic ratio, pluripotency marker expression (e.g., OCT4, NANOG, TRA-1-60, SSEA4), normal karyotype, as well as maintenance of trilineage differentiation potential (i.e., the ability to differentiate to specific cell types of endoderm, mesoderm, and ectoderm). Coupling of expanded PSCs with downstream differentiation protocols provides *in vitro* models for understanding the fundamental basis of genetic diseases, drug discovery, as well as exploring regenerative therapy.

Researchers are sometimes hesitant to change feeder-free culture systems due to concerns about performance in downstream differentiation of PSCs and that differentiation

protocols will need to be updated to accommodate for changes in PSC properties. In this application note, we demonstrate that feeder-free Gibco™ StemFlex™ Medium (1) maintains trilineage differentiation potential following long-term passaging using the flexible feeding schedule (Figure 1), (2) is compatible with downstream differentiation protocols historically used for downstream differentiation of PSCs cultured in Gibco™ Essential 8™ Medium, and (3) is compatible with downstream Gibco™ differentiation kits.

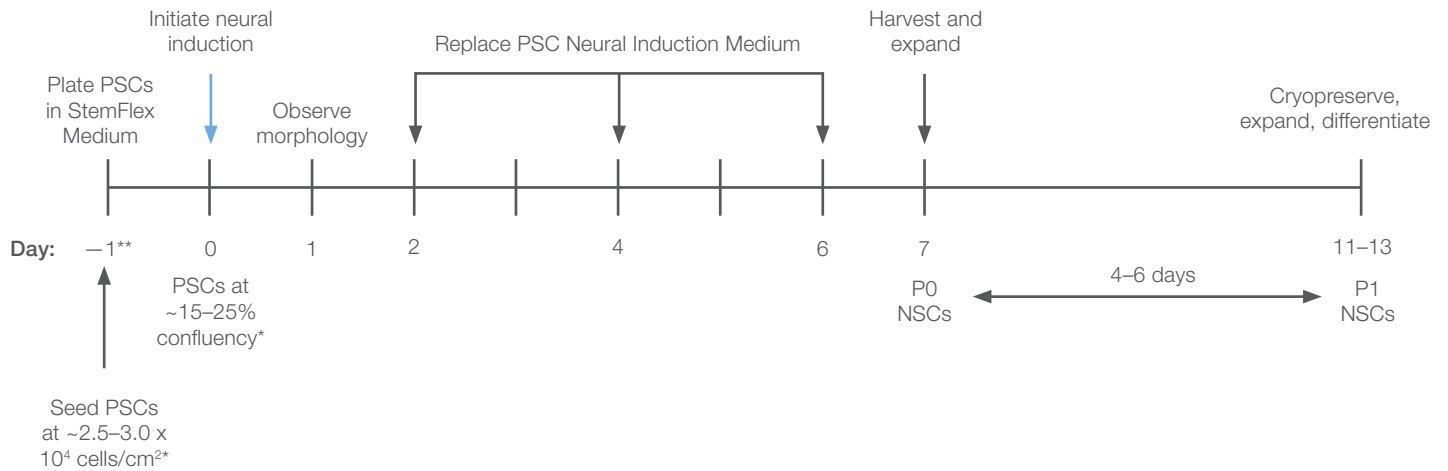
## Suggested workflow

PSCs cultured in StemFlex Medium can be differentiated using Gibco™ PSC Neural Induction Medium (Cat. No. A1647801), Gibco™ PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201), and Gibco™ PSC Definitive Endoderm Induction Kit (Cat. No. A3062601). No changes to the currently recommended workflows are necessary when using StemFlex Medium. Special attention should be given to the suggested PSC seeding densities, as optimal PSC confluency at the time of induction is crucial for successful downstream differentiation. These conditions can be cell line-specific, and may need to be optimized for your PSC line. The recommended workflows for Gibco™ differentiation and induction kits are shown in Figure 2.

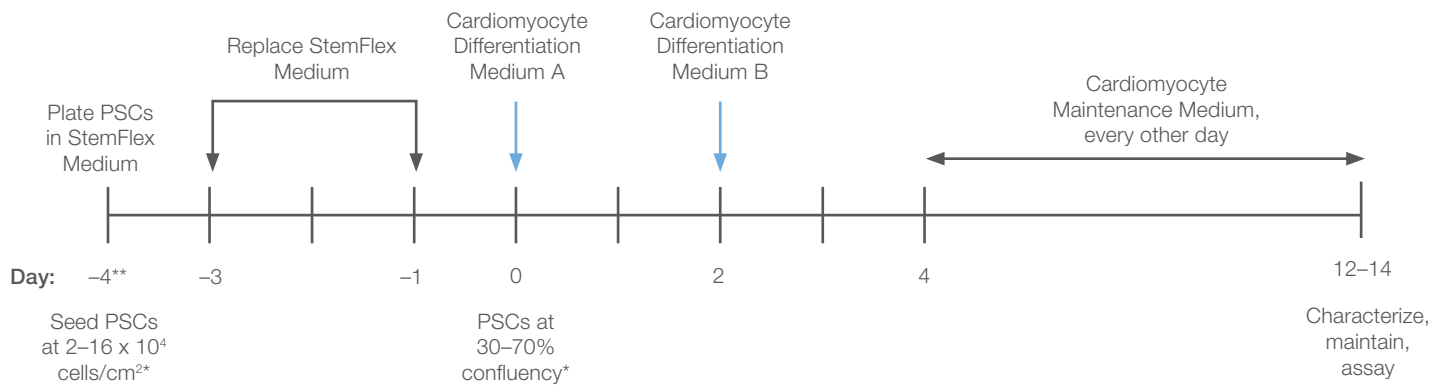


**Figure 1. Recommended weekend-free feeding schedule.** Unlike traditional PSC media, StemFlex Medium eliminates the need to manage cultures daily, enabling a truly weekend-free schedule for expansion and maintenance of PSCs. For additional feeding schedule options, visit [thermofisher.com/stemflex](http://thermofisher.com/stemflex)

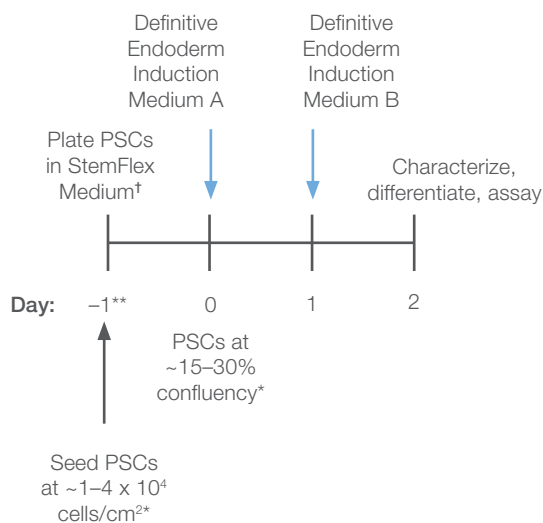
**PSC Neural Induction Medium (Cat. No. A1647801, Pub. No. MAN0013731)**



**PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201, Pub. No. MAN0014534)**



**PSC Definitive Endoderm Induction Kit (Cat. No. A3062601, Pub. No. MAN0016100)**



\* Seeding densities and induction confluencies for PSC differentiation may require optimization for your cell line.

\*\* To achieve optimal confluency for induction, the number of days in culture prior to adding differentiation medium may require adjustment.

† With StemFlex Medium, PSCs can be plated for definitive endoderm induction on either Gibco™ Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Cat. No. A14133) or Gibco™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A31804).

**Figure 2. Schematic workflows for differentiation and induction kits.** Refer to the following manuals available at [thermofisher.com](http://thermofisher.com) for detailed protocols: Pub. No. MAN0013731 for PSC Neural Induction Medium, Pub. No. MAN0014534 for PSC Cardiomyocyte Differentiation Kit, and Pub. No. MAN0016100 for PSC Definitive Endoderm Induction Kit.

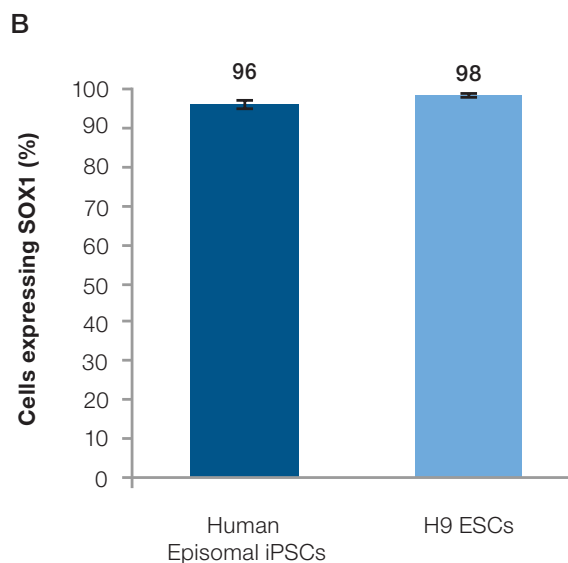
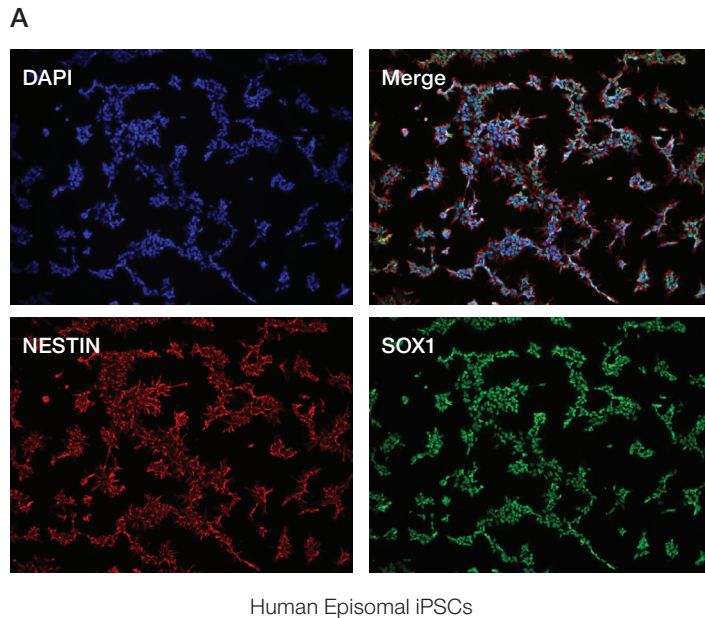
## Results

Prior to differentiation, both Gibco™ Human Episomal iPSCs (Cat. No. A18945) and H9 ESCs were cultured for over 50 passages in StemFlex Medium on Geltrex matrix using the flexible feeding schedule. PSCs were clump passaged with Gibco™ Versene™ Solution (Cat. No. 15040066) twice per week, skipping feeds on one weekday and both weekend days each week.

### Compatibility with PSC Neural Induction Medium

PSC Neural Induction Medium is a serum-free medium that provides high-efficiency neural induction of human PSCs cultured in StemFlex Medium in 7 days. In preparation for neural induction, PSCs were cultured to ~70–80% confluency in StemFlex Medium, clump passaged with Versene Solution, and seeded at 25,000 cells/cm<sup>2</sup> onto a Thermo Scientific™ Nunc™ 6-well plate precoated with Geltrex matrix. Neural induction was initiated once PSCs reached 15–25% confluency. On day 7 of neural induction, newly differentiated neural stem cells (NSCs) were passaged with Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent (Cat. No. A1110501) and Gibco™ RevitaCell™ Supplement (Cat. No. A2644501) and seeded at 100,000 cells/cm<sup>2</sup> onto a Thermo Scientific™ Nunc™ 96-well plate precoated with Geltrex matrix for immunocytochemistry staining and analysis.

In Figure 3A, representative images are shown of NSCs derived from Human Episomal iPSCs using PSC Neural Induction Medium. Cells were stained for the NSC markers NESTIN and SOX1 as well as nuclear DNA using the Invitrogen™ Human Neural Stem Cell Immunocytochemistry Kit (Cat. No. A24354). Figure 3B shows quantitative immunocytochemistry (ICC) data generated using the Thermo Scientific™ CellInsight™ CX5 High Content Screening Platform. NSCs derived from both Human Episomal iPSCs and H9 ESCs cultured in StemFlex Medium show high expression (>95%) of the NSC marker SOX1.



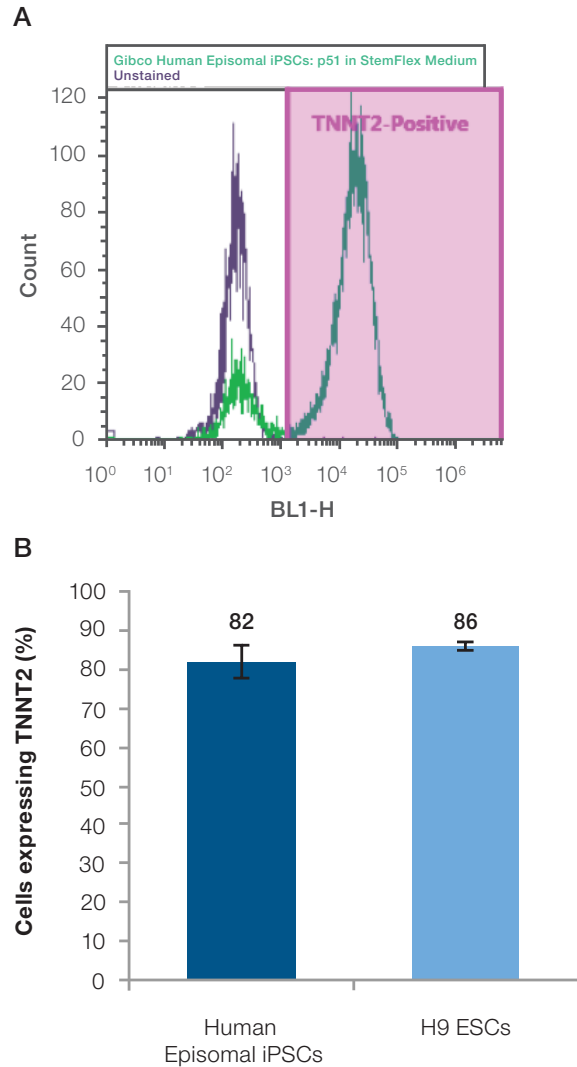
**Figure 3. Induction efficiency with PSC Neural Induction Medium.**

PSCs cultured for >50 passages in StemFlex Medium support maintenance of ectoderm differentiation potential as shown by compatibility with PSC Neural Induction Medium. NSCs were **(A)** stained for the NSC markers NESTIN and SOX1 as well as nuclear DNA (DAPI) and **(B)** assessed for SOX1 expression by quantitative ICC.

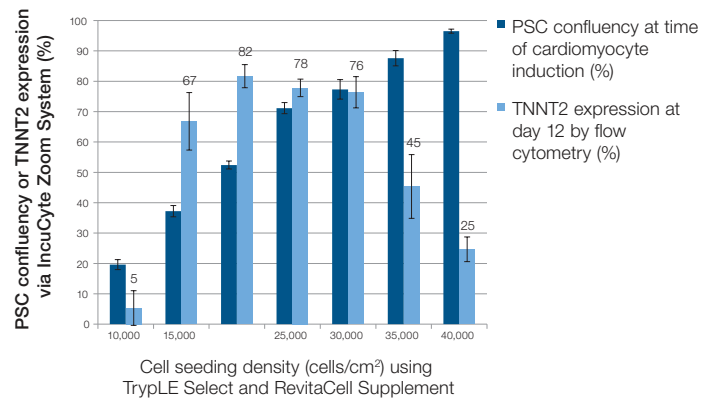
## Compatibility with the PSC Cardiomyocyte Differentiation Kit

The PSC Cardiomyocyte Differentiation Kit consists of a set of serum-free and xeno-free media that enables efficient differentiation of human PSCs to contracting cardiomyocytes in as few as 8 days. In preparation for cardiomyocyte induction, PSCs were cultured to ~70–85% confluency in StemFlex Medium, singularized using Gibco™ TrypLE™ Select Enzyme (Cat. No. 12563011), and seeded with RevitaCell Supplement at 20,000 cells/cm<sup>2</sup> onto a Thermo Scientific™ Nunc™ 12-well plate precoated with Geltrex matrix. Cardiomyocyte induction was initiated once PSCs reached ~50–60% confluency. On day 12, newly differentiated cardiomyocytes were assessed for TNNT2 expression by flow cytometry using the Invitrogen™ Attune™ NxT Flow Cytometer. As shown in Figure 4, cardiomyocyte induction of both Human Episomal iPSCs and H9 ESCs cultured in StemFlex Medium show high expression (>80%) of the cardiomyocyte marker TNNT2 for optimal cell seeding densities.

The data in Figure 5 show that Human Episomal iPSCs cultured in StemFlex Medium can undergo successful cardiomyocyte differentiation at a wide range of induction conditions. For this iPSC line, >65% expression of the cardiomyocyte marker TNNT2 is shown for seeding densities of 15,000–30,000 cells/cm<sup>2</sup> and induction confluencies of ~35–75%.



**Figure 4. Induction efficiency with the PSC Cardiomyocyte Differentiation Kit.** PSCs cultured for >50 passages in StemFlex Medium support maintenance of mesoderm differentiation potential as shown by compatibility with the PSC Cardiomyocyte Differentiation Kit. **(A)** Representative flow cytometry data. **(B)** Percentage of cells expressing the cardiomyocyte marker TNNT2 by flow cytometry.

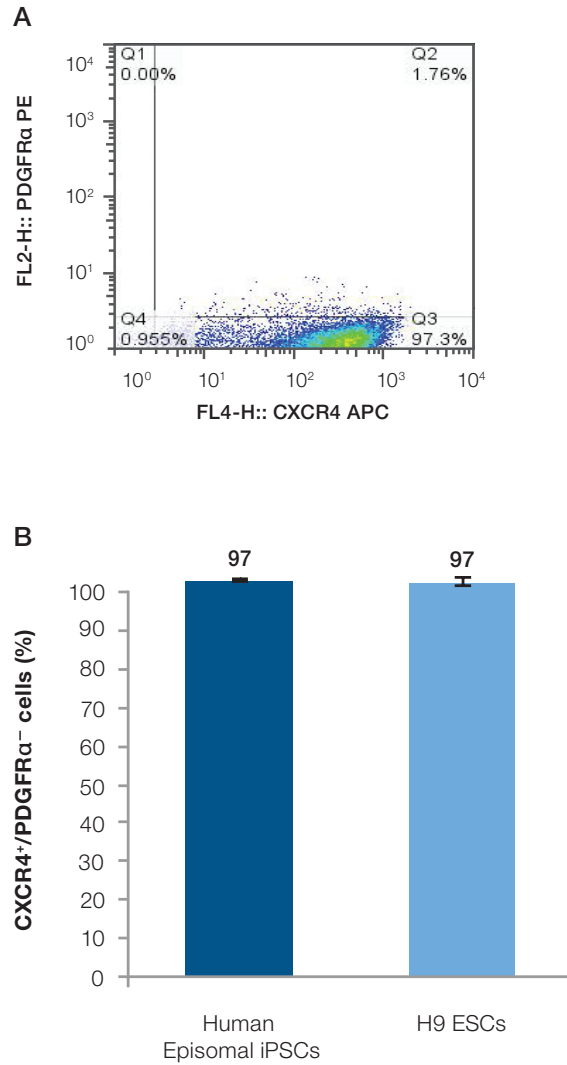


**Figure 5. Differentiation potential at multiple seeding densities.** Human Episomal iPSCs cultured for >50 passages in StemFlex Medium show successful cardiomyocyte differentiation at a range of seeding densities and induction confluencies.

### Compatibility with the PSC Definitive Endoderm Induction Kit

The PSC Definitive Endoderm Induction Kit consists of two xeno-free media that enable efficient induction of human PSCs to definitive endoderm. Unlike other methods that require multiple components and take five or more days, the PSC Definitive Endoderm Induction Kit can generate  $\geq 90\%$  CXCR4<sup>+</sup>/PDGFR $\alpha$ <sup>-</sup> definitive endoderm cells with only two components in just two days.

In preparation for definitive endoderm induction, PSCs were cultured to  $\sim 70\text{--}85\%$  confluency in StemFlex Medium, passaged using StemPro Accutase Cell Dissociation Reagent, and seeded with RevitaCell Supplement at 10,000 cells/cm<sup>2</sup> onto a Nunc 6-well plate precoated with Geltrex matrix. Definitive endoderm induction was initiated once PSCs reached  $\sim 15\text{--}30\%$  confluency. On day 3 of definitive endoderm induction, cells were assessed for expression of CXCR4<sup>+</sup>/PDGFR $\alpha$ <sup>-</sup> by flow cytometry. As shown in Figure 6, definitive endoderm induction of both Human Episomal iPSCs and H9 ESCs cultured in StemFlex Medium show high expression ( $>95\%$ ) of CXCR4.



**Figure 6. Induction efficiency with the PSC Definitive Endoderm Induction Kit.** PSCs cultured for  $>50$  passages in StemFlex Medium support maintenance of endoderm differentiation potential as shown by compatibility with the PSC Definitive Endoderm Induction Kit. **(A)** Representative flow cytometry data. **(B)** Percentage of CXCR4<sup>+</sup>/PDGFR $\alpha$ <sup>-</sup> cells by flow cytometry.



## Conclusions

Together, these data confirm trilineage differentiation potential for two PSC lines cultured for over 50 passages in StemFlex Medium using the flexible feeding schedule. Additionally, the StemFlex Medium system is shown to be compatible with available Gibco™ differentiation products—PSC Neural Induction Medium, the PSC Cardiomyocyte Differentiation Kit, and the PSC Definitive Endoderm Induction Kit. PSCs differentiated following prolonged culture in StemFlex Medium show high expression of respective lineage-specific markers, enabling success in workflows requiring downstream differentiation.

Find out more at [thermofisher.com/stemflex](https://thermofisher.com/stemflex)

**ThermoFisher**  
SCIENTIFIC