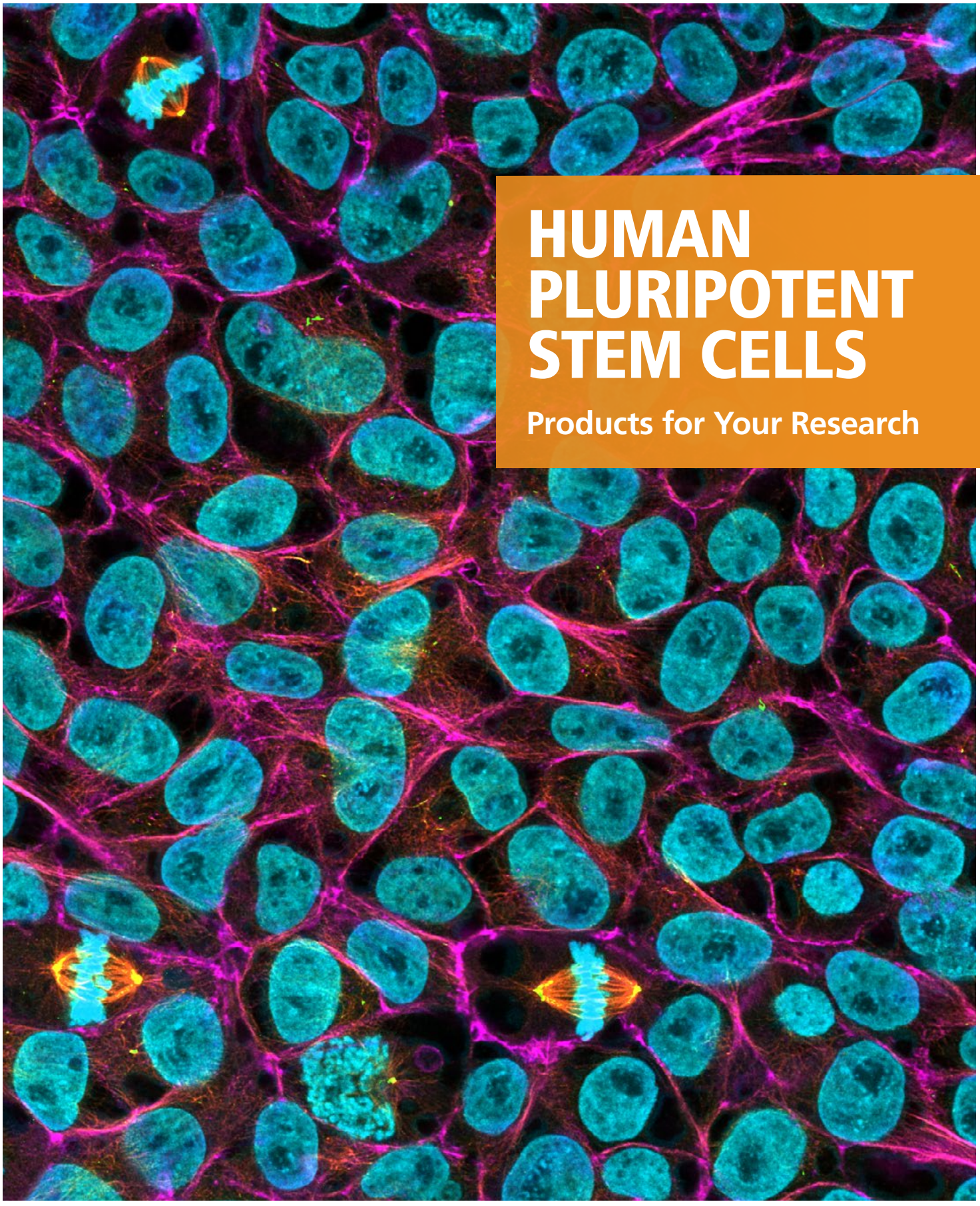

HUMAN PLURIPOTENT STEM CELLS

Products for Your Research

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Reprogramming

ReproRNA™-OKSGM

Generate iPS Cells Using a Non-Integrating Reprogramming Vector

ReproRNA™-OKSGM (Catalog #05931) is a single-stranded RNA replicon vector that contains five reprogramming factors: OCT4, KLF4, SOX2, GLIS1, and c-MYC, as well as a puromycin-resistance gene. This RNA vector reprograms human somatic cells, such as fibroblasts, into induced pluripotent stem (iPS) cells with high efficiency and only requires a single transfection. As shown in Figure 1, using ReproRNA™-OKSGM with ReproTeSR™ (Catalog #05920) reprogramming medium allows for iPS cell colony generation under feeder-free conditions with similar reprogramming efficiency to feeder-based systems. ReproRNA™-derived human iPS cell colonies also express markers of undifferentiated cells and retain a normal karyotype. Subsequently, human iPS cells generated with ReproRNA™-OKSGM can be maintained in a TeSR™ maintenance medium and further differentiated into cells of all three germ layers.

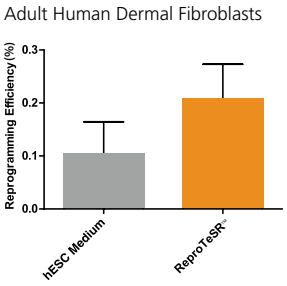


Figure 1. ReproRNA™-OKSGM Vector Efficiently Reprograms Fibroblasts

Human dermal fibroblasts were transfected with the ReproRNA™-OKSGM vector and reprogrammed under feeder-dependent (standard KOSR-containing human embryonic stem cell medium on inactivated mouse embryonic fibroblasts) or feeder-free conditions (ReproTeSR™ on Corning®Matrigel®). The efficiency of reprogramming fibroblasts with ReproRNA™-OKSGM and ReproTeSR™ is comparable to that reported with Sendai virus[1] (n ≥ 6; data shown are mean ± SD).

Learn more at
www.stemcell.com/ReproRNA-OKSGM

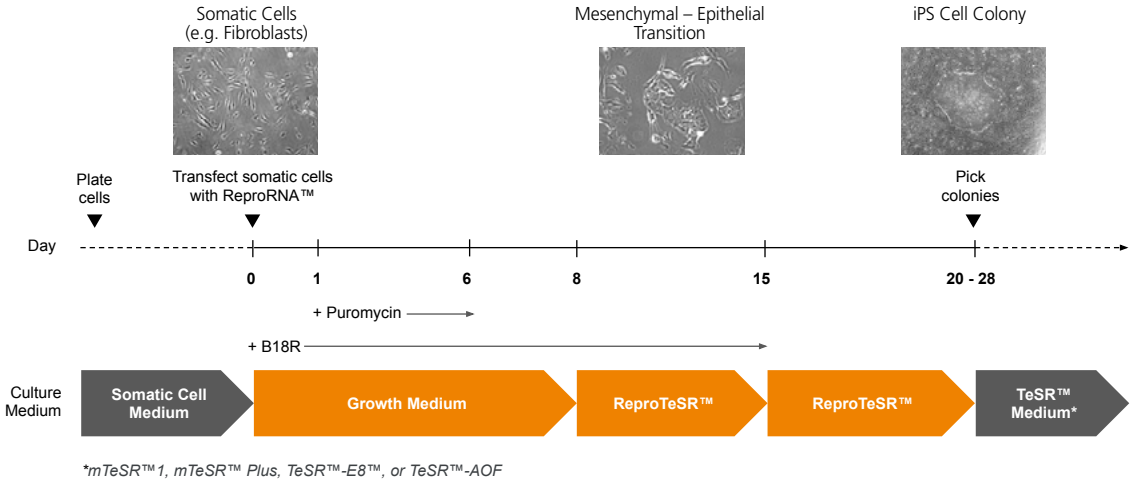


Figure 2. Schematic for Reprogramming with ReproRNA™-OKSGM

Somatic cells are transfected with ReproRNA™-OKSGM at Day 0 and cultured in Growth Medium (containing puromycin). After 5 days of puromycin selection post-transfection, cells are cultured in ReproTeSR™ for the remainder of the reprogramming induction phase until iPS cell colonies emerge. Recombinant B18R Protein is also added during the first 2 weeks after transfection to inhibit the interferon response and increase cell viability. Typically, by Day 20, iPS cell colonies are large enough to be isolated and propagated in a TeSR™ maintenance medium.

ReproTeSR™

Reproducibly Generate High Numbers of Human iPS Cell Colonies

ReproTeSR™ (Catalog #05920) is a complete, defined, xeno-free, and feeder-free reprogramming medium optimized for the generation of human iPS cells. Use ReproTeSR™ during the induction phase of reprogramming to produce more iPS cell colonies than with traditional KOSR-containing human ES cell media. Human iPS cell colonies generated with ReproTeSR™ express undifferentiated cell markers and exhibit more defined borders, compact morphology, and reduced differentiation.

ReproTeSR™ was optimized for reprogramming blood cells and seamlessly integrates with RosetteSep™, SepMate™, EasySep™, and StemSpan™ products for isolating and expanding hematopoietic cells. It can also be used to reprogram other somatic cell types and can be paired with ReproRNA™-OKSGM (Catalog #05931) for reprogramming fibroblasts. iPS cells generated with ReproTeSR™ can be subsequently cultured in a TeSR™ maintenance medium and differentiated with the STEMdiff™ suite of products to cells of all three lineages. Purchase ReproTeSR™ individually or as part of the Erythroid (Catalog #05924) or CD34+ (Catalog #05925) Progenitor Reprogramming Kits.

Integrated Sets of Tools for Reprogramming Human Blood Cells

Erythroid Progenitor Reprogramming Kit

- Enrich cells with RosetteSep™ and SepMate™
- No isolation step required
- Expand erythroid cells with StemSpan™ SFEM II + Erythroid Expansion Supplement
- Reprogram cells with ReproTeSR™

CD34+ Progenitor Reprogramming Kit

- Enrich cells with RosetteSep™ and SepMate™
- Isolate CD34+ cells with EasySep™*
- Expand CD34+ cells with StemSpan™ SFEM II + CD34+ Expansion Supplement
- Reprogram cells with ReproTeSR™

*EasySep™ magnet is not included with the CD34+ Progenitor Reprogramming Kit and must be purchased separately.

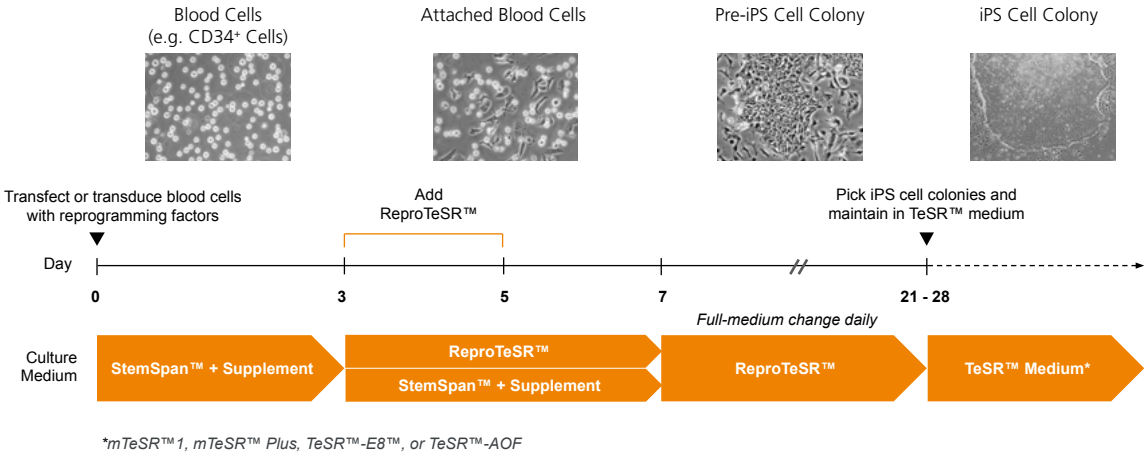


Figure 3. Schematic of ReproTeSR™ Blood Reprogramming Timeline

ReproTeSR™ is used during the entire induction phase of reprogramming (Days 3 to 21). On Days 3 and 5, ReproTeSR™ is added to StemSpan™ growth medium (in a fed-batch manner) to facilitate attachment of transfected cells. Attached cells are further cultured in ReproTeSR™ with daily full media changes until putative iPS cell colonies emerge (Days 21 to 28). iPS cell colonies can then be isolated and propagated in a TeSR™ maintenance medium.

Learn more at www.stemcell.com/ReproTeSR

TeSR™-E7™

Generate iPS Cells from Fibroblasts Without Feeders or Animal Components

TeSR™-E7™ (Catalog #05914) is a defined, animal component-free (ACF) reprogramming culture medium optimized for the generation of human iPS cells without the use of feeders. It is based on the E7 formulation published by the laboratory of Dr. James Thomson.[2] TeSR™-E7™ is specifically formulated to limit fibroblast overgrowth, resulting in colonies with easily recognizable ES cell-like morphology.

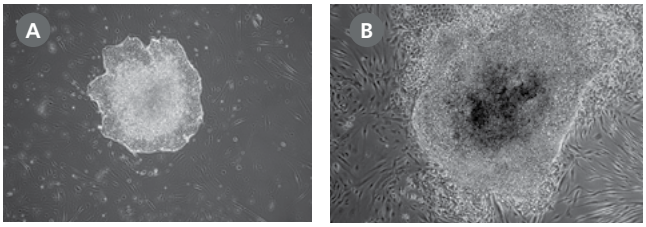


Figure 4. Primary iPS Cell Colonies Derived in TeSR™-E7™ Have Defined Borders and Reduced Differentiation

(A) TeSR™-E7™ yields easily recognizable iPS cell colonies with defined borders. (B) Unqualified components can result in colonies that have poorly defined edges and higher levels of differentiation. Representative colonies from adult human fibroblasts reprogrammed with episomal vectors containing OCT4, SOX2, KLF4, and c-MYC are shown.

Why Use TeSR™-E7™?

- Easily identify and select iPSC colonies by using a medium with pre-screened components that ensures high-quality cells
- Rapidly establish homogeneous iPSC cultures with reduced differentiation and fibroblast growth
- Enjoy reproducibly efficient human iPSC generation with a feeder-free, defined formulation

Learn more at www.stemcell.com/TeSR-E7

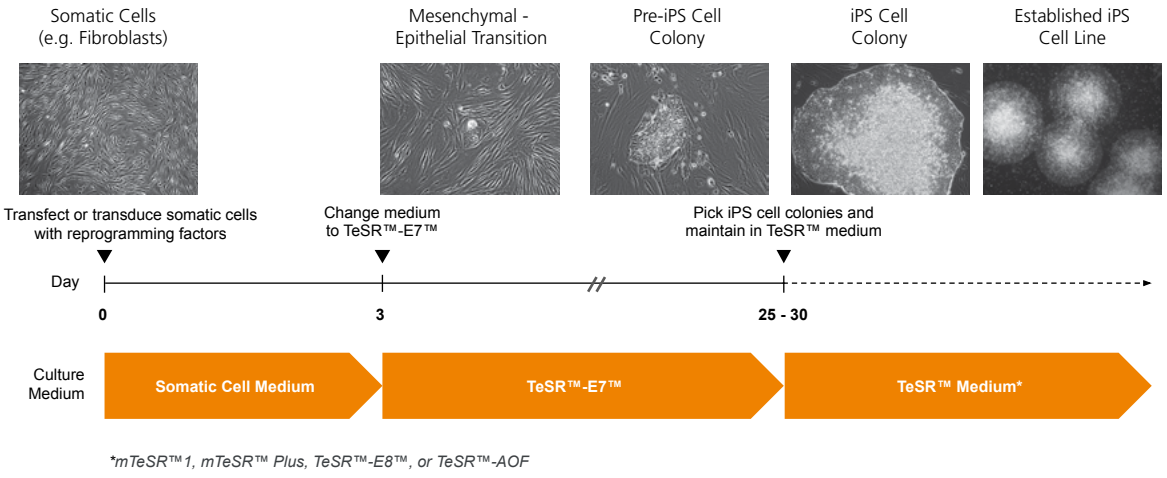


Figure 5. Schematic of Reprogramming Timeline

TeSR™-E7™ can be used during the entire induction phase of reprogramming (Days 3 to 25+). Following reprogramming, iPS cell colonies can be isolated and propagated in feeder-free maintenance systems (e.g. TeSR™ media on Corning® Matrigel® or Vitronectin XF™ matrices). TeSR™ = TeSR™ family of maintenance media.

hPSC Lines and hPSC-Derived Cells

Healthy Control Human iPSC Line, Female, SCTi003-A

Start with a High-Quality iPSC Control Line

Derived from peripheral blood mononuclear cells (PBMCs), the Healthy Control Human iPSC Line, SCTi003-A (Catalog #200-0511) has undergone extensive quality control procedures and been validated with STEMCELL Technologies products for various applications, such as culture scale-up or differentiation to multiple cell types in both 2D and organoid models.

NOTE: For research use or in vitro laboratory-based tissue culture work only. Not approved for application into humans under any circumstances.

Demographic Information

STEMCELL collects donor demographic information ethically, using consent forms and protocols approved by either an institutional review board (IRB), the Food and Drug Administration (FDA), the U.S. Department of Health and Human Services, and/or an equivalent regulatory authority. Donations are performed in the United States in compliance with applicable federal, state, and local laws, regulations, and guidance.

Why Use SCTi003-A?

- Meet regulatory requirements for academic and/or commercial purposes with ethically sourced human iPSCs collected using IRB protocols
- Trust in extensive quality control that meets or exceeds industry standards at every step of the manufacturing process[3]
- Enhance research transparency, and ethical and biological conformity, by using a cell line certified by hPSCreg®
- Confidently integrate human iPSCs into your workflow with a cell line that is compatible with TeSR™ media for maintenance and STEMdiff™ for differentiation

Table 1. Quality Control Procedures Performed on STEMCELL's iPSC Lines

Assessment	Manufacturing Stage			
	Pre-Master	Master Cell Bank	Working Cell Bank	Commercial Cell Bank
Viability	-	√	√	√
Recovery	-	√	√	√
Sterility	-	√	√	√
Viral screen	-	√	-	-
Mycoplasma	-	√	√	√
Cell line identity	-	√	√	√
Residual vector	√	-	-	-
T cell clonality assay*	√	-	-	-
Karyotype	√	√	√	√
20q FISH	-	√	-	-
Copy number variants	-	√	√	√
Whole exome sequencing	-	√	-	-
Undifferentiated marker analysis	-	Yes, 5 passages after thaw	Yes, 3 passages after thaw	Yes, 3 passages after thaw
Trilineage differentiation	√	√	-	-

* Blood-derived iPSC lines only
Note 1: Only iPSC vials from the Commercial Cell Bank are made available for commercial sale.
Note 2: Commercial Cell Bank iPSCs have been characterized for all assessment criteria at varying stages of the manufacturing process.

Extensive quality control procedures are conducted at every stage of the iPSC manufacturing process to ensure cell quality and reproducibility, including assessments for: cell line identity by STR analysis; microbiological sterility by mycoplasma testing, viral screening, and sterility testing; genomic integrity and stability by residual vector testing, T cell clonality, karyotyping, 20q FISH, SNP microarray, and whole exome sequencing; undifferentiated cell marker expression by flow cytometry; and pluripotency by in vitro trilineage differentiation.

Trilineage Differentiation Capabilities

SCTi003-A (Catalog #200-0511), is validated for use with a wide range of products, including a range of STEMdiff™ kits used for differentiation.

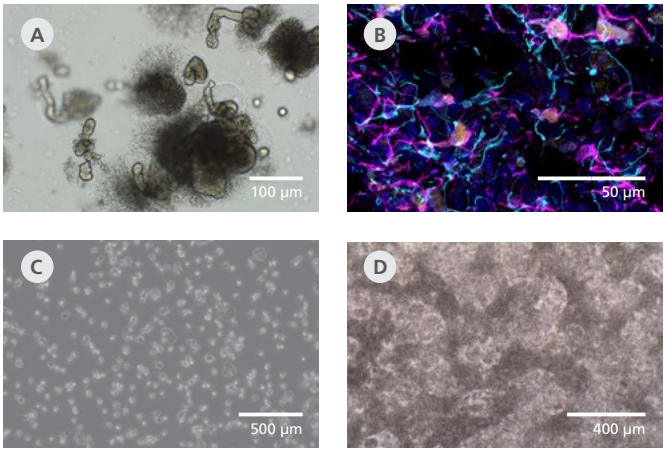


Figure 6. SCTi003-A Human Pluripotent Stem Cells Can Successfully Differentiate into Cell Types from All Three Germ Layers

Line SCTi003-A is validated with a variety of STEMdiff™ differentiation and maturation kits. (A) SCTi003-A iPSCs can be differentiated to intestinal spheroids and embedded in Matrigel® domes for maturation into human intestinal organoids using the STEMdiff™ Intestinal Organoid Kit. Maturing organoids (shown at Day 13) can be passaged and expanded using STEMdiff™ Intestinal Organoid Growth Medium. (B) Neural Organoids stained for DAPI (blue), MAP2 (magenta), NEUN (yellow), and GFAP (cyan) can be differentiated from SCTi003-A using STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit and maintained with STEMdiff™ Neural Organoid Maintenance Kit. (C) iPSC-derived microglia with visible processes and small cytoplasmic-to-nuclear ratios can be generated from SCTi003-A iPSCs via a hematopoietic progenitor cell intermediate using the STEMdiff™ Hematopoietic Kit, with further differentiation using STEMdiff™ Microglia Differentiation and Maturation Kits. (D) Ventricular cardiomyocytes were generated using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit to form an iPSC-derived monolayer that exhibits beating behavior. For more information on the STEMdiff portfolio, visit **www.stemcell.com/stemdiff**.

PRODUCT INFORMATION
Additional data on the SCTi003-A line
www.stemcell.com/scti003-a

RESOURCE
Frequently Asked Questions on iPSCs
www.stemcell.com/ipsc-faq

iPSCdirect™: A Source of Ready-To-Use hPSCs in a Single-Cell Format

Speed up your research with thaw-to-use, single-cell format iPSCdirect™ cells. These specially cryopreserved iPSCs eliminate the need for developing and characterizing cell banks for your iPSC research, as well as the need for keeping iPSCs in long-term culture, both time-consuming and costly processes. Derived from the SCTi003-A cell line, iPSCdirect™ has undergone the same rigorous QC procedures, making it a robust and highly consistent, higher-density version of SCTi003-A, without the upkeep. Each vial contains 10 million viable cells, which are immediately ready for use in downstream applications, such as differentiation using STEMdiff™ media products.

Learn more at www.stemcell.com/ipscdirect

Human iPSC-Derived Neural Progenitor Cells

Ready to use directly after thawing, Human iPSC-Derived Neural Progenitor Cells (NPCs; Catalog #200-0620) are multipotent and suitable for customized downstream differentiation to various CNS cell types, such as forebrain neurons, midbrain neurons, and astrocytes. To ensure high-quality cells, these NPCs were differentiated from the robust, extensively tested SCTi003-A line using serum-free STEMdiff™ SMADi Neural Induction Kit (Catalog #08581). NPCs can be expanded using STEMdiff™ Neural Progenitor Medium (Catalog #05833), allowing for scale-up and reducing the cost of workflows that require large numbers of cells. Cryopreserve expanded NPCs using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838) for flexibility in your experimental schedule. See Page 40 for more information.

Learn more at www.stemcell.com/NPCs

Maintenance Media Overview

Select the Right Maintenance Medium for Your Research

Maintaining high-quality human pluripotent stem cells (hPSCs), including induced pluripotent stem (iPS) and embryonic stem (ES) cells, is critical for success in hPSC research. The TeSR™ family of feeder-free maintenance media is manufactured using rigorously pre-screened materials to ensure the highest levels of batch-to-batch consistency and experimental reproducibility. These media are based on published formulations[2,4-6] from the laboratory of Dr. James Thomson and are tailored to suit your specific needs.

cGMP Media

Minimize Risk in Your Cell Therapy Development

TeSR™-AOF

- Contains no animal-derived raw materials to the secondary level of manufacturing
- Supports high-quality culture morphology, robust attachment, and cell expansion
- Stabilized FGF2 supports high cell quality while allowing for alternate feeding schedules

Versatility for Routine Maintenance and Expansion

mTeSR™ Plus

- Allows for alternate feeding schedules due to enhanced pH buffering and stabilization of FGF2
- Improves upon the trusted mTeSR™1 formulation to provide superior culture morphology as well as cell growth and survival rates
- Maintains cell quality, from iPSC line manufacturing, to preparing hPSCs for downstream differentiation with our STEMdiff™ kits

mTeSR™1

- Used to maintain thousands of hPSC lines in over 60 countries for 15 years
- Contains pre-screened BSA to stabilize medium, aid in lipid/nutrient transport, and protect cultures from cellular toxins and stresses[4]

Why Use the TeSR™ Media Family?

- Minimize variability by choosing feeder-free formulations to limit the presence of undefined components
- Maintain and passage hPSCs with confidence by using the most widely published media family for hPSC culture, with >9000 peer-reviewed publications
- Ensure cell quality by using the TeSR™ medium that best suits your research needs, from clonal selection to scaling up in 3D suspension cultures

PRODUCT INFORMATION
www.tesr.com

Specialized Media

Enhance Single-Cell Passaging

NEW: eTeSR™

- Enhanced buffering, stabilization of FGF2, and optimized metabolites support superior culture morphology and cell growth while allowing for alternate feeding schedules
- Increases yields while reducing stress associated with single-cell passaging or high density cultures during routine maintenance
- Enables easy transition between media for intermediate single-cell culture steps such as cloning or gene editing

Just the Basics

TeSR™-E8™

- Contains only the 8 most critical components required for hPSC maintenance[2,6]
- Uses an animal component-free (ACF) formulation, with no animal-derived raw materials to the primary level of manufacturing
- Has a low-protein formulation, compared to other TeSR™ maintenance media

Suspension Culture

Scale Up Cell Production with Fed-Batch Media

TeSR™-AOF 3D

- Contains no animal-derived raw materials to the secondary level of manufacturing
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 10^9 high-quality hPSCs rapidly without requiring adaptation from 2D culture

mTeSR™3D

- Based on mTeSR™1, optimized for hPSC scale-up
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 109 high-quality, undifferentiated hPSCs in as few as 2 - 3 weeks

TeSR™-E8™3D

- Based on TeSR™-E8™, optimized for hPSC scale-up in low-protein, ACF conditions
- Eliminates the need for medium exchanges on non-passaging days through daily feeds that replenish nutrients
- Enables scale-up to 1 x 10^9 high-quality, undifferentiated hPSCs in as few as 2 - 3 weeks

Coming Soon: eTeSR™

Enhanced Maintenance Medium Optimized for Single-Cell Passaging

eTeSR™ is an enhanced feeder-free cell culture medium, stabilized and optimized to support the maintenance and expansion of human pluripotent stem cells when cultured as single cells. The formulation has been developed to reduce cellular stress associated with single-cell passaging and can be used for routine maintenance or application-specific single-cell culture. eTeSR™ builds upon previous TeSR™ formulations[1,2], the most widely published feeder-free cell culture media family for hPSCs.

eTeSR™ has been specifically developed to support single-cell passaging that typically involves shorter passaging schedules and high densities. To cope with the increased metabolic demand and increased cellular stress associated with this method, eTeSR™ is formulated to stabilize key components, including FGF2, improve buffering capacity, and optimize metabolites, to produce high-quality hPSCs with improved genetic stability compared to other hPSC maintenance media..

eTeSR™ is compatible with both daily and restricted feeding schedules while maintaining high cell quality and equivalent performance, and can also be used with a variety of cell culture matrices, including Corning® Matrigel® hESC-Qualified Matrix and CellAdhere™ Laminin-521 (Catalog #7703).

Each lot of eTeSR™ 10X Supplement is quality-tested in a culture assay using hPSCs.

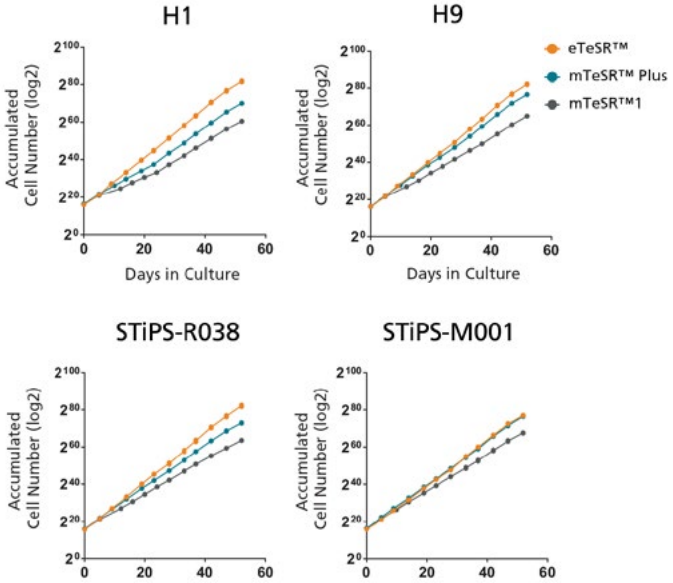


Figure 7. hPSCs Cultured as Single Cells Show Greater Cell Expansion in eTeSR™

Four hPSC lines were single-cell passaged using TrypLE™ and maintained in either mTeSR™1, mTeSR™ Plus, or eTeSR™ on Corning® Matrigel®-coated plates. Cultures were maintained for 11 passages, using daily feeding for mTeSR™1 or a Day 4 and subsequent Day 5 restricted feeding schedule for mTeSR™ Plus and eTeSR™. Accumulated cell numbers were calculated by dividing the number of cells at the end of each passage by the number of cells seeded at passage.

Why Use eTeSR™?

- Support cell quality while allowing for alternative feeding schedules with enhanced buffering, stabilization of key components (including FGF2), and optimized metabolites
- Increase cell yields while reducing stress associated with single-cell passaging or high density cultures during routine maintenance
- Complete your workflow with compatible gene editing, cloning, differentiation, and cryopreservation protocols
- Eliminate spontaneous differentiation

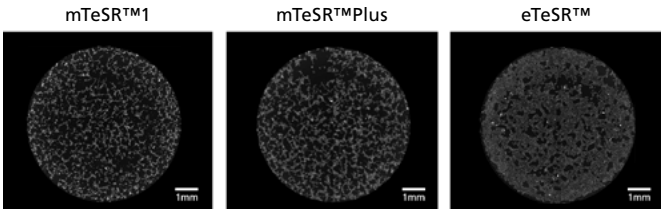


Figure 8. eTeSR™ Improves Attachment Efficiency of hPSCs Seeded as Single Cells

Representative Hoechst staining from the STiPS-R038 hPSC line 24 hours post-seeding. Cells were seeded at 4.7x10^4 cells/cm^2 in either mTeSR™1, mTeSR™ Plus, or eTeSR™ supplemented with 10 µM Y-27632 on Matrigel®-coated 96-well plates. Plates were fixed, stained for Hoechst 33342, and imaged using the ImageXpress® Micro 4 Microscope. Scale bars = 1 mm.

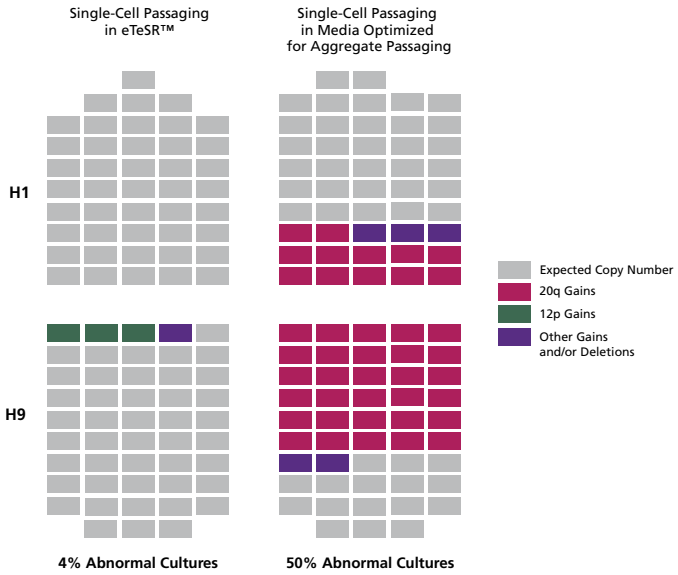


Figure 9. eTeSR Demonstrates Improved Genetic Stability of hPSC Cultures When Maintained Long-Term Using Single-Cell Passaging

A significant reduction in the number of genetic variants was detected in individually cloned H1 and H9 hPSC biological replicates when cultured in eTeSR™ compared to media primarily optimized for aggregate passaging as measured by the hPSC Genetic Analysis Kit and confirmed by FISH. Each box/square represents an individual clone cultured for 20 weeks (30 passages).

mTeSR™ Plus

Enjoy Flexible Feeding Schedules and Enhanced Growth Characteristics with Stabilized, cGMP-Grade Medium

mTeSR™ Plus (Catalog #100-0276) is based on the formula of mTeSR™1, the most-published feeder-free hPSC maintenance medium[2,4-6], and allows for culture versatility that supports high-quality maintenance and expansion of hPSCs.

To enhance cell quality attributes, critical medium components—including FGF2—have been stabilized, while medium pH is more consistent due to enhanced buffering. As a result, this medium supports higher cell numbers with daily feeding, while maintaining consistent quality during restricted feeding schedules.

mTeSR™ Plus is manufactured under relevant cGMPs. With enhanced critical raw material traceability, processes, and quality control validations, mTeSR™ Plus enables a seamless transition from basic research to drug and cell therapy development.

mTeSR™ Plus is compatible with a variety of culture matrices, including Corning® Matrigel® hESC-Qualified Matrix, CellAdhere™ Laminin-521 (Catalog #77003), and Vitronectin XF™ (Catalog #07180).

Each lot of mTeSR™ Plus 5X Supplement is used to prepare complete mTeSR™ Plus medium and is then performance-tested in a culture assay using hPSCs.

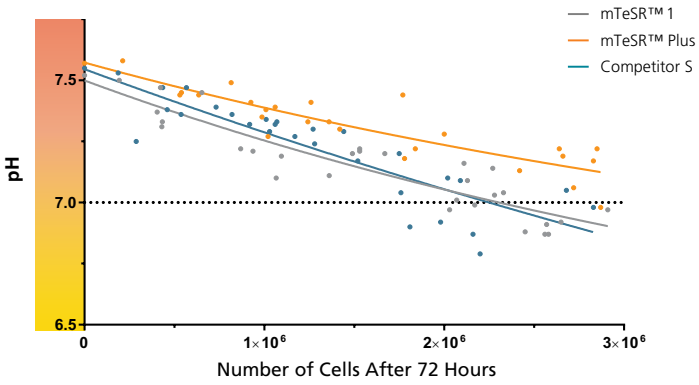


Figure 10. mTeSR™ Plus Maintains Optimal pH Levels Throughout a Weekend-Free Protocol

The pH of spent medium from hPSCs cultured in mTeSR™ Plus is higher than that of hPSCs cultured in mTeSR™1 and another flexible-feeding medium at similar cell densities. pH and cell numbers were measured after a 72-hour period without feeding. The range of cell numbers shown represent different densities that would be observed throughout a typical passage. Cell numbers are from one well of a 6-well plate.

Why Use mTeSR™ Plus?

- Enjoy weekend-free feeding while supporting cell quality, with improved buffering and stabilization of key components
- Achieve superior culture morphology and cell growth characteristics in your aggregate-based cultures
- Complete your workflow with compatible gene editing and differentiation protocols
- Ensure cell safety with a viral-safe medium manufactured under cGMPs

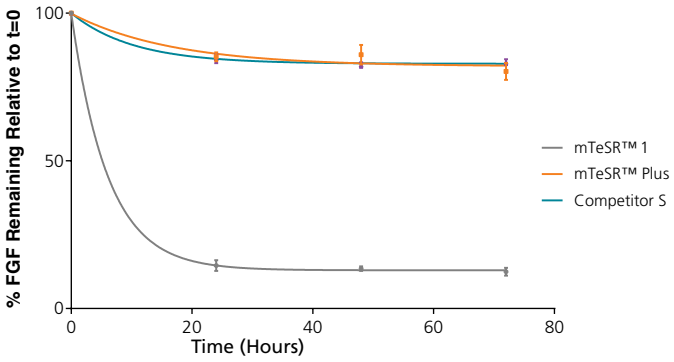


Figure 11. mTeSR™ Plus Maintains Consistent Levels of FGF2 Throughout a Weekend-Free Protocol

FGF2 levels remain high in mTeSR™ Plus when kept at 37°C over a 72-hour time period. Measured by ELISA.

Maintain hPSCs on Your Own Schedule

☑ **Skip 2 days = Double feed**

☑ **Skip 1 day = Regular feed**

The possibilities are endless. Use your regular schedule, or try something new to free up your days.

PASSAGING FREQUENCY	MON	TUE	WED	THU	FRI	SAT	SUN	
7d	P	F	F	F	F	F	F	repeat
7d	P	F	F	F	2F	x	x	repeat
6d	P	x	2F	x	x	F	repeat	
5d	P	F	2F	x	x	repeat		
3d/4d	P	F	x	P	2F	x	x	repeat
Fill Out Your Own								

P = Passage; F = Single Feed; 2F = Double Feed

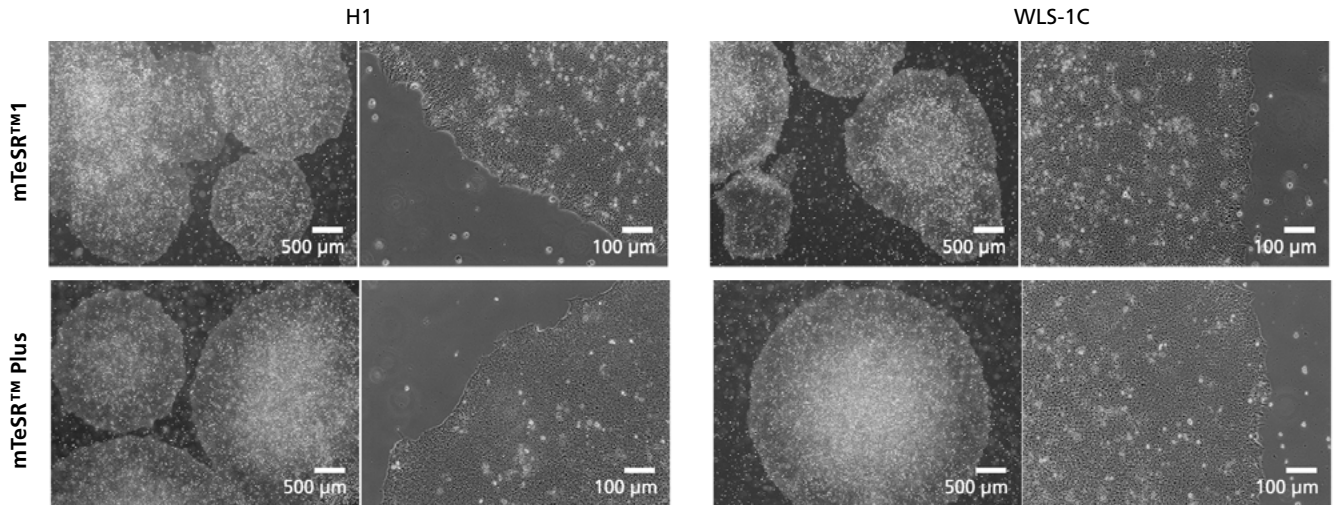


Figure 12. Normal Human ES and iPS Cell Morphology Is Observed in mTeSR™ Plus Cultures

Images depict undifferentiated human ES (H1) and iPS (WLS-1C) cells cultured on Corning® Matrigel® matrix in mTeSR™1 with daily feeds or in mTeSR™ Plus with restricted feeds. Cells retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type after 10 passages. Densely packed cells and multi-layering are prominent when cells are ready to be passaged.

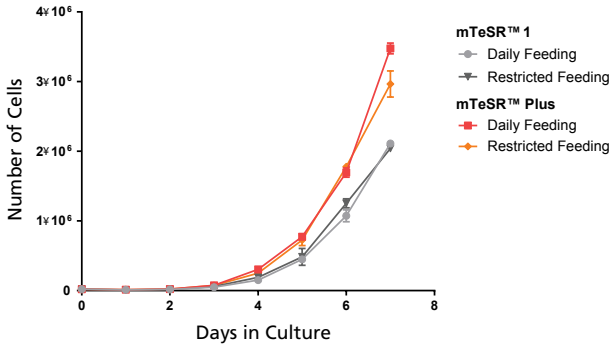


Figure 13. mTeSR™ Plus Supports Higher Cell Numbers, Even with Restricted Feeding

Growth curves were obtained for human ES (H9) cells cultured in mTeSR™1 or mTeSR™ Plus on Corning® Matrigel® matrix over 7 days with either daily feeds or restricted feeds. Growth curves were determined by seeding 20,000 cells per well of a 6-well plate as aggregates and counting the cell numbers each day in duplicate wells.

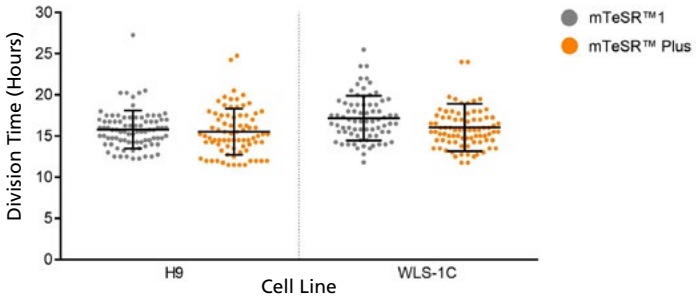


Figure 14. hPSCs Maintained in mTeSR™ Plus Demonstrate Equivalent Cell Division Times to Cells Maintained in mTeSR™1

Human ES and iPS cells (H9 and WLS-1C) cultured in mTeSR™1 or mTeSR™ Plus were dissociated to single cells and seeded at 20,000 cells/cm^2 on Matrigel®-coated plates. The cells were imaged every 20 minutes on an IncuCyte ZOOM® for three days with a medium exchange the day after seeding. Individual cell division times were determined using single cell tracking. Data points include first, second, and third cell divisions.

Learn more at www.stemcell.com/mTeSRPlus

Minimize Risk in Your Cell Therapy Development

Our Commitment to Quality and Good Manufacturing Practices

Choosing a cell and tissue culture medium is a critical step in ensuring the quality and performance of your cultures. For cell therapy manufacturers, this choice can have additional impacts on the safety of your cell therapy. Minimize risk by using animal origin-free TeSR™-AOF as part of a high-compliance maintenance workflow that also includes dissociation reagents (Gentle Cell Dissociation Reagent, ReLeSR™), matrices (CellAdhere™ Laminin-521), and cryopreservation reagents (CryoStor® CS10). For more information on how we can support your regulatory needs, including navigating requirements for using TeSR™-AOF in your cell therapy applications, visit **www.stemcell.com/regulatory-support** or contact your STEMCELL representative.

TeSR™-AOF

Maintain hPSCs for Cell Therapy Development Using cGMP-Grade, Animal Origin-Free, Stabilized Media

Reduce risk and obtain greater numbers of higher quality cells for your human pluripotent stem cell (hPSC)-derived cell therapy development with TeSR™-AOF (Catalog #100-0401), manufactured under relevant cGMPs.

With no raw materials of animal or human origin to the secondary level of manufacturing, enjoy more straightforward traceability and enhanced viral safety compared to media that are only animal origin-free to the primary level of manufacturing.

Use TeSR™-AOF to consistently culture viral-safe, high-quality hPSCs on a schedule that works for you—with whatever cell lines you choose.

To enhance cell quality attributes, particularly during restricted feeds, critical medium components have been stabilized, including FGF2 (also known as basic FGF; bFGF). As a result, TeSR™-AOF allows for both daily and restricted feeding schedules while maintaining cell quality and performance.

TeSR™-AOF is compatible with a variety of culture matrices, including Corning® Matrigel® hESC-Qualified Matrix, Vitronectin XF™ (Catalog #07180), and CellAdhere™ Laminin-521 (Catalog #77003).

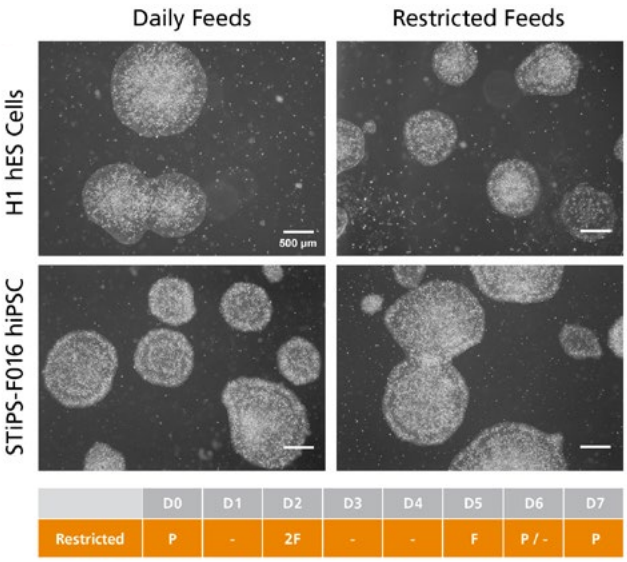


Figure 16. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Exhibit Comparable Colony Morphology

hPSCs were maintained on Vitronectin XF™ for five passages. Phase-contrast images were taken on Day 7 after seeding. For restricted feeds, hPSCs were fed with a double volume (4 mL) of medium on Day 2 after passage, followed by two consecutive skipped days of feeds, with a final single-volume feed (2 mL) on Day 5, prior to passaging on Day 6 or 7. hPSCs maintained in TeSR™-AOF exhibit hPSC-like morphology, forming densely packed, round colonies with smooth edge morphology.

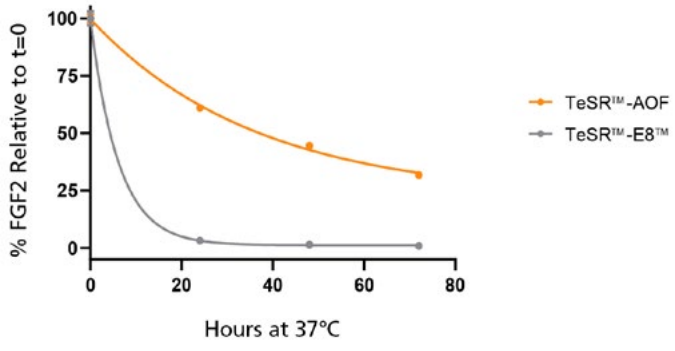


Figure 15. Native bFGF Levels Are Stabilized at 37°C in TeSR™-AOF

TeSR™-AOF and TeSR™-E8™ were incubated at 37°C for 24, 48, and 72 hours. FGF2 levels were measured by Meso Scale Discovery (MSD) immunoassay; data were normalized to t = 0 levels for TeSR™-E8™ and TeSR™-AOF, respectively. FGF2 levels in TeSR™-AOF remain at 36.7 ± 5.61% of t = 0 levels at 72 hours when incubated at 37°C. Data representative of n = 3 biological replicates ± SD.

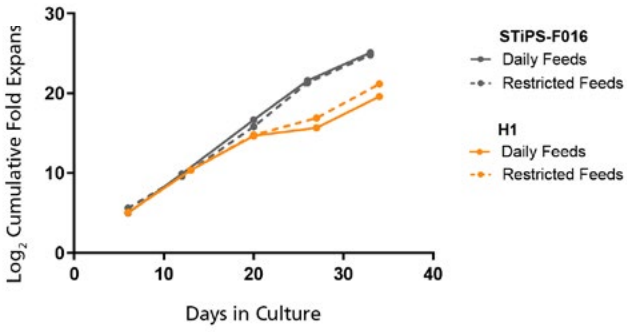
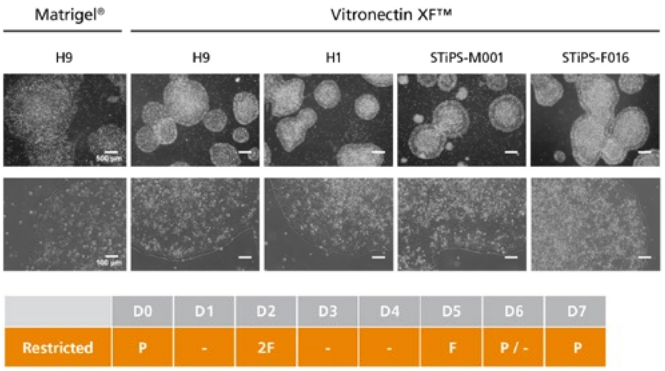


Figure 17. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Have Comparable Expansion Rates

hPSCs were maintained on Vitronectin XF™ for five passages. At the end of each passage, cell counts were obtained using the Nucleocounter® NC-200™ ChemoMetec automated cell counter to count DAPI-stained nuclei. The $\log_2$ transformed cumulative fold expansion was plotted against time in culture (days).



	D0	D1	D2	D3	D4	D5	D6	D7
Restricted	P	-	2F	-	-	F	P / -	P

Figure 18. hPSCs Cultured in TeSR™-AOF with Restricted Feeding Demonstrate Classic hPSC Colony Morphology

hPSCs maintained in TeSR™-AOF were passaged as aggregates with ReLeSR™ passaging reagent every 6 - 7 days for more than 10 passages. hPSCs maintained in TeSR™-AOF exhibit hPSC-like morphology, forming densely packed, round colonies with smooth edge morphology. Homogeneous cell morphology characteristic of hPSCs are observed, including large nucleoli and scant cytoplasm.

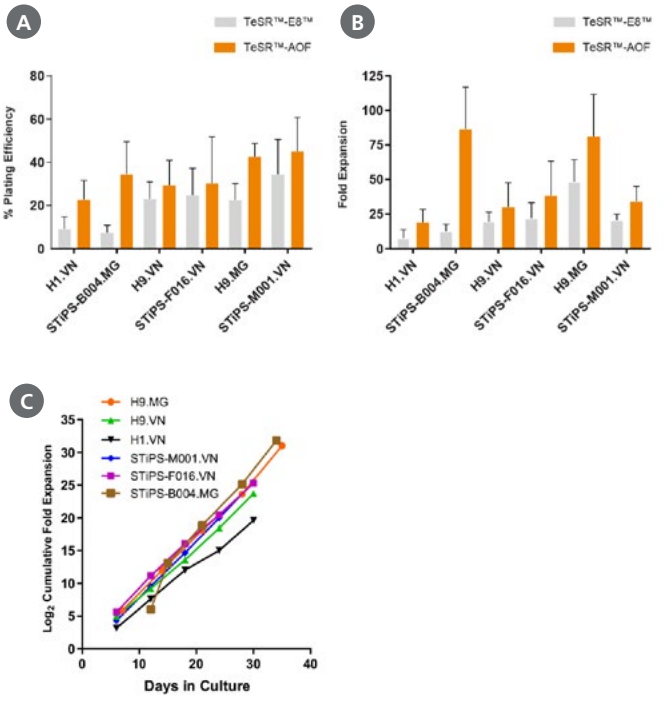


Figure 19. hPSCs Maintained in TeSR™-AOF Have Improved Attachment and Higher Overall Expansion Compared to Low-Protein Medium

(A) hPSCs cultured in TeSR™-AOF demonstrate a higher plating efficiency compared to hPSCs maintained in low-protein medium (TeSR™-E8™). Plating efficiency is calculated by seeding a known number of aggregates and comparing to the number of established colonies on Day 7. (B) hPSCs maintained in TeSR™-AOF exhibit a higher average fold expansion per passage compared to TeSR™-E8™. (C) hPSCs cultured in TeSR™-AOF demonstrate consistent expansion and minimal cell-line-to-cell-line variability between ES and iPS cell lines assessed. Cumulative fold expansion was measured from passage 1 to 5. Data represented as mean plating efficiency or fold expansion across 10 passages ± SD. MG = Matrigel®; VN = Vitronectin XF™.

Why Use TeSR™-AOF?

- Reduce risk in your choice of ancillary materials by selecting a medium with no animal raw materials to the secondary level of manufacturing
- Consistently culture hPSCs with high-quality colony morphology, robust attachment, and high cell expansion
- Enjoy the flexibility to use alternate feeding schedules without sacrificing cell quality, with stabilization of key components, including FGF2

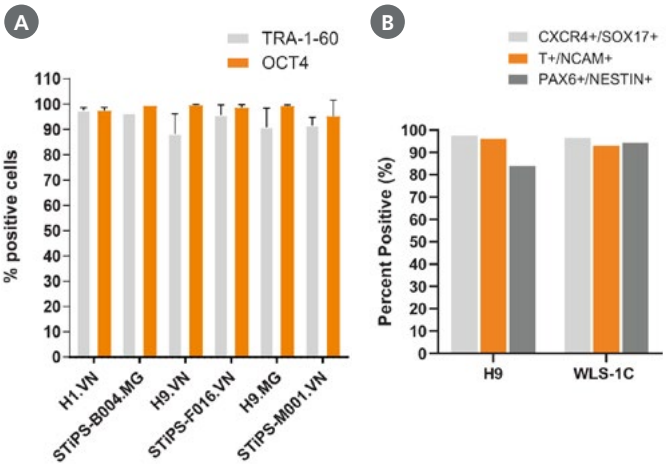


Figure 20. hPSCs Cultured in TeSR™-AOF Express Markers of the Undifferentiated State and Differentiate to the Three Germ Layers

(A) hPSCs maintained in TeSR™-AOF exhibit high levels of TRA-1-60 and OCT4 by flow cytometry at passage 5 and 10. Across n = 6 cell lines, the average TRA-1-60 expression was 93.4 ± 3.37%, and percentage of OCT4-positive cells was 98.3 ± 1.55%. Data shown represent cells at an average of passage 5, and 10 flow cytometry results for each cell line. MG = Matrigel®; VN = Vitronectin XF™. (B) Efficient differentiation to the three germ layers was demonstrated in one hES and one hiPS cell line maintained for > 5 passages in TeSR™-AOF. Cultures were processed for flow cytometry and assessed for $CXCR4^+/SOX17^+$ cells on Day 5 following differentiation using the STEMdiff™ Definitive Endoderm Kit. Cultures were processed for flow cytometry and assessed for Brachyury $(T)^+/OCT4^-$ cells on Day 5 following differentiation in STEMdiff™ Mesoderm Induction Medium. Cultures were processed for flow cytometry and assessed for $PAX6^+/Nestin^+$ cells on Day 7 following monolayer differentiation using STEMdiff™ Neural Induction Medium.

Learn more at www.stemcell.com/TeSR-AOF

mTeSR™1

Maintain hPSCs in cGMP-Grade, Feeder-Free hPSC Medium

mTeSR™1 (Catalog #85850) is a serum-free and complete cell culture medium that has been used to successfully maintain thousands of hPSC lines, with established protocols for applications ranging from gene editing and bioreactor expansion to lineage-specific differentiation. mTeSR™1 is designed for use in cell therapy research applications, manufactured following the recommendations of USP <1043> on ancillary materials, and available for use under an approved US FDA Investigational New Drug (IND) application.

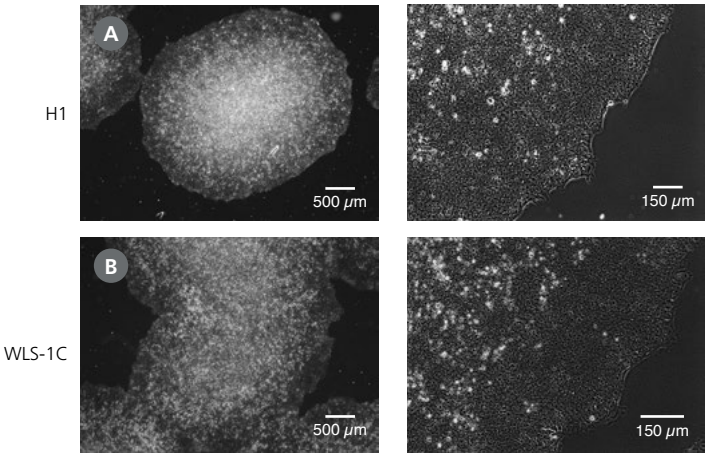


Figure 21. Normal Human ES and iPS Cell Morphology Is Observed in mTeSR™1 Cultures

Undifferentiated (A) H1 human ES and (B) WLS-1C human iPS cells cultured on Corning® Matrigel® matrix in mTeSR™1 retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of this cell type after 10 passages. Densely packed cells and multilayering are prominent when cells are ready to be passaged.

Learn more at www.stemcell.com/mTeSR1

TeSR™-E8™

Maintain hPSCs in Animal Component-Free, Feeder-Free hPSC Medium

TeSR™-E8™ (Catalog #05990) is based on the E8 formulation[2,6] developed by the laboratory of Dr. James Thomson, the lead research group behind the design of mTeSR™1. TeSR™-E8™ contains only the most critical components required for maintenance of hPSCs, providing a simpler medium for hPSC culture. This medium can be used with Vitronectin XF™ (Catalog #07180) for a completely xeno-free system.

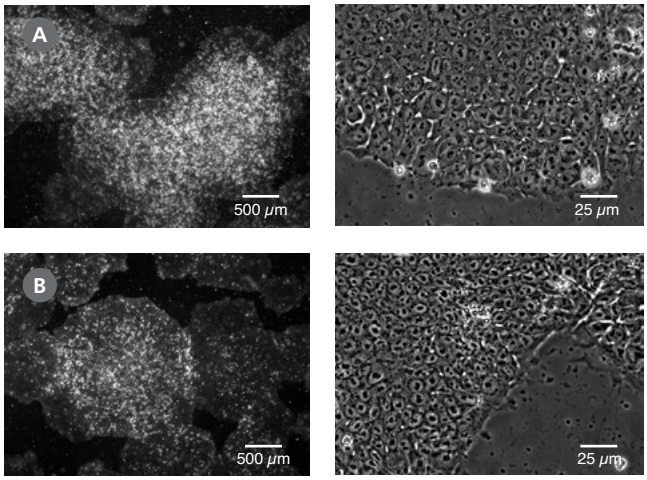


Figure 22. Normal Human ES and iPS Cell Morphology Is Observed in TeSR™-E8™ Cultures

Undifferentiated (A) human ES (H9) and (B) human iPS (WLS-1C) cells cultured on Corning® Matrigel® in TeSR™-E8™ retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio, characteristic of this cell type. Densely packed cells and multilayering are prominent when cells are ready to be passaged.

Learn more at www.stemcell.com/TeSR-E8

Scale Up

Expand Large Numbers of hPSCs in 3D Suspension Culture

Suspension culture of hPSCs as 3D aggregates provides a convenient method to produce large numbers of high-quality, undifferentiated hPSCs with reduced labor and costs. hPSCs expanded in the TeSR™ family suspension culture systems have robust growth, maintain high expression of pluripotent stem cell markers, and retain trilineage differentiation ability.

Why Use Suspension Culture?

- Simplify your culture system with serum-free media that do not require microcarriers or external matrices
- Rapidly generate billions of hPSCs in as few as 2 - 3 weeks
- Save time and money with a fed-batch strategy that does not require full medium changes

Why Use TeSR™-AOF 3D?

- Minimize risk associated with your ancillary materials by selecting a medium with no animal raw materials to the secondary level of manufacturing
- Reduce time and labor with a fed-batch feeding strategy that does not require full-medium changes
- Scale up high-quality hPSCs rapidly without requiring adaptation from 2D culture

TeSR™-AOF 3D

Use TeSR™-AOF 3D (Catalog #100-0720) to safely generate large numbers of high-quality hPSCs for your cell banking and cell therapy manufacturing applications. TeSR™-AOF 3D has been designed to support rapid scale-up without requiring adaptation from 2D culture, while also reducing time and labor with a fed-batch feeding strategy that does not require full medium changes. And, as you might be thinking about the eventual therapeutic applications of your work, TeSR™-AOF 3D contains no materials of animal or human origin to at least the secondary level of manufacturing, eliminating the need for viral safety testing.

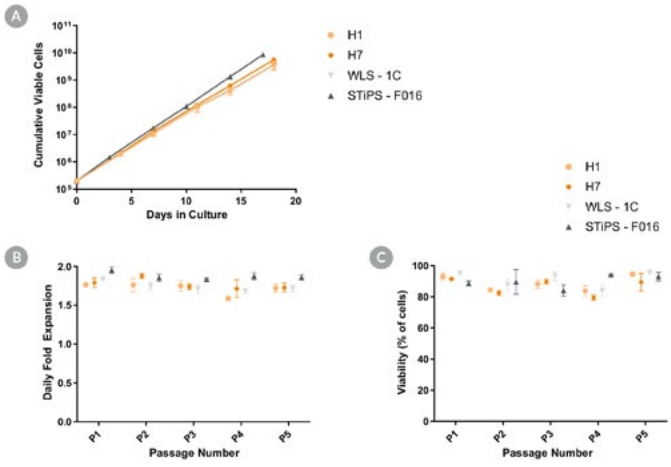


Figure 23. Growth of Human Pluripotent Stem Cells (hPSCs) in TeSR™-AOF 3D

TeSR™-AOF 3D supports the expansion and high viability of hPSCs over multiple passages in aggregate suspension culture. Shown are (A) cumulative viable cells, (B) daily fold expansion, and (C) end-of-passage viabilities in human ES cell lines (H1 and H7) and human iPS cell lines (WLS-1C and STiPS-F016) over 5 passages. Error bars represent ± SD, n = 3.

Learn more at www.stemcell.com/TeSR-AOF-3D

PBS-MINI Bioreactor

Rapidly Scale Up Your 3D hPSC Culture

Reliably and rapidly scale up your 3D cell cultures and suspensions with the PBS-MINI Bioreactor (Catalog #100-1005). The gentle yet efficient mixing provided by the Vertical-Wheel™ impeller enables the expansion of shear-sensitive cells without anti-foaming agents or shear protectants. Ideal for hPSCs cultured in the TeSR™ 3D family of media, the compact, sealed base unit and the 0.1 (Catalog #100-1006) and 0.5 (Catalog #100-1007) MAG Single-Use Vessels can be used inside incubators. Conveniently control your culture system with a speed dial and digital display, and visualize cells in low-light conditions using built-in LED lights.

Learn more at www.stemcell.com/PBS-MINI

mTeSR™3D

Based on mTeSR™1, mTeSR™3D (Catalog #03950) is optimized for the expansion and scale-up of hPSCs. It is optimized as a fed-batch culture system, in which required nutrients are added daily, eliminating the need for daily medium exchanges.

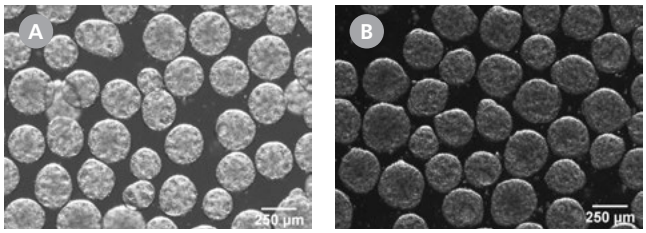


Figure 24. Morphology of hPSC Aggregates Cultured in mTeSR™3D

Characteristic morphology of suspension-cultured hPSC aggregates includes: approximately spherical shape, edges that are clear but not perfectly smooth, and a mottled or pock-marked appearance. Aggregates should be approximately 350 - 400 µm by the end of the passage. Shown are (A) human ES cell line H7 and (B) human iPS cell line STiPS-F016 cultured in mTeSR™3D.

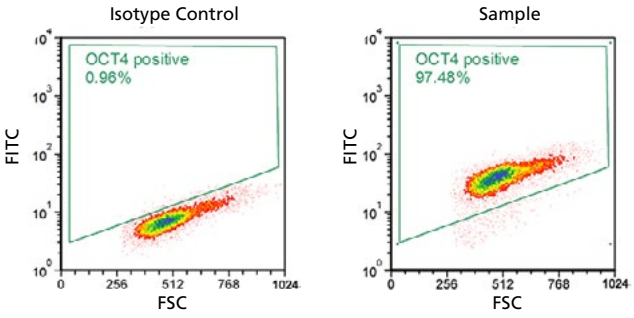


Figure 25. OCT4 Expression of hPSCs Cultured in mTeSR™3D

hPSCs expanded in mTeSR™3D maintain expression of pluripotent stem cell markers. Shown are representative plots of OCT4 expression after 7 passages in mTeSR™3D.

Learn more at www.stemcell.com/mTeSR3D

TeSR™-E8-3D

TeSR™-E8-™3D (Catalog #3990) is a low protein, animal component-free medium based on TeSR™-E8™. The system contains only the most critical components for hPSCs, providing a simpler culture medium for robust, large-scale hPSC expansion. It uses a fed-batch feeding strategy that replenishes nutrients daily while reducing labor and costs.

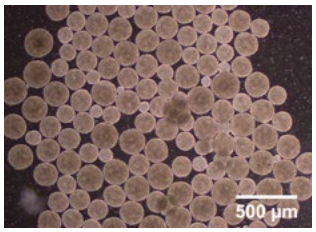


Figure 26. Morphology of hPSC Aggregates Cultured in TeSR™-E8™3D

Characteristic morphology of suspension-cultured hPSC aggregates includes: approximately spherical shape, edges that are clear but not perfectly smooth, and a mottled or pock-marked appearance. Aggregates should be approximately 350 - 400 µm by the end of the passage. Shown are human ES cell line H1 cultured in TeSR™-E8™3D.

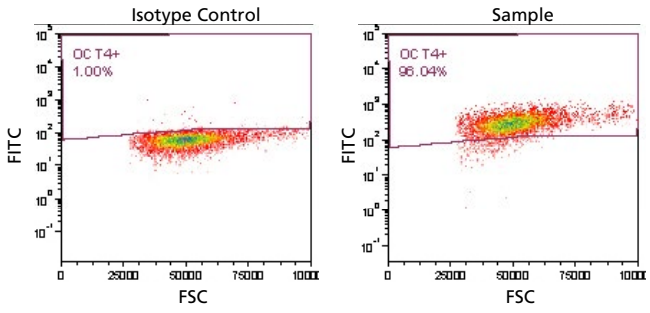


Figure 27. OCT4 Expression of hPSCs Cultured in TeSR™-E8™3D

hPSCs expanded in TeSR™-E8™3D maintain expression of pluripotent stem cell markers. Shown are representative plots of OCT4 expression after 10 passages in TeSR™-E8™3D.

Learn more at www.stemcell.com/TeSR-E8-3D

Naïve Induction and Maintenance

RSeT™ Feeder-Free Medium

Maintain Feeder-Free, Naïve-Like hPSCs in Defined Medium

RSeT™ Feeder-Free Medium (Catalog #05975) is a serum-free medium that reverts primed hPSCs and maintains cells in a naïve-like state without the need for basic fibroblast growth factor (bFGF) or feeder cells. RSeT™ Feeder-Free Medium produces robust cultures with a naïve-like morphology and increased expression of key naïve-associated transcripts. This improved formulation enables efficient reversion to a naïve-like state as early as passage 1, without the variability and burden associated with using feeder cells.

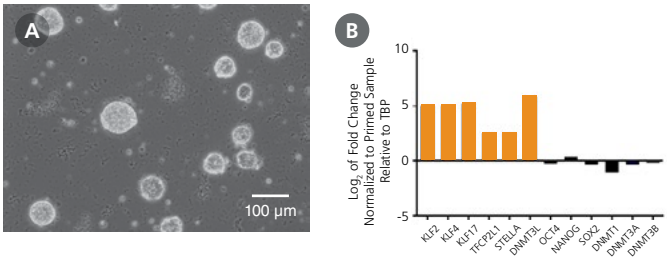


Figure 28. hPSCs Maintained in RSeT™ Feeder-Free Medium Are Reverted to a Naïve-Like State and Express High Levels of Naïve-Associated Genes

(A) A representative image of hPSCs that reverted to a naïve-like state after being cultured in RSeT™ Feeder-Free Medium for 1 passage. During reversion, colonies change from a flat morphology to a domed morphology characteristic of naïve-state hPSCs. (B) Expression of naïve-associated genes (KLF2, KLF4, KLF17, TFCP2L1, STELLA, and DNMT3L) in hPSCs that were reverted to a naïve-like state in RSeT™ Feeder-Free Medium. Expression levels were measured by qPCR and normalized to levels in primed hPSCs.

Why Use RSeT™ Feeder-Free Medium?

- Reproducibly maintain naïve-like hPSCs with a serum-free, bFGF-free formulation that contains pre-screened quality components
- Reduce cost and variability with easy-to-use, feeder-independent culture system
- Efficiently revert cells to a naïve-like state with stable domed morphology, naïve gene expression profiles, and low levels of spontaneous differentiation, without the need for exogenous genes

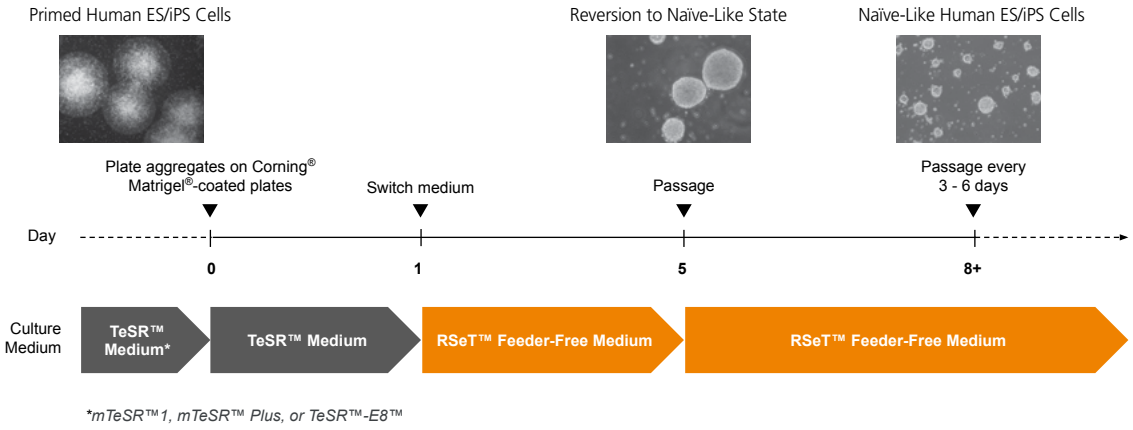


Figure 29. Schematic of Reversion of Primed to Naïve-Like hPSCs with RSeT™ Feeder-Free

Primed hPSCs are plated as aggregates in a TeSR™ medium (mTeSR™ Plus, mTeSR™1, or TeSR™-E8™). On Day 1, TeSR™ medium is replaced with RSeT™ Feeder-Free Medium, and the medium is exchanged every other day. By Day 4 or 5, the colonies are generally large enough to be passaged. During the initial culture in RSeT™ Feeder-Free Medium, colonies expand and begin to adopt a tightly packed, highly domed morphology characteristic of naïve-like stem cells with smooth and refractive colony edges as early as passage 1. Developed under license from the Weizman Institute of Science.[7]

Learn more at www.stemcell.com/RSeT-FeederFree

NaïveCult™

Achieve Serum- and Transgene-Free Induction and Expansion of Reset Naïve hPSCs

NaïveCult™ (Catalog #05580) is a serum-free media system that generates transgene-free, reset naïve hPSCs from primed hPSCs and allows for their continual maintenance. NaïveCult™ contains pre-screened quality components to work consistently across multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines.

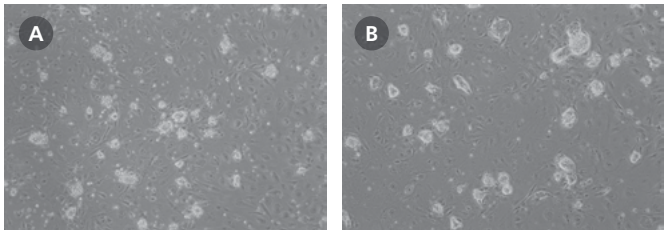


Figure 30. Human ES and iPS Cells Cultured in NaïveCult™ Show Characteristic Morphology of Naïve-State hPSCs

Representative images of human (A) H9 ES cells at passage 7 and (B) WLS-1C iPS cells at passage 9 that were reverted to a naïve state using the NaïveCult™ Induction Kit and subsequently cultured in NaïveCult™ Expansion Medium. During reversion, colonies change from a flat morphology to a tightly packed and uniformly domed morphology with refractive edges characteristic of naïve-state hPSCs.[8-10]

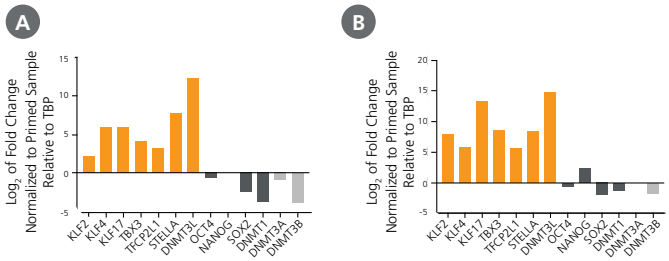
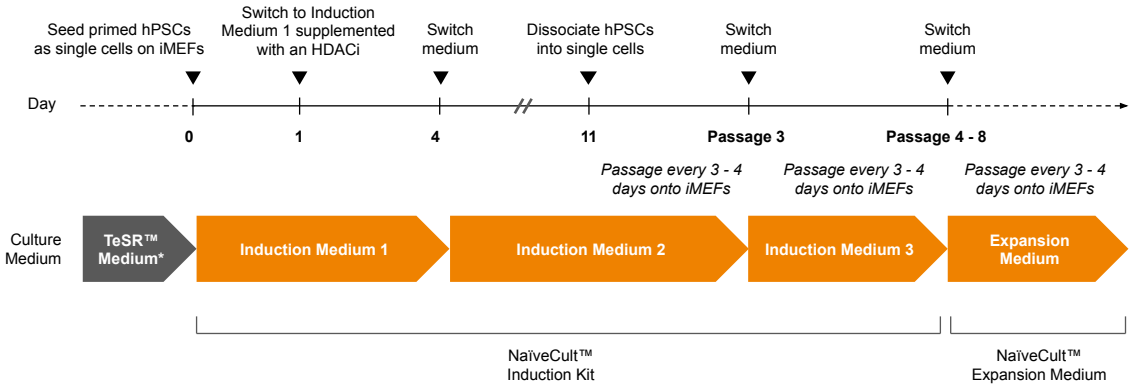


Figure 31. hPSCs Cultured in the NaïveCult™ Media System Express High Levels of Factors Associated with Naïve hPSCs[7-9]

Human (A) H9 ES cells and (B) WLS-1C iPS cells were reverted using the NaïveCult™ Induction Kit and maintained in NaïveCult™ Expansion Medium. Expression levels were measured by quantitative PCR (qPCR) and normalized to levels in primed hPSCs.



Note: From Day 0 onward, culture under hypoxic conditions (5% O_2, 5% CO_2). Perform full medium changes daily.
**mTeSR™1, mTeSR™ Plus, or TeSR™-E8™*

Figure 32. Schematic of Reversion of Primed to Naïve-Like hPSCs in NaïveCult™

Primed hPSCs are plated on irradiated mouse embryonic fibroblasts (iMEFs) and treated with ROCK inhibitor (10 µM Y-27632) for 24 hours in hypoxic conditions. Background differentiation will decrease between passage 3 and 8. At this time, cells can be transferred into NaïveCult™ Expansion Medium for long-term maintenance and expansion. Developed under license from Cambridge Enterprises.[8]

Learn more at www.stemcell.com/NaiveCult

hPSC Naïve State qPCR Array

The hPSC Naïve State qPCR Array (Catalog #07521) provides a validated 90-gene assay to characterize the state of hPSCs in the spectrum from naïve to primed pluripotency. Data analysis is streamlined with our flexible online app (**www.stemcell.com/qPCRanalysis**).

Learn more at www.stemcell.com/naive-array

Matrices

Cell culture matrices support the growth and differentiation of human pluripotent stem cells (hPSCs), including human embryonic stem (ES) and induced pluripotent stem (iPS) cells, by mimicking the in vivo extracellular matrix. When used with TeSR™ maintenance media, they provide a robust culture system for cell maintenance under feeder-free conditions.

Vitronectin XF™

Grow and Differentiate hPSCs Under Serum-Free, Feeder-Free Conditions

Vitronectin XF™ (Catalog #07180), developed and manufactured by Nucleus Biologics, is a defined, xeno-free cell culture matrix that supports the growth and differentiation of hPSCs. Use with TeSR™-E8™ (Catalog #05990) media for a completely xeno-free culture system and complete control over the culture environment, resulting in more consistent cell populations and reproducible results in downstream applications. Human ES and iPS cells cultured on Vitronectin XF™ retain pluripotency and normal colony morphology, without the need for an adaptation step. Pair with Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0485) or ReLeSR™ (Catalog #05872) when passaging to maintain high-quality cultures.

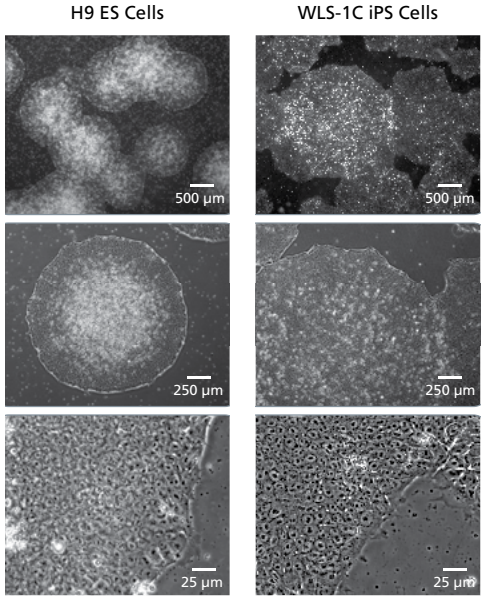


Figure 33. Human ES and iPS Cells Exhibit Normal Morphology When Cultured on Vitronectin XF™ Cell Culture Matrix in TeSR™-E8™

Undifferentiated human ES (H9) and iPS (WLS-1C) cell cultures exhibit normal morphology when cultured on Vitronectin XF™. Colonies are round, tightly packed and multilayered, with a high nucleus-to-cytoplasm ratio. Cells were transferred directly from Matrigel® hESC-Qualified Matrix without an adaptation step.

Note: Colonies grown in TeSR™-E8™ have a more condensed and round morphology when grown on Vitronectin XF™ matrix, compared to colonies grown on Matrigel® hESC-Qualified Matrix, which are more diffuse and irregularly shaped.

Learn more at www.stemcell.com/Vitronectin-XF

Why Use Vitronectin XF™ and CellAdhere™ Laminin-521?

- Decrease sources of variability in your experiment with a recombinant human protein matrix
- Use with any TeSR™ family medium to maintain hPSCs
- Create a completely xeno-free system when used with TeSR™2, TeSR™-E8™, or TeSR™-AOF

CellAdhere™ Laminin-521

Maintain hPSCs Long-Term in Feeder-Free Conditions

CellAdhere™ Laminin-521 (Catalog #77003) is a defined and xeno-free cell culture matrix that supports the growth and differentiation of ES and iPS cells under feeder-free conditions. Laminin-521 is expressed and secreted by hPSCs in the inner cell mass of the embryo and can therefore be used to create a biologically relevant hPSC culture environment in vitro.

For consistent cell populations and reproducible results in downstream applications, use CellAdhere™ Laminin-521 with TeSR™ maintenance media to provide a defined culture substrate for cell maintenance. Compared to other matrices, CellAdhere™ Laminin-521 increases single-cell attachment and survival and does not require the addition of apoptotic inhibitors during plating.

NOTE: While it is possible to passage hPSCs as single cells, this practice may lead to genetic aberrations in the culture. If passaging as single cells, check the karyotype frequently.

Learn more at www.stemcell.com/Laminin-521

Dissociation Reagents

ReLeSR™ and Gentle Cell Dissociation Reagent

Passage hPSCs, Enzyme-Free

ReLeSR™ (Catalog #05872) selectively detaches undifferentiated cells from hPSC cultures, eliminating manual selection and scraping. Passaging hPSCs with ReLeSR™ enables the easy generation of optimally sized aggregates while eliminating the hassle and variability of manual selection. By removing the need for scraping, ReLeSR™ more readily enables the use of culture flasks and other closed vessels, thus facilitating culture scale-up and automation.

Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0485) is an enzyme-free reagent suitable for the dissociation of hPSCs into cell aggregates for routine passaging or into a single-cell suspension.

ReLeSR™ and GDCR are both manufactured under relevant cGMPs and can be used as part of a high-compliance hPSC maintenance workflow, such as with TeSR™-AOF (Catalog #100-0401) medium and CellAdhere™ Laminin-521 (Catalog #77003).

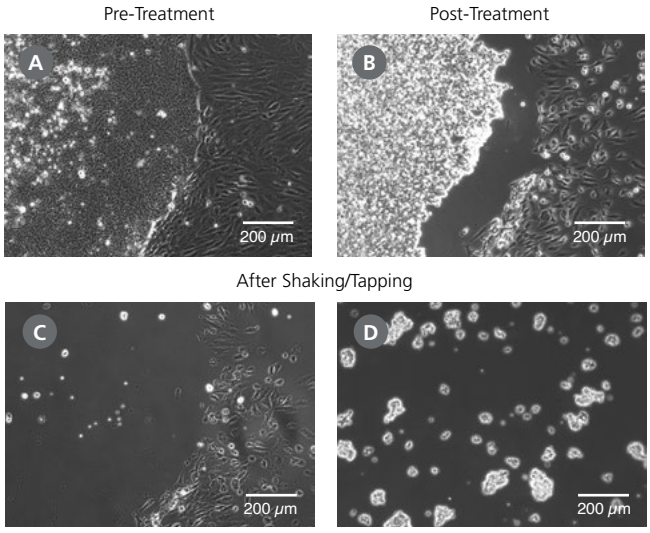


Figure 34. ReLeSR™ Selectively Detaches Undifferentiated Cells from hPSC Cultures Without Manual Selection and Generates Optimally Sized Aggregates

(A) An hPSC culture ready for passaging. Note the presence of differentiated cells at the edge of the undifferentiated hPSC colony. (B) Following incubation with ReLeSR™, the undifferentiated hPSC colony starts to lift off of the cultureware. The differentiated cells remain attached to the cultureware. (C) Following shaking/tapping of the cultureware, the undifferentiated cells completely lift off of the cultureware. (D) The undifferentiated hPSC colony is broken up into optimally sized aggregates for replating.

Learn more at www.stemcell.com/ReLeSR

Learn more at www.stemcell.com/GCDR

ACCUTASE™

For Creation of Single-Cell Suspensions

ACCUTASE™ (Catalog #07920) is a cell detachment solution of proteolytic and collagenolytic enzymes, and is useful for the routine detachment of cells from standard tissue culture plasticware and adhesion-coated plasticware. ACCUTASE™ does not contain mammalian or bacterial-derived products.

Dispase

For Enzymatic Dissociation

Dispase (Catalog #07446) is a commonly used enzyme preparation recommended for passaging hPSCs maintained in feeder-free conditions on Corning® Matrigel®.

Genome Editing

CloneR™2

Enhance the Cloning Efficiency and Single-Cell Survival of hPSCs

Generate clonal human pluripotent stem cell (hPSC) lines that maintain their genomic integrity and downstream differentiation potential with this defined, serum-free supplement. By using CloneR™2 (Catalog #100-0691), you can increase the cloning efficiency and survival of human embryonic stem (ES) and induced pluripotent stem (iPS) cells under high-stress conditions, including seeding at low or high densities, post-thaw recovery, and when creating monolayers ahead of downstream differentiation. For your gene-editing workflows, add CloneR™2 to improve ES and iPS cell survival following electroporation and during clonal deposition (see data below).

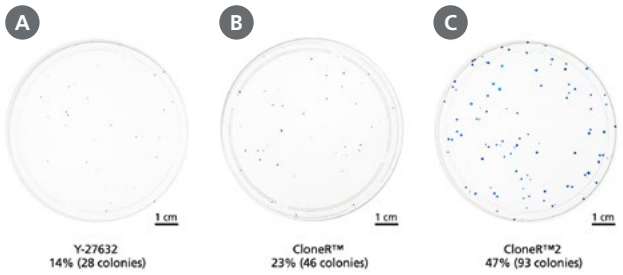


Figure 35. CloneR™ and CloneR™2 Supplements Improve Cloning Efficiency and Colony Size

hPSCs display a considerable increase in cloning efficiency when cloned using (B) CloneR™ compared to using (A) Y-27632 compound. (C) CloneR™2 further improves cloning efficiency and increases colony size when compared to either Y-27632 compound or CloneR™. Shown are examples of H9 hESCs in 10-cm dishes, plated at 200 cells per dish (~4 cells/cm²) in mTeSR™ Plus on Vitronectin XF™.

Why Use CloneR™2?

- Generate more colonies for selection days sooner
- Consistently generate clones with similar high performance across culture systems and cell lines
- Plate your cells more efficiently at all densities and after high-stress events such as electroporation or thawing
- Save time by going straight to single cells, with no adaptation phase required

Learn more at www.stemcell.com/CloneR2

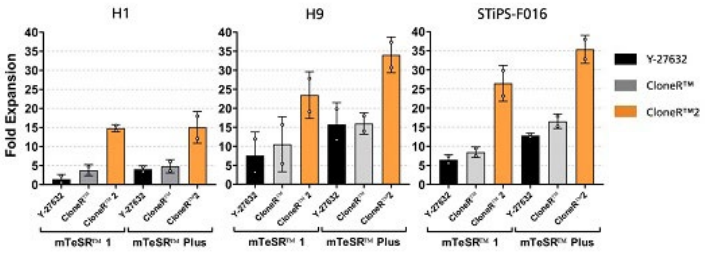


Figure 36. CloneR™2 Improves Recovery After Electroporation

Three hPSC lines were electroporated, then plated in mTeSR™1 and mTeSR™ Plus containing Y-27632, CloneR™, or CloneR™2. Cultures were maintained in complete TeSR™ media (without cloning supplement) after 24 hours and analyzed after 5 days. In all 3 cell lines, CloneR™2 dramatically improved cell survival and expansion when used as a supplement in the first 24 hours immediately following electroporation compared to both Y-27632 and CloneR™ (n = 2 replicates per cell line).

Also Consider: CloneR™

CloneR™ (Catalog #05888) is the original serum-free supplement formulated for enhancing the cloning efficiency and single-cell survival of hPSCs, especially under clonal and low-density seeding conditions.

ArciTect™

Genome Edit hPSCs with a CRISPR-Cas9 System

The ArciTect™ product family is a ribonucleoprotein (RNP)-based CRISPR-Cas9 genome editing system for hPSCs. Whether you are seeking purified Cas9 proteins, customizing guide RNA targeting, estimating editing efficiency, or optimizing transfection protocols, the ArciTect™ toolkit contains qualified solutions for every step in the hPSC genome editing workflow. Refer to the Technical Bulletin: Genome Editing of Human Pluripotent Stem Cells (Document #27084) for a protocol optimized and validated for use with ArciTect™.

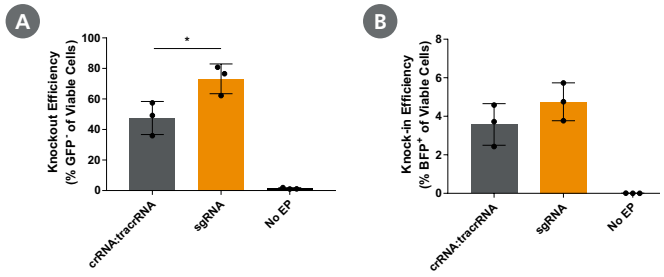


Figure 37. Efficient Genetic Knockout and Knock-In in Human Pluripotent Stem Cells Using the ArciTect™ CRISPR-Cas9 System

1C-eGFP hPSC lines were cultured in mTeSR™1 supplemented with CloneR™ for 24 hours after electroporation with CRISPR-Cas9 RNP complexes containing ArciTect™ Cas9 Nuclease and either ArciTect™ crRNA:tracrRNA duplexes or sgRNA targeting GFP, co-delivered with ssODN encoding nucleotides to convert GFP to BFP. (A) Knockout (% GFP- cells) and (B) knock-in (% BFP+ cells) efficiency were measured by flow cytometry 3 days after electroporation; n = 3. Control samples were not electroporated (no EP). Error bars represent standard deviation.

Learn more at www.ArciTect.com

Cell Quality Characterization

hPSC Genetic Analysis Kit

Detect Most Karyotypic Abnormalities in Human ES and iPS Cells Using qPCR

The hPSC Genetic Analysis Kit (Catalog #07550) contains primer/probe mixes to detect the majority of karyotypic abnormalities reported in human embryonic stem (ES) and induced pluripotent stem (iPS) cells. This qPCR-based kit enables the genetic screening of multiple human ES and iPS cell lines in a rapid and cost-effective manner, and includes enough material to analyze 20 individual samples in triplicate. Our online hPSC Genetic Analysis Tool (**www.stemcell.com/geneticanalysisapp**) is designed to help with data analysis and interpretation: simply input qPCR data and the tool will perform statistical analyses, assist with data interpretation, and provide visual representation of the data.

Why Use the hPSC Genetic Analysis Kit?

- Detect the most common karyotypic abnormalities observed in hPSC cultures
- Generate results within one day
- Enable more frequent screening with this cost-effective kit
- Interpret results easily with the online hPSC Genetic Analysis Tool

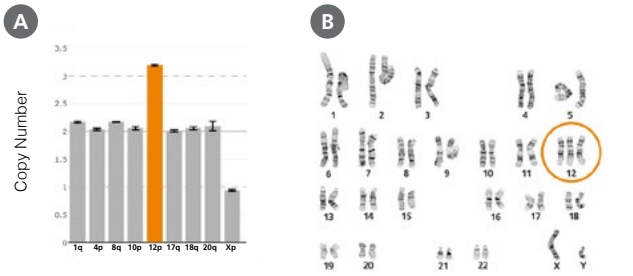


Figure 38. The hPSC Genetic Analysis Kit Identifies Chromosome 12 Trisomy

Chromosome 12 trisomy in the WLS-1C human iPS cell line is (A) detected using the hPSC Genetic Analysis Kit and (B) confirmed by G-banding.

Figure 39. The hPSC Genetic Analysis Kit Identifies Chromosome 20q11.21 Duplication

Chromosome 20q duplication in the WLS-1C human iPS cell line is (A) detected using the hPSC Genetic Analysis Kit, (B) undetected by G-banding, and (C) confirmed by fluorescent in situ hybridization using probes for 20p11 (green) and 20q11.21 (red).

Learn more at
www.stemcell.com/GeneticAnalysisKit

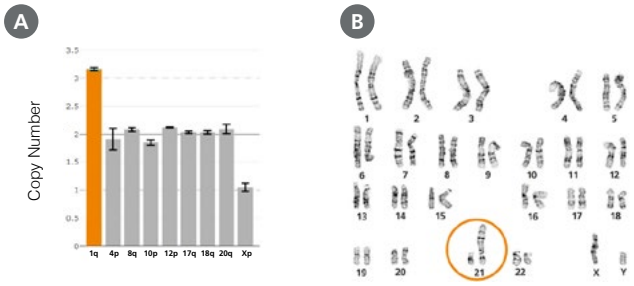


Figure 40. The hPSC Genetic Analysis Kit Identifies Chromosome 1 Duplication via Unbalanced Translocation

Unbalanced rearrangement of chromosome 1 in the WLS-1C human iPS cell line, in which an extra copy of the long (q) arm of chromosome 1 translocated to the short arm (p) of chromosome 21, was (A) detected using the hPSC Genetic Analysis Kit and (B) confirmed by G-banding.

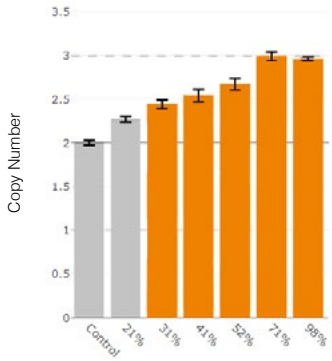


Figure 41. The hPSC Genetic Analysis Kit Identifies Abnormalities in Cultures with Approximately 30% Mosaicism

Genetically normal WLS-1C human iPS cells were mixed in the indicated ratios with WLS-1C human iPS cells containing a chromosome 20q duplication. Cultures with approximately 30% genetically abnormal cells exhibit a significantly enriched population of 20q11.21 duplication (orange bars).

STEMdiff™ Trilineage Differentiation Kit

Validate Pluripotency with Directed Differentiation

The STEMdiff™ Trilineage Differentiation Kit (Catalog #05230) provides a simple cell culture assay to functionally and reproducibly validate the ability of human ES and iPS cells to differentiate to the three germ layers. This kit includes reagents and protocols to perform parallel in vitro directed differentiation experiments for each germ layer, clearly establishing trilineage differentiation potential within one week. Clear, quantitative assay results evaluated by immunocytochemistry, flow cytometry, or transcriptome analysis make the STEMdiff™ Trilineage Differentiation Kit a valuable tool for establishing the pluripotency of human ES and iPS cell lines.

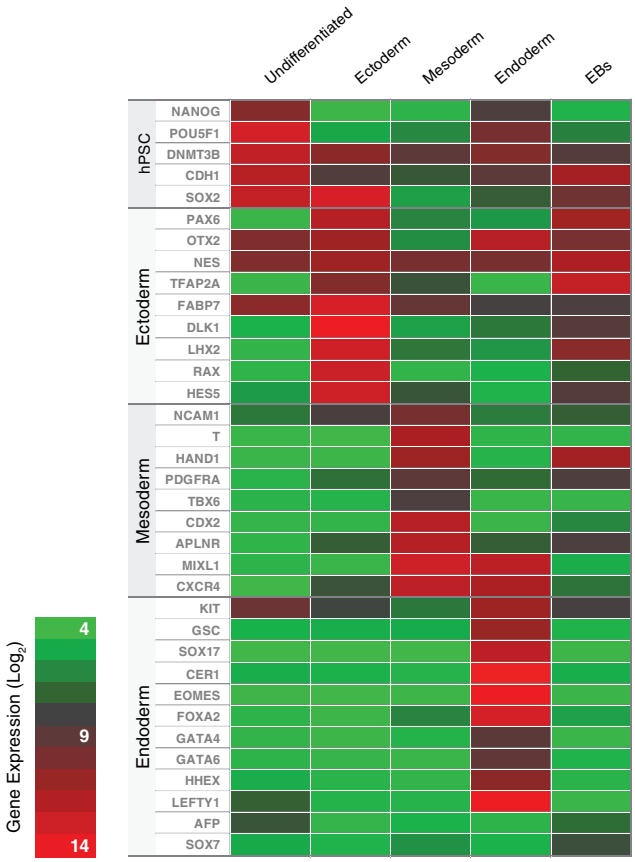


Figure 42. Molecular Analysis of Cultures Differentiated with the STEMdiff™ Trilineage Differentiation Kit Shows Strong Separation of Lineage-Specific Markers

H9 cells were maintained in mTeSR™1 and subsequently differentitated in vitro using either directed differentiation with the STEMdiff™ Trilineage Differentiation Kit or spontaneous differentiation in embryoid bodies (EBs) using a 10-day protocol in serum-containing medium. Undifferentiated cells, differentiated ectoderm, mesoderm, and endoderm cells from the directed differentiation kit and EBs were then subjected to a microarray-based transcriptome analysis to evaluate expression levels of key germ layer markers. Cells differentiated using the STEMdiff™ Trilineage Differentiation Kit showed clear upregulation of appropriate germ layer-specific markers, whereas the same cells differentiated spontaneously in EBs did not show significant upregulation of mesoderm or endoderm markers.

Learn more at www.stemcell.com/TrilineageKit

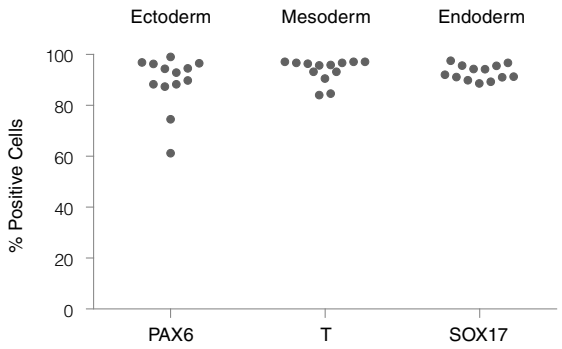


Figure 43. The STEMdiff™ Trilineage Differentiation Kit Promotes Efficient Differentiation to All Three Germ Layers

Pluripotent stem cells (both iPS and ES cells represented) were maintained in mTeSR™1, differentiated using the STEMdiff™ Trilineage Differentiation Kit, and subjected to flow cytometry analysis (n = 13 biological replicates, including 5 distinct cell lines). The markers used for flow cytometry for each germ layer are listed below the x-axis.

hPSC Trilineage Differentiation qPCR Array

The hPSC Trilineage Differentiation qPCR Array (Catalog #07515) provides a validated 90-gene assay to assess gene expression associated with undifferentiated hPSCs or their derivatives undergoing the early stages of differentiation, plus housekeeping controls and a synthetic DNA positive control. Data analysis is streamlined with our flexible online app (**www.stemcell.com/qPCRanalysis**).

Learn more at www.stemcell.com/trilineage-array

Cryopreservation

Increase Post-Thaw Recovery and Viability with Serum-Free Cryopreservation Media

mFreSR™ and FreSR™-S

Conventional methods for cryopreservation of human pluripotent stem cells (hPSCs) use fetal bovine serum, introducing an undefined component into the culture system. FreSR™ cryopreservation media are defined, serum-free, and optimized for use with cells cultured with TeSR™ maintenance media. Cells stored in FreSR™ media have higher recovery and maintain higher viability post-thaw than cells frozen via conventional methods using serum.[11-14] mFreSR™ (Catalog #05855) serum-free medium is optimized for cryopreservation of hPSCs as aggregates. FreSR™-S (Catalog #05859) animal component-free media is optimized for cryopreservation of cells in single-cell suspension and provides faster post-thaw recovery of hPSC cultures compared with conventional freezing methods.

Why Use mFreSR™ and FreSR™-S?

- Obtain more cells with high post-thaw viability and recovery
- Enjoy seamless compatibility with hPSCs cultured in TeSR™ media
- Reduce viral contamination risk with animal component-free FreSR™-S, optimized for single-cell suspensions

Learn more at www.stemcell.com/mFreSR

Learn more at www.stemcell.com/FreSR-S

CryoStor® CS10

Storage and cryopreservation of cells and tissues are important parts of the workflow for biological research. CryoStor® CS10 (Catalog #07930) is animal component-free, cGMP-manufactured with USP grade components, and designed to maintain high viability and maximize hPSC cell recovery after long-term storage. CryoStor® CS10 contains 10% dimethyl sulfoxide (DMSO) and provides a safe and protective environment for cells and tissues during the freezing, storage, and thawing processes.

Learn more at www.stemcell.com/CryoStor-CS10

ThawSTAR® CFT2 Automated Thawing System

Standardize Your Thawing Performance Through Automation

Increase confidence in your cell thawing workflow by using the ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650) to ensure sample sterility and consistent thawing performance.

ThawSTAR® CFT2—a sensor-based, water-free instrument—reduces the risk of contamination and delivers cell thawing profiles similar to those of a water bath. Using this convenient and compact system, you can thaw your cells directly in the biosafety cabinet, in ~2.5 minutes.

Built for quality control, ThawSTAR® CFT2 easily integrates into cell thawing processes within research and clinical settings requiring a higher level of compliance. You can document instrument performance and create an audit trail by using ThawSTAR® CFT2 confirmation vials before and after each thaw session. Completing the system, the portable ThawSTAR® CFT2 Transporter (Catalog #100-0642) protects your cells from transient warming events during handling and transport of frozen vials from long-term storage to the downstream thawing step.

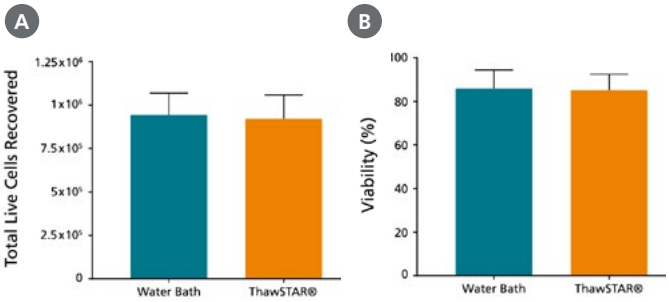


Figure 44. Frozen hPSCs Thawed Using the ThawSTAR® CFT2 Automated Thawing System Show High Recovery and Viability

hPSCs cryopreserved in CryoStor® CS10 at a concentration of 1 x 10^6 cells/vial were retrieved from liquid nitrogen one week after storage. When thawed using the ThawSTAR® CFT2 Automated Thawing System or a water bath, (A) the mean live cell recovery was 9.05 x 10^5 vs. 9.35 x 10^5 cells, respectively and (B) the mean viability was 83.04% vs. 82.93%, respectively. The hPSCs were from 3 different cell lines (M001, 1C, and H9) and were tested in triplicates. Cell recovery and viability was assessed using a Nucleoview™ counter.

Learn more at www.stemcell.com/ThawSTAR-CFT2

Differentiation

STEMdiff™ Pluripotent Stem Cell Differentiation Media

When working with hPSCs, inconsistent differentiation is a common challenge, even with detailed and established differentiation protocols.[15,16] Use STEMdiff™ to reproducibly differentiate from multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines to cell types and organoids originating from all three embryonic germ layers. For gene-edited or patient-derived hPSC lines, these optimized media and protocols enable the generation of a variety of cell types with the same genotype. The STEMdiff™ family of products is part of our complete system of reagents for hPSC culture and is compatible with TeSR™ maintenance media, including mTeSR™1 (Catalog #85850) and mTeSR™ Plus (Catalog #100-0276).

Explore the following pages for tools to support neural, hematopoietic, immune, cardiac, renal, intestinal, pulmonary, pancreatic, and customizable cell and organoid differentiation.

Learn more at www.STEMdiff.com

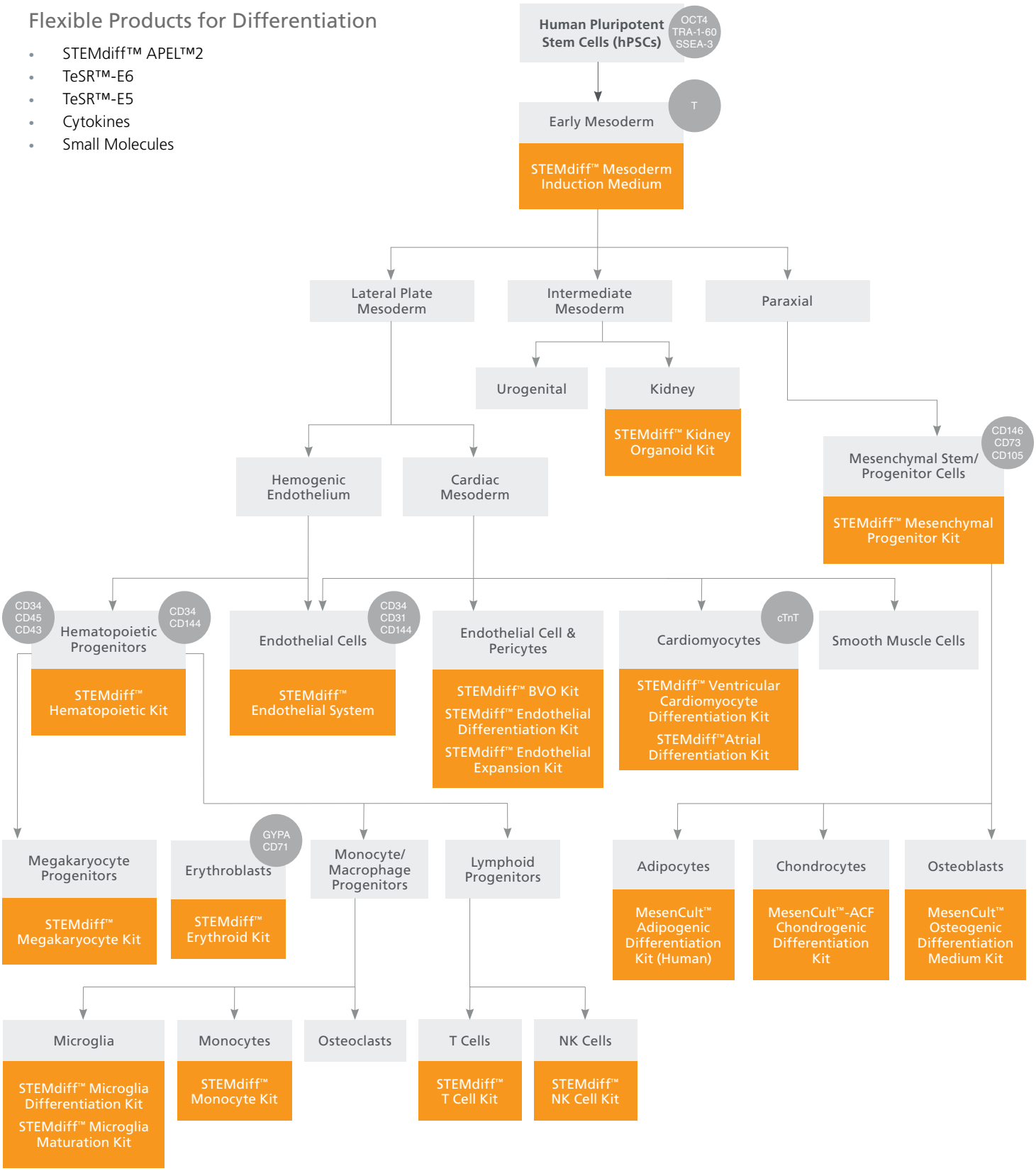
Why Use STEMdiff™?

- Reduce experimental variability with formulations optimized under rigorous quality controls
- Differentiate across multiple ES and iPS cell lines
- Standardize your differentiation to cells from all three germ layers with simplified kit formats
- Generate and bank progenitor cell types for experimental flexibility or as a reliable cell source for customized downstream differentiation

Mesoderm Differentiation Pathways

Flexible Products for Differentiation

- STEMdiff™ APEL™2
- TeSR™-E6
- TeSR™-E5
- Cytokines
- Small Molecules



STEMdiff™ Mesoderm Induction Medium

Differentiate to Early Mesoderm, Xeno-Free

STEMdiff™ Mesoderm Induction Medium (MIM; Catalog #05220) is a defined, xeno-free medium for generation of early mesoderm cells from human embryonic stem (ES) and induced pluripotent stem (iPS) cells. Protocols for mesodermal differentiation can be difficult and inconsistent. Using the short and simple STEMdiff™ MIM monolayer protocol enables efficient and reproducible differentiation of multiple human ES and iPS cell lines.

STEMdiff™ MIM produces a cell population enriched for early mesoderm, as indicated by positive expression of Brachyury (T), MIXL1, and NCAM markers (Figure 45).

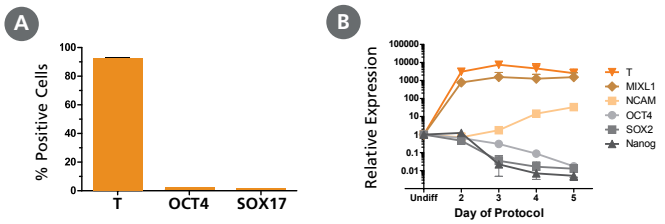


Figure 45. STEMdiff™ MIM Efficiently Generates a Homogenous Population of Early Mesoderm Cells

(A) Data showing marker expression characteristic of early mesoderm (positive Brachyury (T) expression and negative OCT4 and SOX17 expression) on Day 5 of the protocol. Data expressed as a mean percentage of cells expressing each marker ± SD, n = 33 (T, OCT4); n = 5 (SOX17). (B) Expression of undifferentiated cell markers (OCT4, SOX2, NANOG) and early mesoderm markers (T, MIXL1, NCAM), measured by qPCR and normalized to levels in undifferentiated cells; n = 2.

Learn more at www.stemcell.com/STEMdiff-MIM

STEMdiff™ Mesenchymal Progenitor Kit

Derive Functional Mesenchymal Progenitor Cells

The STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240) is optimized for the efficient and reproducible derivation of mesenchymal progenitor cells (MPCs) from human ES or iPS cells. This kit contains animal component-free (ACF) induction medium, expansion medium, and attachment substrate for the derivation and expansion of MPCs. It uses a simple monolayer protocol to generate MPCs under feeder-free conditions in three weeks. Human ES or iPS cell-derived MPCs are capable of long-term expansion (Figure 46). The derived MPCs are characterized by strong expression of cell-surface markers CD73, CD90, and CD105, and lack expression of CD45.

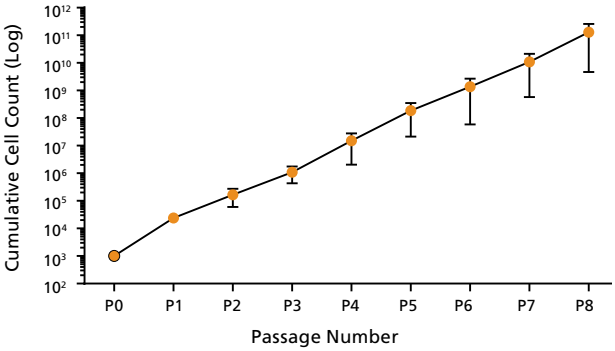


Figure 46. hPSC-Derived MPCs Generated Using the STEMdiff™ Mesenchymal Progenitor Kit Exhibit a High Rate of Cell Expansion in MesenCult™-ACF Plus Medium

The average cell expansion of human MPCs generated from hPSCs using the STEMdiff™ Mesenchymal Progenitor Kit. Error bars represent standard error of mean (SEM; n = 5).

Learn more at www.stemcell.com/STEMdiff-MPC

STEMdiff™ Endothelial Differentiation Kit

Efficiently Differentiate hPSCs to Endothelial Cells

The STEMdiff™ Endothelial Differentiation Kit (Catalog #08005) includes attachment substrate, animal component-free (ACF) endothelial induction medium, and endothelial expansion medium. It is optimized for differentiating hPSCs to endothelial-like cells on Corning® Matrigel®. This kit is designed to be used immediately after early mesoderm induction with STEMdiff™ Mesoderm Induction Medium (Catalog #05220).

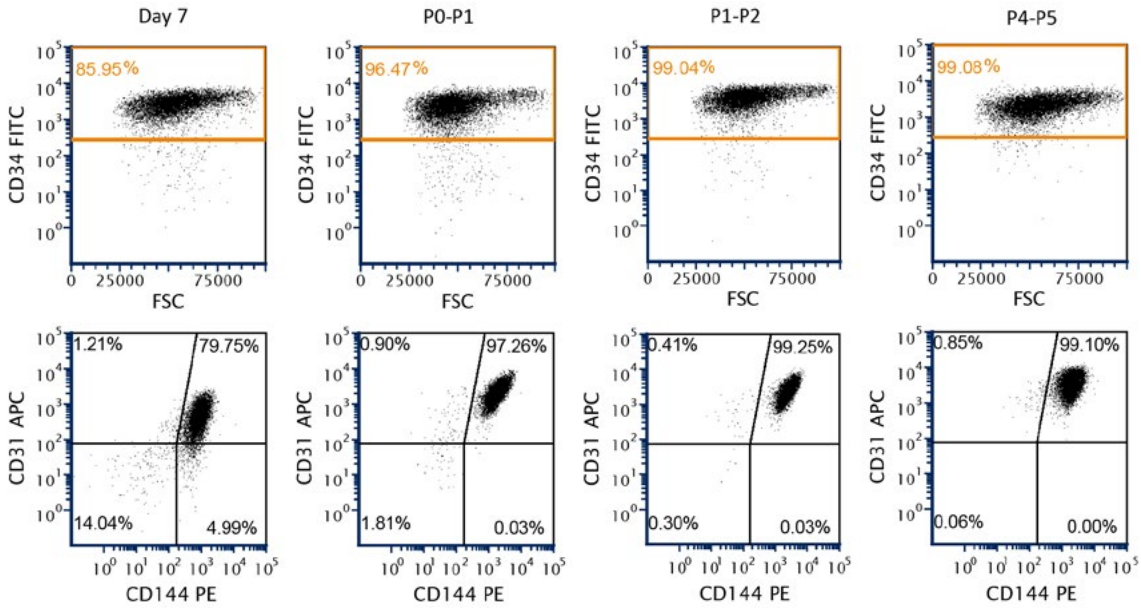


Figure 47. A Representative Flow Cytometric Analysis of Endothelial Marker Expression in hPSC-Derived Endothelial Cells

hPSC (H9 cell line)-derived endothelial cells were obtained at Day 7 using STEMdiff™ Endothelial Induction Medium. Greater than 85% of the cells were $CD34^+$ and had high levels of CD31 and CD144 expression. With subsequent passages (up to passage 5), the proportion of cells expressing endothelial markers (CD34, CD31, and CD144) increased.

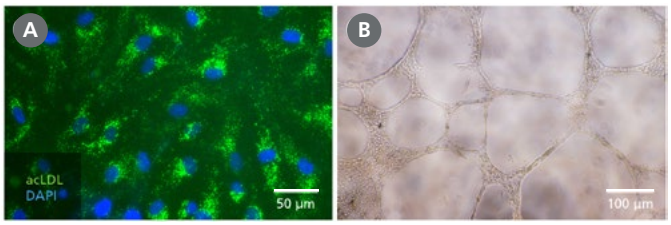


Figure 48. STEMdiff™ Endothelial Differentiation Kit Generates Functional hPSC-Derived Endothelial Cells

(A) Endothelial cells generated from hPSCs (F016 cell line) using the STEMdiff™ Endothelial Differentiation Kit take up acetylated LDL when plated at 10,000 cells/cm^2. (B) Cells are able to form tubular networks in vitro in a tube formation assay when plated at 20,000 cells/well in a 96-well plate for 24 hrs.

Learn more at www.stemcell.com/endo-diff

STEMdiff™ Blood Vessel Organoid Kit

Blood vessels are a fundamental part of all organ systems and have critical roles in multiple diseases, including diabetes, Alzheimer's disease, and cancer. The blood vasculature is composed of endothelial cells that form luminal tubes and pericytes covering the endothelial wall. In vitro models of vascular biology involve co-culturing endothelial cells with pericytes but do not fully recapitulate their three-dimensional (3D) organization and functionality.

STEMdiff™ Blood Vessel Organoid Kit (Catalog #100-0651) is a serum-containing kit for differentiation of hPSC-derived blood vessel organoids (BVOs) in a five-stage protocol, with the option to scale up for high-throughput screening in a 96-well format. BVOs generated using this kit have CD31⁺/CD34⁺/CD144⁺/KDR⁺ endothelial cells and PDGFR-β⁺/CD146⁺/SMA⁺/NG-2⁺ pericytes. These self-organizing hPSC-derived BVOs are able to form functional, perfusable blood vessels in vivo and can be used to study vascular dysfunction associated with various pathologies. The organoids can also be maintained in STEMdiff™ Blood Vessel Organoid Maturation Medium (Catalog #100-0658) for long-term assays*.

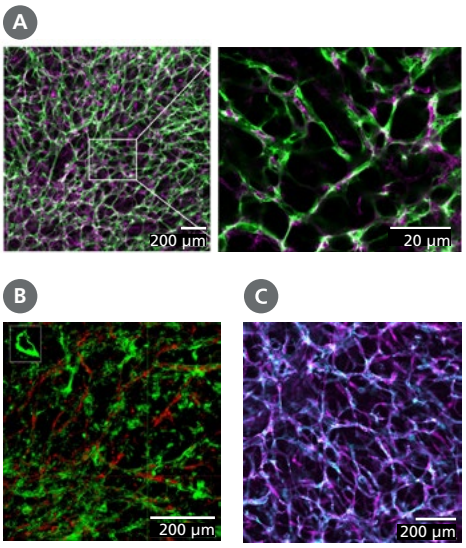


Figure 49. Vascular Networks Mature into Stable Blood Vessels When Cultured Within the Extracellular Matrix in STEMdiff™ Blood Vessel Maturation Medium

(A) hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (green) and hPDGFRβ⁺ cells (magenta); small quadrant shows tight endothelial and pericyte interactions. (B) hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (red) and deposited collagen IV (green; 3D reconstruction of optical Z stacks); small quadrant shows blood vessel lumen. (C) hPSC-derived blood vessel organoids are composed of hCD31⁺ cells (blue) and alpha-smooth muscle actin cells (magenta).

*STEMdiff™ Blood Vessel Organoid Maturation Medium is available for individual sale.

STEMdiff™ Hematopoietic System

Generate Hematopoietic Progenitor Cells, Immune Cells, and Blood Cells

The STEMdiff™ Hematopoietic Kit (Catalog #05310) consists of serum-free basal medium and supplements designed for the generation of hematopoietic progenitor cells (HPCs). Optimized for a standardized, 12-day differentiation protocol, this kit supports robust differentiation of hPSCs into HPCs that can be identified by the expression of CD34 and CD45, and by the ability to form hematopoietic colonies of multiple lineages in colony-forming unit (CFU) assays with MethoCult™ medium.

The resulting HPCs may be used for downstream assays or quantified in a CFU assay with MethoCult™ SF H4636 (Catalog #04636) medium, designed specifically for use with hPSC-derived HPCs, or MethoCult™ H4435 Enriched (Catalog #04435) medium. HPCs generated with the STEMdiff™ Hematopoietic Kit may be further differentiated using the STEMdiff™ Microglia Differentiation Kit (Catalog #100-0019) or STEMdiff™ Monocyte Kit (Catalog #05320). HPCs and downstream cells in the erythroid lineage may be obtained directly using the STEMdiff™ Erythroid Kit (Catalog #100-0074), and HPC and immune cell types in the lymphoid lineages may be obtained using the STEMdiff™ NK (Catalog #100-0170) and T Cell (Catalog #100-0194) Kits.

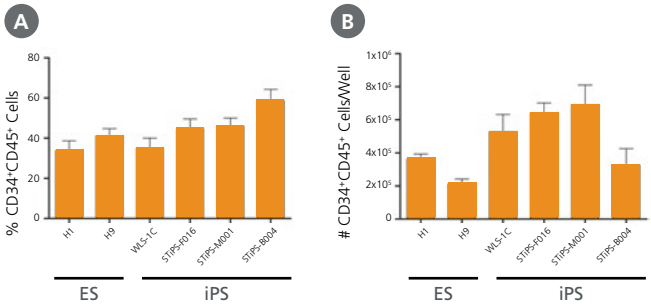


Figure 50. Efficient and Robust Generation of CD34⁺CD4⁺ HPCs

Human ES and iPS cells were cultured for 12 days in single wells of 12-well plates using the STEMdiff™ Hematopoietic Kit. At the end of the culture period, cells in suspension were harvested, stained, and analyzed by flow cytometry for the expression of hematopoietic cell surface markers CD34 and CD45. (A) Percentages and (B) total numbers of CD34⁺CD45⁺ cells in cultures of human ES or iPS cells are shown for 6 cell lines. Data shown as mean ± SEM; n ≥ 3.

Learn more at www.stemcell.com/STEMdiffHeme

WEBINAR

Modeling the Structural and Functional Features of Blood Vasculature with Blood Vessel Organoids

www.stemcell.com/bvo

STEMdiff™ Megakaryocyte Kit

STEMdiff™ Megakaryocyte Kit (Catalog #100-0901) is designed for the serum-free and feeder-free differentiation of human embryonic stem (hES) and induced pluripotent stem (iPS) cells to megakaryocytes expressing CD41a and CD42b. This optimized two-dimensional and two-stage protocol is capable of generating high yields of megakaryocytes per hPSC in 17 days. The resulting megakaryocytes show high ploidy and platelet-shedding ability and are also amenable to large-scale culture.

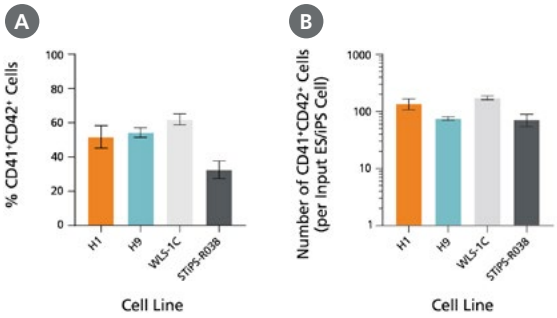


Figure 51. hPSC-Derived HPCs Efficiently Expand and Differentiate to CD41a$^+$CD42b$^+$ Megakaryocytes

hPSC-derived HPCs on Day 12 were cultured for 5 additional days in Medium MK2 to promote differentiation into mature MKs. The graph shows frequencies and numbers of CD41a$^+$CD42b$^+$ MKs per input cell for two hES cell lines (H1 and H9) and two hiPS cell lines (WLS-1C and STiPS-R038). The average frequency of viable CD41a$^+$CD42b$^+$ cells on Day 17 ranged between 56% and 77%. The average yield of CD41a$^+$CD42b$^+$ MKs generated per input cell ranged between 223 and 425. Data are shown as mean ± SEM (n = 12 for H1, n = 29 for H9, n = 27 for WLS-1C, n = 12 for STiPS-R038).

Learn more at
www.stemcell.com/megakaryocyte-diff

STEMdiff™ Erythroid Kit

Differentiate hPSCs to erythroid progenitor cells (erythroblasts) expressing Glycophorin A and CD71. hPSCs are induced toward erythroid-biased hematopoietic progenitor cells, and then further differentiated to erythroid progenitor cells (Day 10 - 24). Cells generated using the STEMdiff™ Erythroid Kit (Catalog #100-0074) can be further matured into normoblasts and reticulocytes once moved to appropriate culture conditions for maturation.

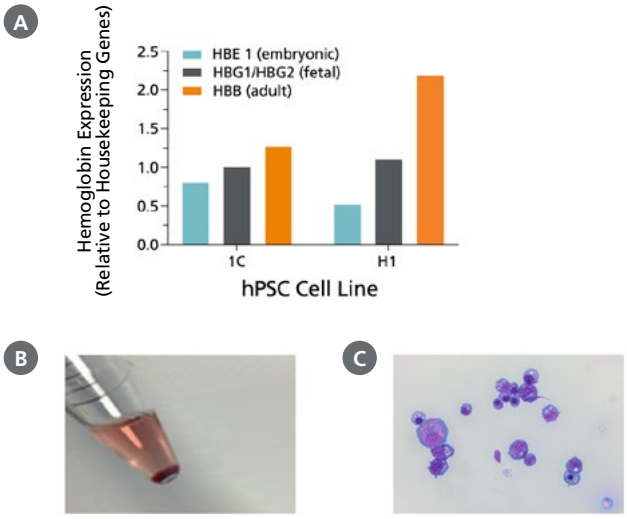


Figure 52. hES and hiPS Cell-Derived Erythroid Cells Are Hemoglobinized and Display Typical Erythroid Morphology

(A) Erythroid cells generated with the STEMdiff™ Erythroid Kit express a mix of primitive (embryonic) and definitive (fetal and adult) hemoglobin. Shown are the results of qPCR analysis for globin gene expression after 24 days of culture. (B) A picture of the cell pellet shows that cells produced in culture are hemoglobinized. (C) Cells display typical basophilic erythroblast morphology after 24 days of culture using the STEMdiff™ Erythroid Kit (40X magnification; May-Grunwald Giemsa stain).

Learn more at www.stemcell.com/erythro-diff

STEMdiff™ NK Cell Kit

Feeder-free and serum-free conditions provided by the STEMdiff™ NK Cell Kit (Catalog #100-0170) ensure a robust differentiation of hPSC-derived NK cells for developing adoptive immunotherapies in cancer patients as well as for research into the basic biology of these cells.

STEMdiff™ T Cell Kit

Obtain high yields of CD4⁺CD8⁺ double-positive (DP) T cells by differentiating from hPSCs in feeder-free and serum-free conditions with the STEMdiff™ T Cell Kit (Catalog #100-0194). Additionally, generate CD8⁺ single-positive (SP) T cells with an optional protocol.

Why Use the STEMdiff™ NK Cell and T Cell Kits?

- Differentiate embryonic stem (ES) and induced pluripotent stem (iPS) cells into T cells or NK cells with high yield and frequency
- Produce approximately 230 CD56⁺ NK cells or 60 CD4⁺CD8⁺ double-positive (DP) T cells per input hPSC-derived CD34⁺ cell
- Reduce variability by producing uniform aggregates for embryoid body (EB) formation with AggreWell™
- Eliminate variation introduced by serum and stromal cell lines by using serum- and feeder-free conditions
- Avoid extra passaging steps required with stromal cell-based culture

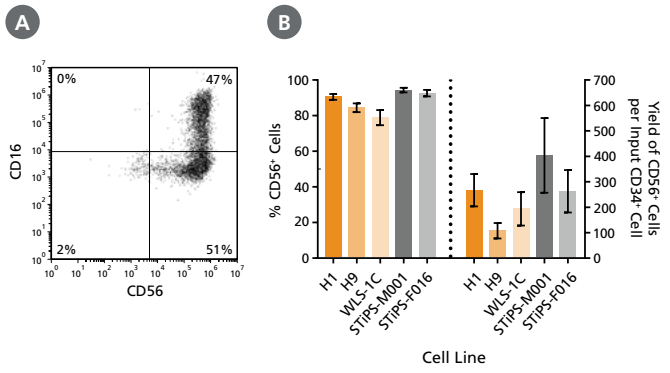


Figure 53. hPSCs Differentiate into CD56⁺ NK Cells After 40 Days of Culture

hPSCs were cultured using the STEMdiff™ NK Cell Kit for a total of 40 days. Cells were harvested and analyzed for expression of CD56 and CD16 by flow cytometry. (A) Representative flow cytometry plot is shown for ES (H1)-derived cells. (B) After 40 days of culture, the average frequency of viable CD56⁺ NK cells from hPSC-derived CD34⁺ cells ranged between 79% and 94%. The average yield of CD56⁺ cells produced per hPSC-derived CD34⁺ cell was between 108 and 404. Data are shown as mean ± SEM (n = 7 - 18).

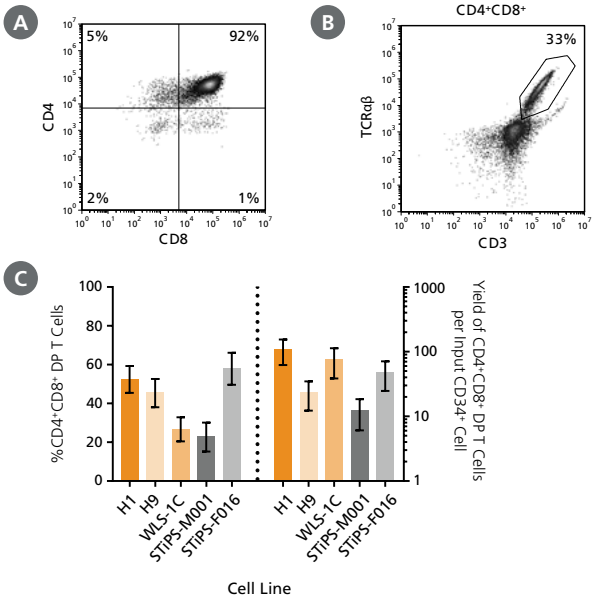


Figure 54. CD4⁺CD8⁺ DP T Cells Can Be Generated from Human hPSCs After a Total of 40 Days of Culture with the STEMdiff™ T Cell Kit

CD4⁺CD8⁺ DP T cells were differentiated from hPSCs using the STEMdiff™ T Cell Kit. Cells were harvested and analyzed for expression of CD3, CD4, CD8, and TCRαβ by flow cytometry. (A,B) Representative flow cytometry plots are shown for ES (H1)-derived cells. (C) The average frequency of viable CD4⁺CD8⁺ DP T cells on Day 28 ranged between 23% and 58%, and the average yield of DP T cells produced per input hPSC-derived CD34⁺ cell was between 12 and 108. Data are shown as mean ± SEM (n = 6 - 17).

TECHNICAL BULLETIN

Generation of Natural Killer Cells from Human Pluripotent Stem Cells

www.stemcell.com/STEMdiffProtocol-NK

Learn more at www.stemcell.com/STEMdiff-NK

Learn more at www.stemcell.com/STEMdiff-T

STEMdiff™ Monocyte Kit

Feeder-free and serum-free conditions provided by the STEMdiff™ Monocyte Kit (Catalog #05320) ensure a robust differentiation of hPSC-derived monocytes. Further differentiation to dendritic cells or macrophages can be achieved using ImmunoCult™ Dendritic Cell Culture Kit (Catalog #1095) or ImmunoCult™-SF Macrophage Medium (Catalog #10961), respectively.

For differentiation to microglia, please see page 46.

Why Use the STEMdiff™ Monocyte Kit?

- Generate up to 7 million CD14⁺ monocytes per plate in just 14 - 23 days
- Eliminate variation introduced by serum and feeder cells by using serum- and feeder-free conditions
- Produce monocytes in a simple monolayer culture for easier harvest of suspended cells

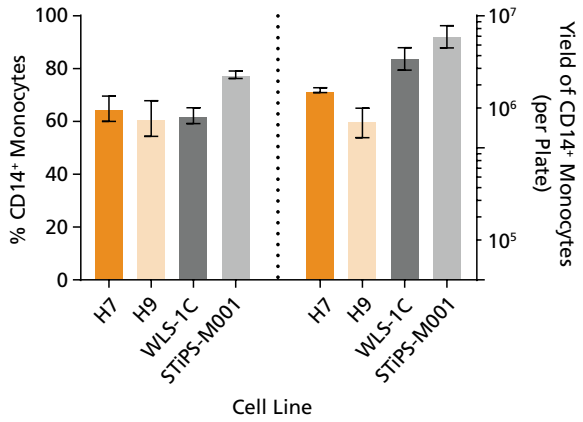


Figure 55. STEMdiff™ Monocyte Kit Enables Robust and Efficient Generation of CD14⁺ Monocytes

hPSCs were differentiated using the STEMdiff™ Monocyte Kit and harvested every 2 - 3 days between Days 17 and 23. The average frequency of viable CD14⁺ monocytes at the peak harvest was 61 - 78% and the average yield of CD14⁺ monocytes produced per 6-well plate was between 1.6 x 10^6 and 7.1 x 10^6 cells.

Learn more at
www.stemcell.com/STEMdiff-Monocyte

STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit

Efficiently and reproducibly generate functional, phenotypically pure ventricular cardiomyocytes from hPSCs for use in downstream applications such as disease modeling, drug discovery, and cardiotoxicity screening. The STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Catalog #05010) consists of defined, serum-free basal media optimized for a standardized, 15-day differentiation protocol. Achieve robust differentiation of hPSCs into ventricular cardiomyocytes, which can be identified by the expression of a key marker, cardiac troponin T (cTnT) (Figure 56). Contracting hPSC-derived cardiomyocytes can be seen as early as Day 8. This kit is formulated for use in feeder-free conditions, optimized for the differentiation of hPSCs maintained in mTeSR™1 (Catalog #05850) or TeSR™-E8™ (Catalog #05940), and compatible with multiple human embryonic stem (hES) and induced pluripotent stem (hiPS) cell lines.

Complete Workflow

The STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit is part of the STEMdiff™ Ventricular Cardiomyocyte System, which is itself composed of specialized media products that optimize the entire hPSC-derived cardiomyocyte research workflow, including differentiation and maintenance, dissociation and replating, and cryopreservation. Following differentiation, hPSC-derived ventricular cardiomyocytes can be maintained long-term using the STEMdiff™ Cardiomyocyte Maintenance Kit (Catalog #05020), which enables the standardized harvesting of hPSC-derived cardiomyocytes that are ready for use in downstream applications such as flow cytometry, immunocytochemistry, calcium imaging, electrophysiology, and cryopreservation. These cardiomyocytes can be cryopreserved using STEMdiff™ Cardiomyocyte Freezing Medium (Catalog #05030) to maintain the viability of cardiomyocytes after thawing or during dissociation, harvesting, or replating.

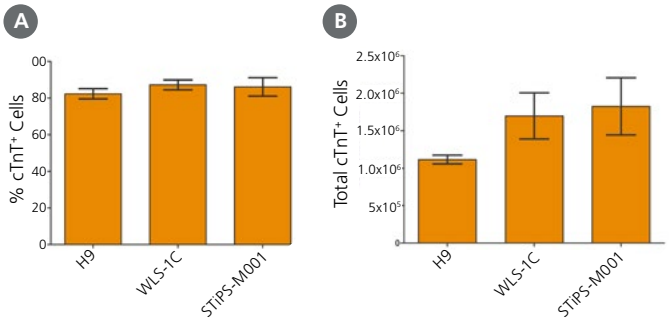


Figure 56. Efficient and Robust Generation of cTnT-Positive Ventricular Cardiomyocytes

hPSCs were cultured for 15 days in single wells of 12-well plates using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. At the end of the culture period, cells were harvested and analyzed by flow cytometry for expression of cell marker cTnT. (A) Percentages and (B) total numbers of cells expressing cTnT in cultures of human ES (H9) or iPS (WLS-1C and STiPS-M001) cells are shown. Data shown as mean ± SEM; n = 3.

Learn more at www.stemcell.com/cardio-diff

STEMdiff™ Myogenic Progenitor Supplement Kit

Generate hPSC-Derived Myogenic Progenitors and Myotubes

STEMdiff™ Myogenic Progenitor Supplement Kit (Catalog #100-0151) consists of serum-free supplements intended for use with DMEM/F12 to differentiate hPSCs to myogenic progenitor cells. The latter, which are characterized by myogenic cell markers such as CD56 and CD82, can be culture-expanded for more than five passages using the MyoCult™-SF Expansion Supplement Kit (Human; Catalog #05980) and further differentiated to functional multinucleated MyHC+ myotubes with high efficiency using the MyoCult™ Differentiation Kit (Human; Catalog #05965). These myotubes can be used for various downstream applications and analyses.

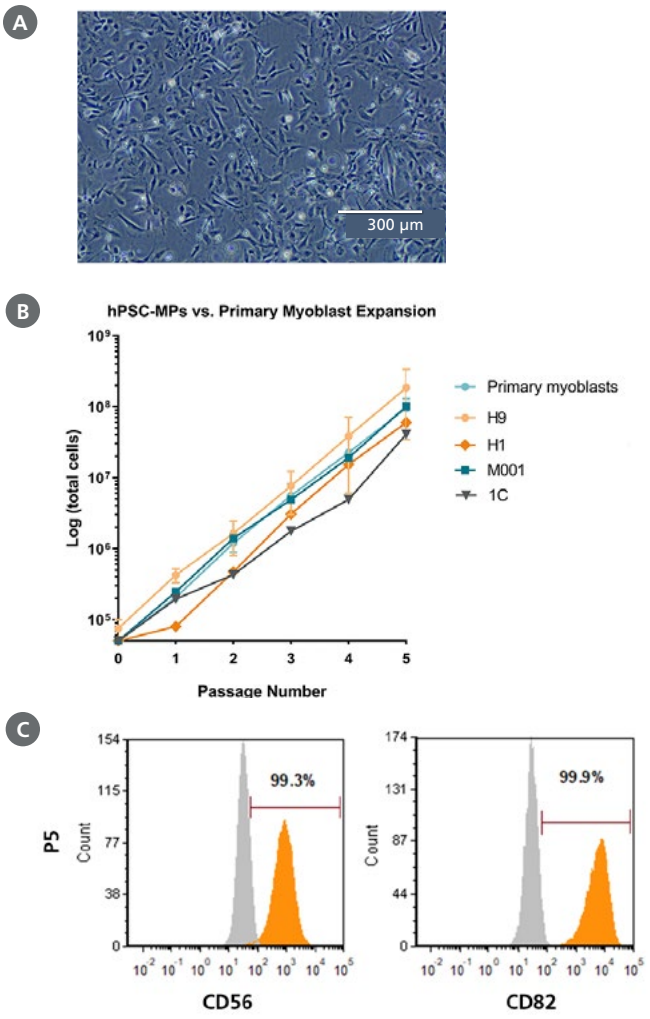


Figure 57. STEMdiff™ Myogenic Progenitor Kit Generates Expandable hPSC-Derived Myogenic Progenitors

(A) Representative image of proliferating sub-cultured hPSC-derived myogenic progenitors generated using the STEMdiff™ Myogenic Progenitor Kit. (B) Expansion rates of hPSC-derived myogenic progenitors (hSPC-MP) over 5 passages across multiple hPSC lines are comparable to human primary myoblasts. Error bars represent standard error of mean, n = 3. (C) hPSC-derived myogenic progenitors harvested at passage 5 expressed human myoblast markers CD56 and CD82.

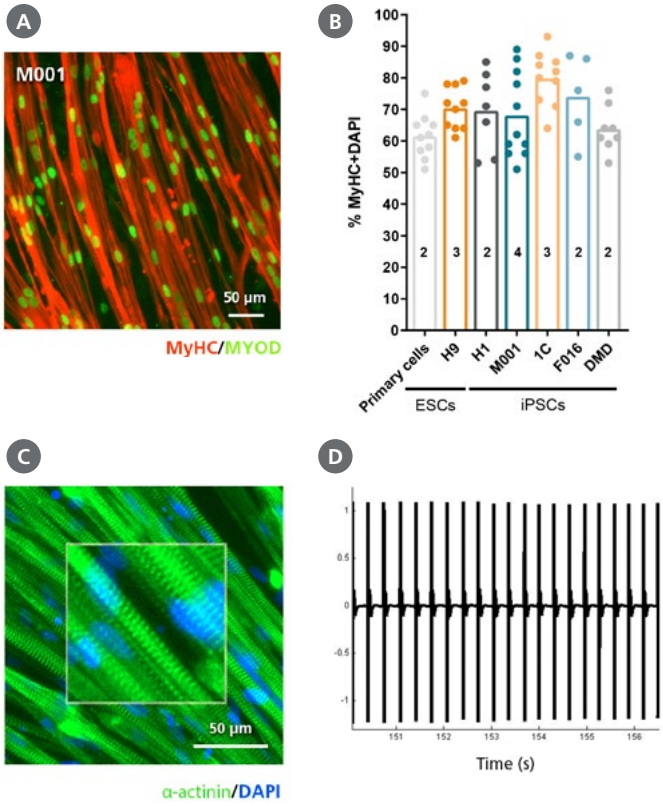


Figure 58. hPSC-Derived Myotubes Generated Using the STEMdiff™ Myogenic Progenitor Kit Are Efficiently Differentiated and Functionally Contractile

hPSC-derived myogenic progenitors were generated from the M001 cell line using the STEMdiff™ Myogenic Progenitor Kit and then induced to differentiate into myotubes using MyoCult™ Differentiation Medium (Human). (A) After 8 days, myotubes were fixed and stained for MyHC and MyoD. (B) Multiple hPSC lines differentiated and induced using this method exhibited high fusion indices similar to primary human myoblasts (numbers in bars represent the n number and dots represent technical replicates). (C) hPSC-derived myotubes were stained for alpha-actinin and displayed organized sarcomeric structures as indicated by the zoomed-in area. (D) Spontaneous field potential recordings of hPSC-derived myotubes using a microelectrode assay plate indicated that the derived myotubes are contractile.

Learn more at www.stemcell.com/myo-diff

STEMdiff™ Kidney Organoid Kit

Differentiate Kidney Organoids from hPSCs

Directed differentiation of hPSCs into kidney organoids allows researchers to work with an in vitro model culture system that has direct relevance to the developing human kidney. Kidney organoids form large (~150 - 400 µm), branched structures containing endothelial cells, podocytes, and epithelial cells of the proximal and distal tubules, mimicking nephron-like structure and segmentation. Kidney organoids modeling both health and disease in specific genetic backgrounds can be created by reprogramming patient-derived cells. These in vitro models can be further manipulated by introducing or correcting mutations through CRISPR-Cas9 gene editing prior to differentiation. This approach has successfully been used to model polycystic kidney disease and podocyte organization during development.[17,18] Like other hPSC-derived organoid systems, kidney organoids resemble the first trimester kidney and display markers of the developing kidney as well as markers of differentiation.[19,20]

The STEMdiff™ Kidney Organoid Kit (Catalog #05160) enables growth of tubular kidney organoids from ES and iPS cells in 21 days. These organoids are suitable for a wide range of experimental contexts, including developmental and cell biology, disease modeling, drug screening and nephrotoxicity assessment, and cell therapy research.

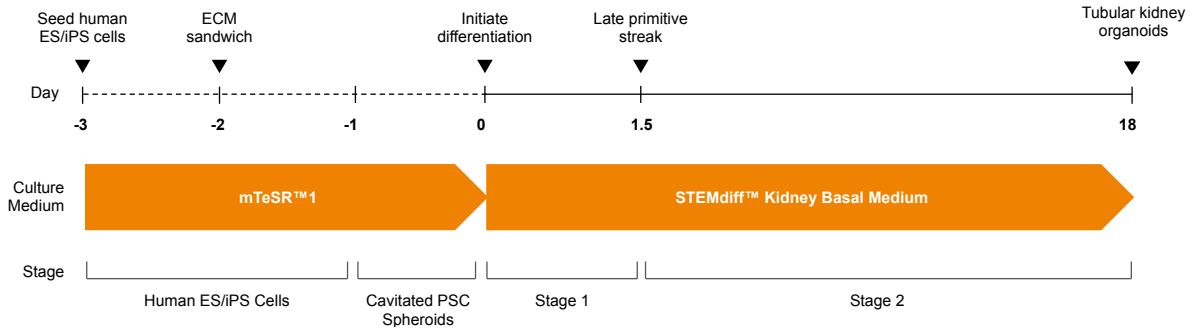


Figure 59. Schematic for Differentiation from hPSCs to Human Kidney Organoids with the STEMdiff™ Kidney Organoid Kit

hPSC cultures progress through a simple three-stage process to generate kidney organoids. hPSCs are plated and overlaid with Corning® Matrigel® to form cavitated spheroids. These are induced toward the late primitive streak and intermediate mesoderm, forming tubular kidney organoids by Day 18 of differentiation.

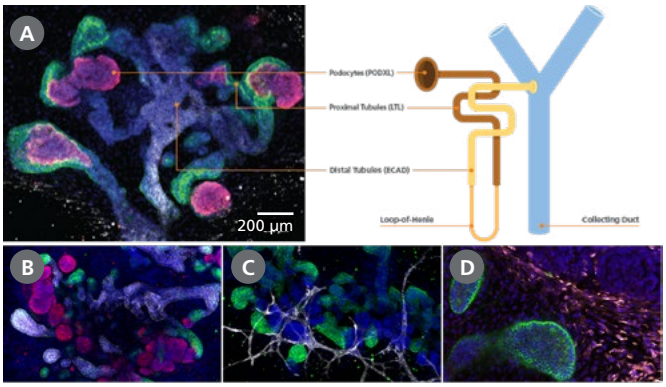


Figure 60. Kidney Organoids Display Distinct Domains of the Developing Nephron

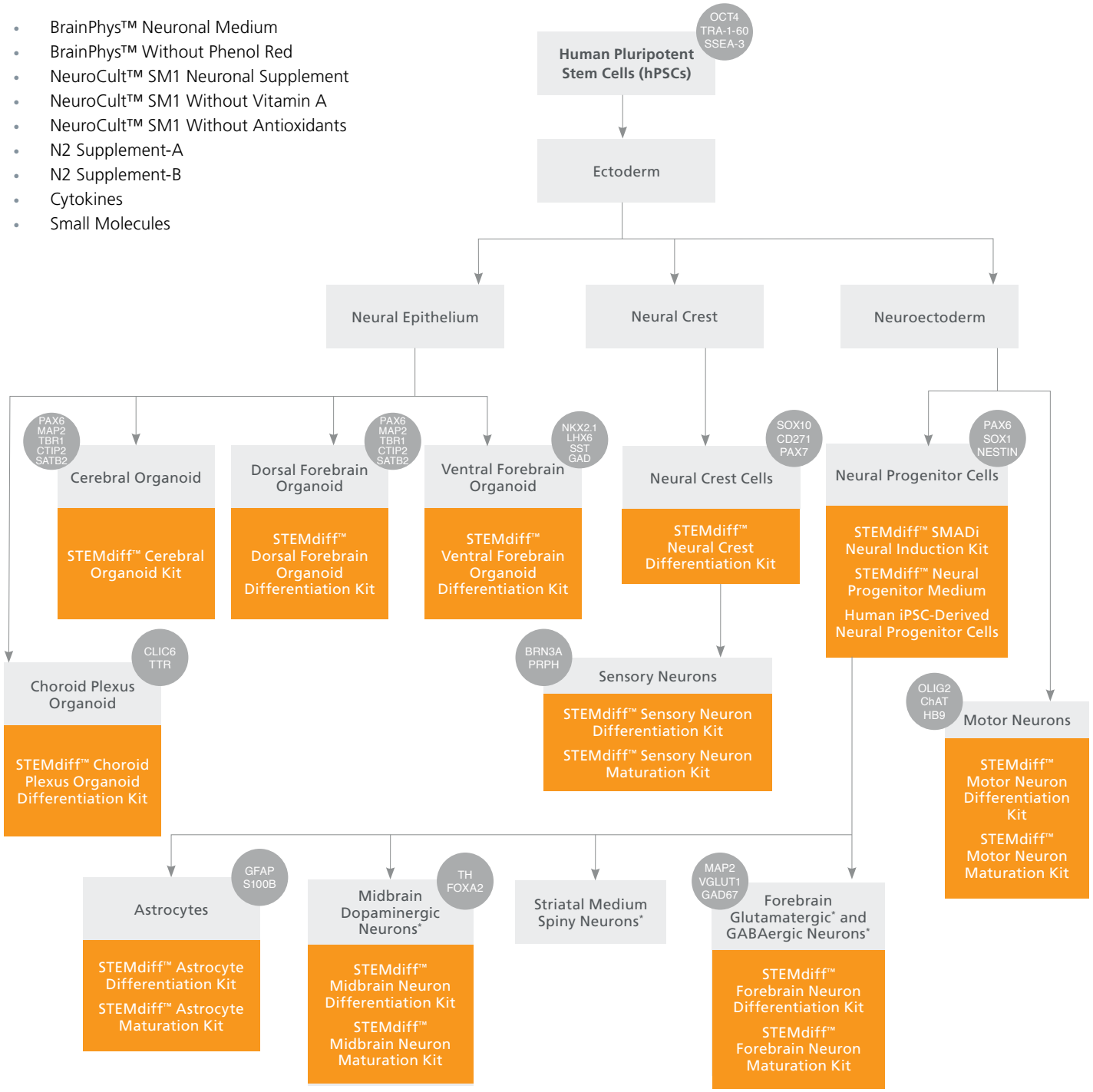
hPSC-derived kidney organoids generated using the STEMdiff™ Kidney Organoid Kit incorporate cells and organization mimicking the structure and segmentation of the developing nephron. (A, B) Branched, tubular organoids display markers of proximal tubules (LTL, green), distal tubules (ECAD, white), and podocytes (PODXL, red), while DAPI (blue) shows the nuclei of all cells, including (C) endothelial cells (CD31, white) and (D) mesenchyme (VIM, white; Meis 1/2/3, red). Scale bar = 200 µm.

Learn more at www.stemcell.com/STEMdiffKidney

Ectoderm Differentiation Pathways

Flexible Products for Differentiation

- BrainPhys™ Neuronal Medium
- BrainPhys™ Without Phenol Red
- NeuroCult™ SM1 Neuronal Supplement
- NeuroCult™ SM1 Without Vitamin A
- NeuroCult™ SM1 Without Antioxidants
- N2 Supplement-A
- N2 Supplement-B
- Cytokines
- Small Molecules



Other Products for Differentiation

- STEMdiff™ Microglia Differentiation Kit

*Flexible products for differentiation can be used for these cell types.

STEMdiff™ Neural System

Differentiate hPSCs to Neural Progenitor Cells, Neurons, and Glia

The STEMdiff™ SMADi Neural Induction Kit (Catalog #08581) combines STEMdiff™ Neural Induction Medium (Catalog #05835) with STEMdiff™ SMADi Neural Induction Supplement, which directs differentiation by blocking TGF-β and BMP-dependent SMAD signaling, resulting in efficient neural induction of even hard-to-differentiate cell lines.

Neural progenitor cells (NPCs) can be generated using the STEMdiff™ SMADi Neural Induction Kit with either an embryoid body (EB) protocol or monolayer culture protocol. STEMdiff™ Neural Rosette Selection Reagent (Catalog #05832) allows rapid and efficient isolation of neural rosettes to enrich for CNS-type NPCs.

NPCs generated using the STEMdiff™ SMADi Neural Induction Kit can be efficiently expanded and cryopreserved in serum-free STEMdiff™ Neural Progenitor Medium (Catalog #05833) and STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838), respectively.

NPCs cultured in STEMdiff™ Neural Progenitor Medium display typical NPC morphology (Figure 61D) and can be consistently expanded three- to five-fold upon each passage to generate a large number of cells. NPCs generated using the STEMdiff™ SMADi Neural Induction Kit can be differentiated to functional neuronal subtypes using the lineage-specific STEMdiff™ differentiation and maturation kits.

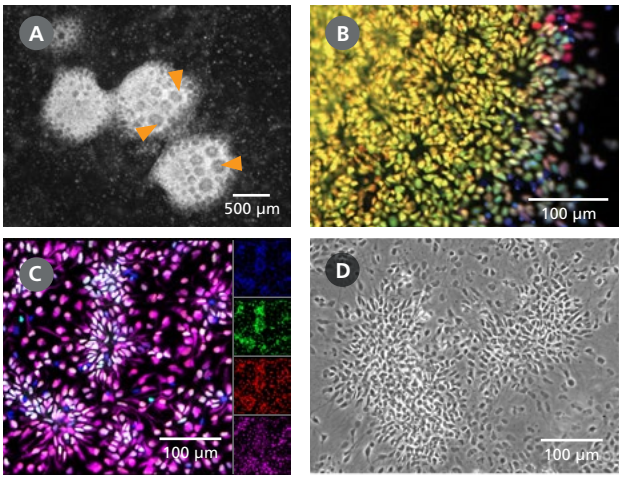


Figure 61. Neural Induction Using the STEMdiff™ SMADi Neural Induction Kit and STEMdiff™ Neural Progenitor Medium Generates Neural Rosettes and Enriches for CNS-type Neural Progenitor Cells

Starting hPSCs were maintained in mTeSR™1 and differentiated using an EB protocol. (A) Morphologically distinct neural rosettes (arrowheads) are clearly visible two days after replating EBs. (B,C) NPCs express CNS-type NPC markers PAX6 (B,C; green), SOX1 (B,C; red), and Nestin (C; purple). Nuclei are counterstained with DAPI. (D) NPCs maintained in STEMdiff™ Neural Progenitor Medium (C) display typical NPC morphology (shown at Day 6 of passage 1).

TECH TIP
Designing Your Neural Induction and Differentiation Workflow

TRAINING
Free Virtual On-Demand Neural Induction Course

Learn more at
www.stemcell.com/STEMdiff-NIM-SMADi

STEMdiff™ Forebrain Differentiation and Maturation Kits

A mixed population of excitatory and inhibitory forebrain-type (FOXG1+) neurons can be generated using the serum-free STEMdiff™ Forebrain Neuron Differentiation Kit (Catalog #08600) and STEMdiff™ Forebrain Neuron Maturation Kit (Catalog #08605). The basal medium for the maturation kit is BrainPhys™ (Catalog #05790), a neuronal medium designed to support electrical activity and neuronal maturation for functional neurons.

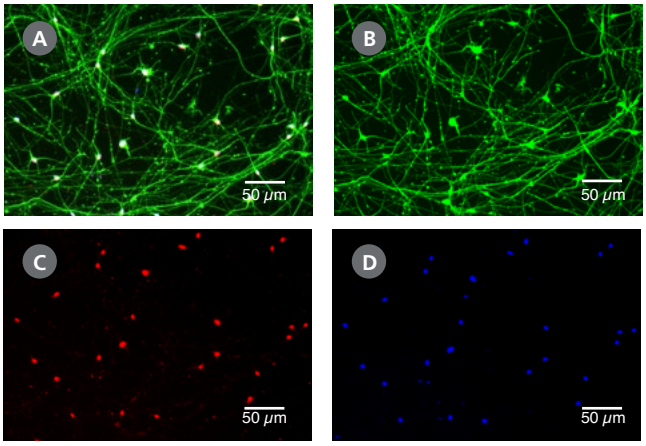


Figure 62. Downstream Differentiation of Neural Progenitor Cells to Neurons Is Possible Using the STEMdiff™ Differentiation and Maturation Kits

(A) NPCs generated from STiPS-R038 hPSCs in mTeSR™1 using the STEMdiff™ SMADi Neural Induction Kit EB protocol were differentiated and matured to cortical neurons using STEMdiff™ Forebrain Neuron Differentiation Kit for 7 days and STEMdiff™ Forebrain Neuron Maturation Kit for 14 days. The resulting cultures contain a highly pure population of (B) class III β-tubulin-positive neurons (green) with less than 10% GFAP-positive astrocytes (not shown). (C) The generated neurons are also positive for FOXG1 expression (red), indicating a forebrain-type identity. (D) Nuclei are labeled with Hoechst (blue).

Learn more at
www.stemcell.com/STEMdiff-Neuron

STEMdiff™ Midbrain Differentiation and Maturation Kits

Dopaminergic neurons can be generated using the serum-free STEMdiff™ Midbrain Neuron Differentiation Kit (Catalog #100-0038) and STEMdiff™ Midbrain Neuron Maturation Kit (Catalog #100-0041). The midbrain-patterned cell population produced contains FOXA2-, LMX1A-positive neuronal precursors, yielding neurons that can be maintained long-term in culture (Figure 63).

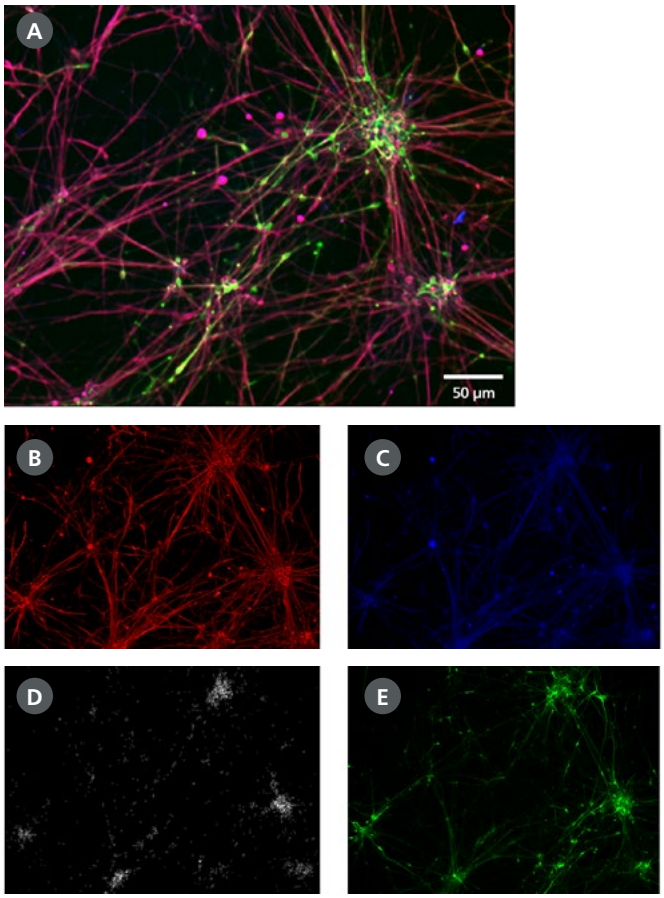


Figure 63. Midbrain-Type Neurons Express Tyrosine Hydroxylase and Dopamine Transporters (DAT) After Differentiation and Maturation in STEMdiff™ Midbrain Neuron Kits

(A) NPCs generated from H9 hPSCs in mTeSR™1 using the STEMdiff™ SMADi Neural Induction Kit monolayer protocol were differentiated and matured to midbrain-type neurons using the STEMdiff™ Midbrain Neuron Differentiation Kit for 12 days and STEMdiff™ Midbrain Neuron Maturation Kit for 14 days. The resulting cultures contain a population of (B) class III β-tubulin-positive neurons (red), which (C) express DAT in blue, and (E) more than 15% tyrosine hydroxylase-positive cells (green). (D) Nuclei are labeled with DAPI (white).

Learn more at
www.stemcell.com/STEMdiff-Dopa

STEMdiff™ Astrocyte Differentiation and Maturation Kits

Generate a highly pure population of astrocytes using the STEMdiff™ Astrocyte Differentiation Kit (Catalog #100-0013) and STEMdiff™ Astrocyte Maturation Kit (Catalog #100-0016). Matured astrocytes are functional, as assayed by calcium imaging (data not shown) and can be used for co-culture applications.

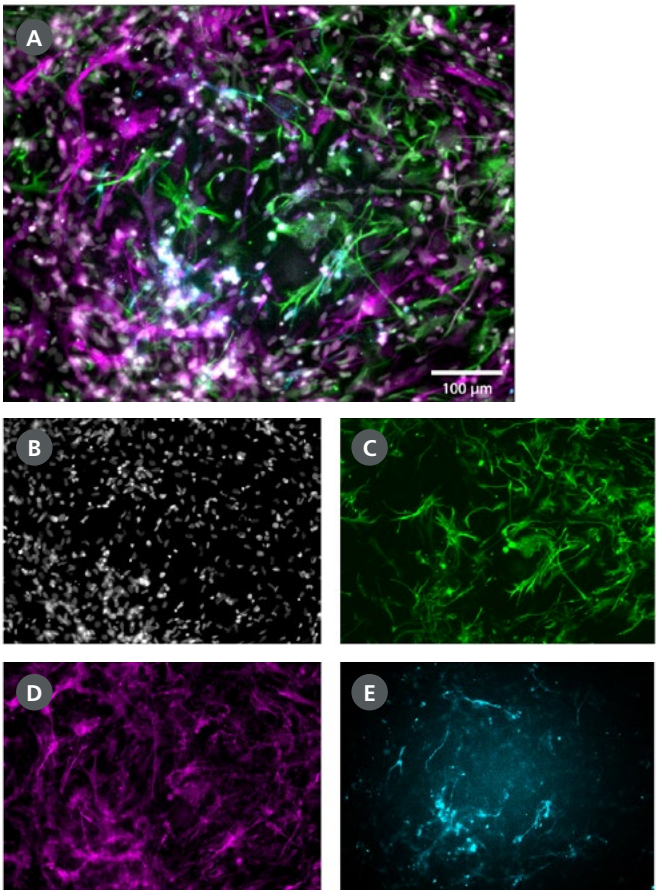


Figure 64. Cortical-Type Astrocytes Are Generated After Culture in STEMdiff™ Astrocyte Differentiation and Maturation Kits

(A) NPCs generated from hPSCs in TeSR™-E8™ using the STEMdiff™ SMADi Neural Induction Kit embryoid body (EB) protocol were differentiated and matured to cortical-type astrocytes after culturing with the STEMdiff™ Astrocyte Differentiation Kit for 3 weeks followed by STEMdiff™ Astrocyte Maturation Kit for 3 weeks. (B) Nuclei are labeled with DAPI (gray). The resulting cultures contain a highly pure population of astrocytes, which are (C) more than 60% GFAP-positive (green) and (D) more than 70% S100B-positive (magenta), with (E) fewer than 15% neurons (DCX-positive cells, cyan).

Learn more at
www.stemcell.com/STEMdiff-Astro

STEMdiff™ Motor Neuron Kits

Generate pure in vitro populations of motor neurons from hPSCs in only 14 days using the STEMdiff™ Motor Neuron Differentiation Kit (Catalog #100-0871). These motor neurons can be further matured with BrainPhys™-based STEMdiff™ Motor Neuron Maturation Kit (Catalog #100-0872). The resultant motor neuron populations exhibit high-level expression of expected motor neuron markers.

Why Use the STEMdiff™ Motor Neuron Kits?

- Generate motor neurons from human induced pluripotent stem cells in only 14 days
- Streamline motor neuron culture with a simple, scalable workflow
- Produce physiologically relevant results with integrated BrainPhys™ Neuronal Medium, supporting neuronal activity and maturation
- Model the complexities of cell-cell interactions by pairing with compatible differentiation kits for co-culture applications

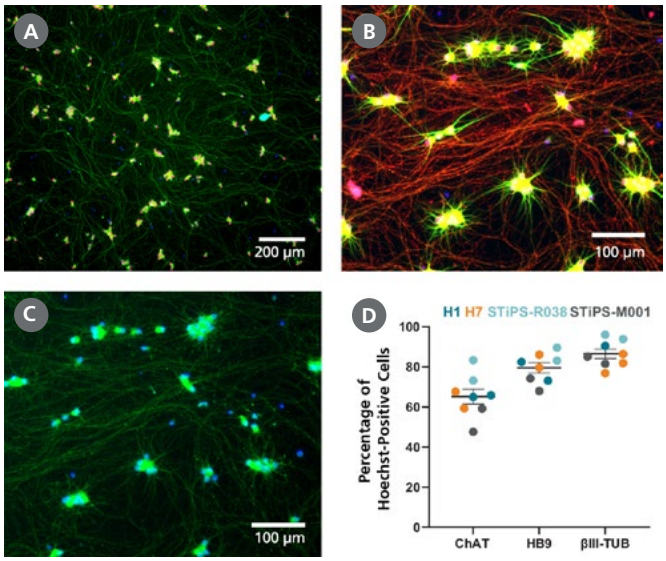


Figure 65. hPSC-Derived Motor Neurons Can Be Further Matured in STEMdiff™ Motor Neuron Maturation Medium

Motor neuron progenitors derived from a variety of lines were matured using the STEMdiff™ Motor Neuron Maturation Kit. (A) Mature motor neuron cultures contain a population of cells expressing neuronal identity marker βIII-TUB (green), mature motor neuron markers HB9 (red), (B) SYNAPSIN (red), and MAP2 (green), as well as (C) cholinergic neuron marker ChAT (green). Nuclei are labeled with Hoechst (blue). (D) The percentage expression of ChAT, HB9 & βIII-TUB in the resulting cultures, derived from 2 hES (H1 and H7) and 2 hiPS (STiPS-R038 and STiPS-M001) cell lines, were quantified. This differentiation generated ChAT+ (65.16% ± 3.737%, mean ± SEM; n = 4 cell lines, 2 replicates per condition), HB9+ (79.58% ± 2.570%, mean ± SEM) & βIII-TUB+ (86.56% ± 2.331%, mean ± SEM) motor neurons. Numbers are % positive of total Hoechst-positive cells.

Human iPSC-Derived Neural Progenitor Cells

Integrate quality into your neural workflow from the start with high-quality, ready-to-use Human iPSC-Derived Neural Progenitor Cells (NPCs; Catalog #200-0620). These cryopreserved central nervous system (CNS)-type progenitors are differentiated from the robust, extensively tested human induced pluripotent stem cell (iPSC) control line, SCTi003-A (Catalog #200-0511; see Page 7 for more information), derived from healthy female donor peripheral blood mononuclear cells (PBMCs). Ready to use directly from thawing, these human NPCs are multipotent, suitable for customized downstream workflows, and compatible with the STEMdiff™ neural system to generate various CNS cell types, such as forebrain neurons, midbrain neurons, and astrocytes. NPCs can be expanded using STEMdiff™ Neural Progenitor Medium (Catalog #05833), allowing for scale-up and reducing the cost of workflows that require large numbers of cells. Cryopreserve expanded NPCs using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838) for flexibility in your experimental schedule.

This research-use-only (RUO) product has been consented for both academic and commercial use. SCTi003-A is derived from cells that are ethically sourced using Institutional Review Board (IRB)-approved consent forms and protocols. It is karyotypically stable, demonstrates trilineage differentiation potential, expresses undifferentiated cell markers, and was reprogrammed using a non-integrating reprogramming technology. Registration with hPSCreg® ensures ethical and biological conformity based on community standards.

NOTE: For research use or in vitro laboratory-based tissue culture work only. Not approved for application into humans under any circumstances

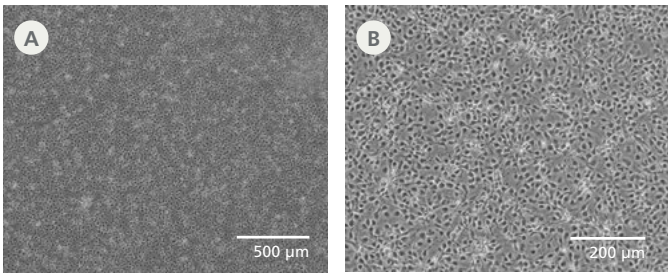


Figure 66. Human iPSC-Derived Neural Progenitor Cells Exhibit High-Quality Morphology Characteristic of Multipotent Central Nervous System Progenitor Cells

Cryopreserved Human iPSC-Derived Neural Progenitor Cells were thawed and plated onto Corning® Matrigel®-coated plates at 200,000 cells/cm^2. NPCs were incubated for 24 hours in STEMdiff™ Neural Progenitor Medium at 37°C and subsequently analyzed by brightfield microscopy. NPCs display the small, teardrop-shaped morphology expected for NPCs. (A) 10X magnification, (B) 20X magnification.

Learn more at www.stemcell.com/NPCs

Why Use Human iPSC-Derived Neural Progenitor Cells?

- Expand immediately post-thaw with STEMdiff™ Neural Progenitor Medium
- Save time by starting your differentiation workflow with a highly characterized neural progenitor intermediate
- Differentiate into forebrain neurons and/or astrocytes using the STEMdiff™ neural system
- Ensure relevance with neuron-astrocyte co-culture generated with the same genetic background
- Obtain high-quality NPCs, derived from the highly characterized control line, SCTi003-A

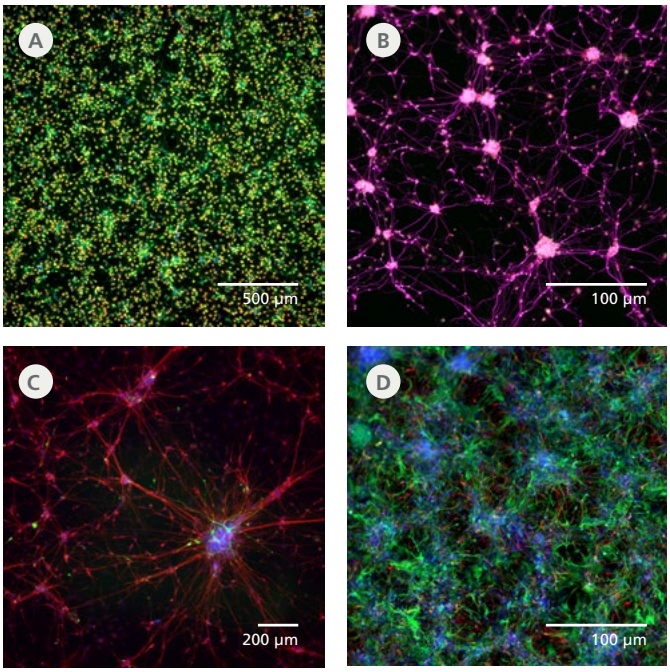


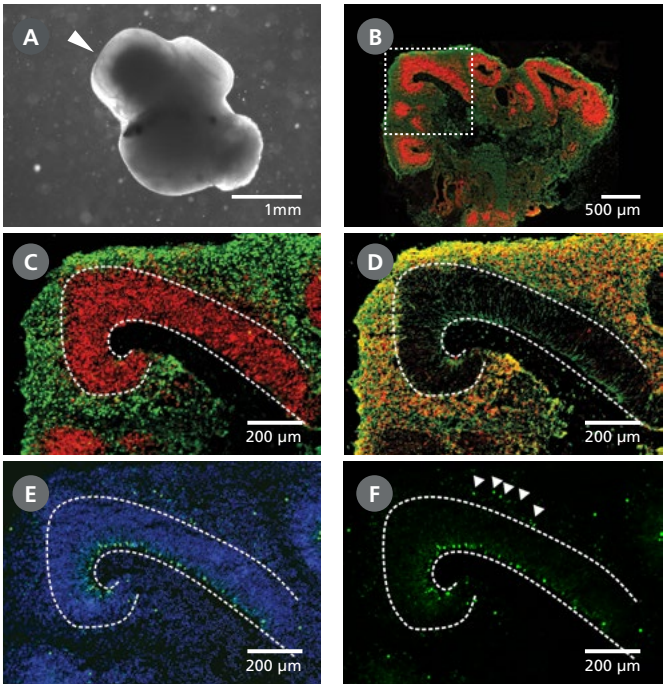
Figure 67. Human iPSC-Derived Neural Progenitor Cells Can Effectively Differentiate into Forebrain Neurons, Midbrain Neurons, and Astrocytes

Human iPSC-Derived Neural Progenitor Cells generated from SCTi003-A iPSCs were thawed, established in culture, and fixed for immunocytochemistry. (A) The NPCs express neural progenitor markers SOX1 (red) and PAX6 (green). (B) NPCs cultured with the STEMdiff™ Forebrain Neuron Kit produce forebrain neuron cell populations expressing neuronal identity marker βIII-TUB (magenta). (C) NPCs cultured with the STEMdiff™ Midbrain Neuron Kit produce midbrain neuron cell populations expressing neuronal identity marker βIII-TUB (red) and dopaminergic neuron marker TH (green). (D) NPCs cultured with the STEMdiff™ Astrocyte Kit produce astrocyte populations expressing astrocyte marker S100β (green) and GFAP (red).

STEMdiff™ Cerebral Organoid Kit

Cerebral organoids are three-dimensional in vitro cultures that recapitulate the developmental processes and organization of the developing human brain. The STEMdiff™ Cerebral Organoid Kit (Catalog #08570) is designed to generate unpatterned, multi-layered neural organoids from human ES and iPS cells.

For extended periods of organoid culture, the kit components required for organoid maturation are available separately as the STEMdiff™ Cerebral Organoid Maturation Kit (Catalog #08571). To facilitate embedding of 3D aggregates, this media is compatible with the Organoid Embedding Sheet (Catalog #08579).



Why Use the STEMdiff™ Cerebral Organoid Kit?

- Generate unpatterned organoids capable of spontaneous differentiation to produce multiple brain regions within the same organoid
- Culture under flexible conditions with either matrix droplet embedding or liquid matrix
- Enjoy increased efficiency of organoid formation with a formulation based on a popular published protocol[21]
- Generate new or modified organoid models with this highly compatible platform

Learn more at www.stemcell.com/COKit

Figure 68. Cerebral Organoids Contain Multiple Layered Regions That Recapitulate the Cortical Lamination Process Observed During In Vivo Human Brain Development

(A) A representative phase-contrast image of a whole cerebral organoid at Day 40 generated using the STEMdiff™ Cerebral Organoid Kit. Cerebral organoids at this stage are made up of phase-dark structures that may be surrounded by regions of thinner, more translucent structures that display layering (arrowheads). (B) Immunohistological analysis on cryosections of cerebral organoids reveals cortical regions within the organoid labeled by the apical progenitor marker PAX6 (red) and neuronal marker β-tubulin III (green). (C-F) Inset of boxed region from (B). (C) PAX6$^+$ apical progenitors (red, enclosed by dotted line) are localized to a ventricular zone-like region. β-tubulin III$^+$ neurons (green) are adjacent to the ventricular zone. (D) CTIP2, a marker of the developing cortical plate, co-localizes with β-tubulin III$^+$ neurons in a cortical plate-like region. Organization of the layers recapitulates early corticogenesis observed during human brain development. (E) Proliferating progenitor cells labeled by Ki-67 (green) localize along the ventricle, nuclei are counterstained with DAPI (blue). (F) An additional population of Ki-67$^+$ cells is found in an outer subventricular zone-like region (arrowheads).

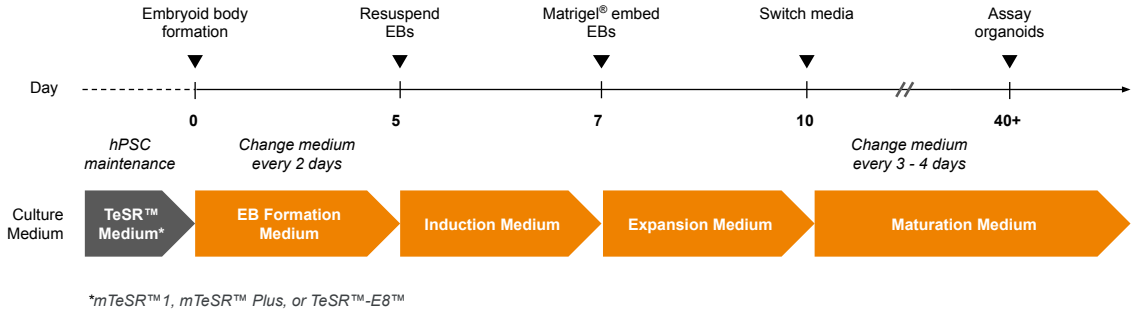


Figure 69. Schematic for Generating Unpatterned Neural Organoids Using the STEMdiff™ Cerebral Organoid Kit

The protocol for generating human cerebral organoids using the STEMdiff™ Cerebral Organoid Kit involves EB formation followed by neural induction. After embedding in extracellular matrix, the neuroepithelia proliferate and expand. Organoids are then matured and can be maintained for extended periods over 40 days with the STEMdiff™ Cerebral Organoid Maturation Kit. Based on the protocol published by MA Lancaster and JA Knoblich.[21]

STEMdiff™ Dorsal and Ventral Forebrain Organoid Kits

Robustly generate three-dimensional, patterned brain organoid cultures from human pluripotent stem cells without matrix embedding. The STEMdiff™ Dorsal (Catalog #08620) and Ventral (Catalog #08630) Forebrain Organoid Differentiation Kits are serum-free cell culture media that work well with embryoid bodies (EBs) generated with AggreWell™ (Catalog #34811) to differentiate brain-region-specific organoids that are representative of the developing human forebrain.

The STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit generates tissue of the early developing dorsal pallium, while the STEMdiff™ Ventral Forebrain Organoid Differentiation Kit generates tissue of the early developing ventral subpallium.

For extended periods of organoid culture (> 50 days), the components required for organoid maintenance are available as the STEMdiff™ Neural Organoid Maintenance Kit (Catalog #08571).

Learn more at www.stemcell.com/DFOrganoid

Learn more at www.stemcell.com/VFOrganoid

Why Use the STEMdiff™ Dorsal and Ventral Forebrain Organoid Kits?

- Reduce handling and media waste with fusion-free growth media
- Obtain greater analytic sensitivity for disease phenotypes with reproducible morphology between lines and individual organoids
- Eliminate matrix embedding steps with the matrix-free formulation and protocol
- Achieve long-term culture survival and reduced caspase-3 expression for neurotoxicity and neurodegenerative models
- Combine modular region-patterned organoids to generate advanced AssemBloids™ for disease modeling and regenerative applications

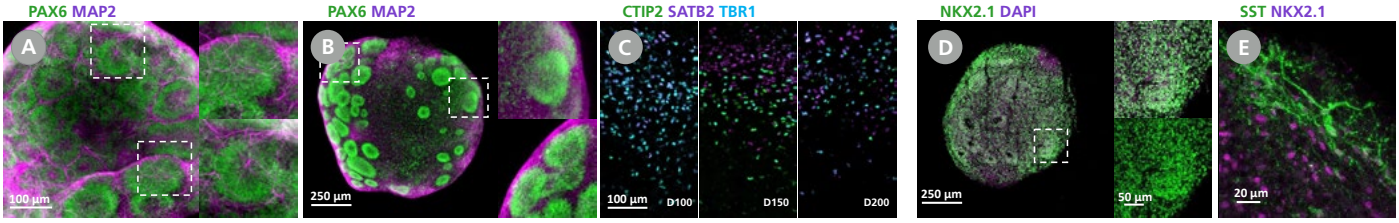


Figure 70. Dorsal Forebrain Organoids Exhibit Cortical Layering, and Both Dorsal and Ventral Organoids Express Markers Characteristic of Their Respective Brain Regions

(A) Day 25 dorsal forebrain organoids display multiple cortical-like regions marked by radialized PAX6+ cells surrounded by MAP2 neurons. (B) Day 50 dorsal forebrain organoids continue to display multiple cortical-like regions marked by PAX6 and MAP2. (C) Dorsal forebrain organoids cultured for 100 - 200 days show increasing separation of deep-layer neurons (CTIP2, TBR1) from upper-layer neurons (SATB2). (D) Ventral forebrain organoids at Day 25 exhibit a high level of expression of NKX2.1. (E) Somatostatin (SST)-positive GABAergic interneurons can be seen by Day 75.

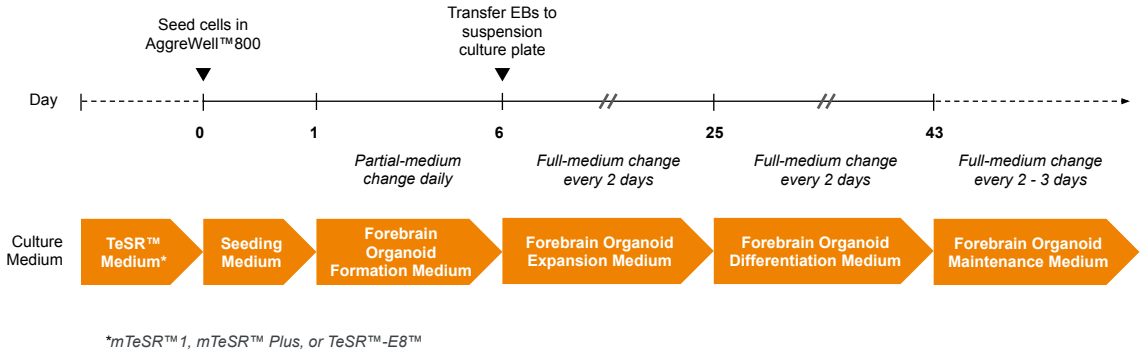


Figure 71. Schematic for the STEMdiff™ Dorsal and Ventral Forebrain Organoid Differentiation Kits

Human ES or iPS cell-derived dorsal forebrain or ventral organoids can be generated in 43 days. Embryoid bodies can be created in 6 days with AggreWell™800 plates. The EBs are then cultured in suspension, allowing growth and subsequent patterning to the dorsal forebrain. For patterning to ventral forebrain, the protocol differs only by a supplement added to Forebrain Organoid Expansion Medium. For the long-term maintenance and further maturation of dorsal and forebrain organoids, see the Product Information Sheet. Adapted from protocols by Sergiu Paşca.[22]

STEMdiff™ Choroid Plexus Organoid Kits

Take an in vitro approach to human neural biomarker discovery and CNS permeability with hPSC-derived organoids patterned to the choroid plexus. After a maturation period, organoids generated using the STEMdiff™ Choroid Plexus Differentiation Kit (Catalog #100-0824) feature cystic structures filled with a fluid resembling cerebrospinal fluid (CSF) and surrounded by an epithelial layer expressing ependymal markers (TTR, CLIC6, AQP1).

For extended periods of organoid culture (> 40 days), the components required for organoid maturation can be purchased as the STEMdiff™ Choroid Plexus Organoid Maturation Kit (Catalog #100-0825). To facilitate embedding of 3D aggregates, this media is compatible with the Organoid Embedding Sheet (Catalog #08579).

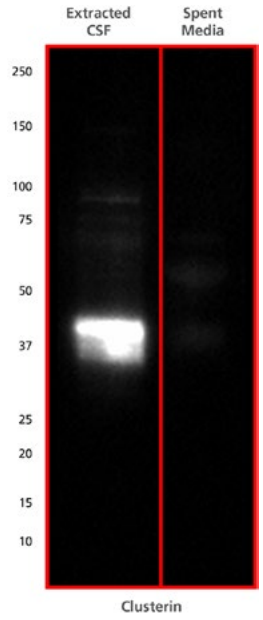


Figure 72. Fluid Extracted from Cysts in Choroid Plexus Organoids Is Enriched with Clusterin Protein, a Marker of Cerebrospinal Fluid (CSF)

Day 40 choroid plexus organoids were generated from hPSCs using the STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits. CSF-like fluid was extracted from cysts contained in Day 40 choroid plexus organoids using a 28G syringe. A western blot was performed on the extracted fluid to detect clusterin and shows a band between the 37 and 50 kDa molecular weight marker. Clusterin is a soluble secreted chaperone protein and biomarker relevant to Alzheimer's disease[23] found in high abundance in CSF.

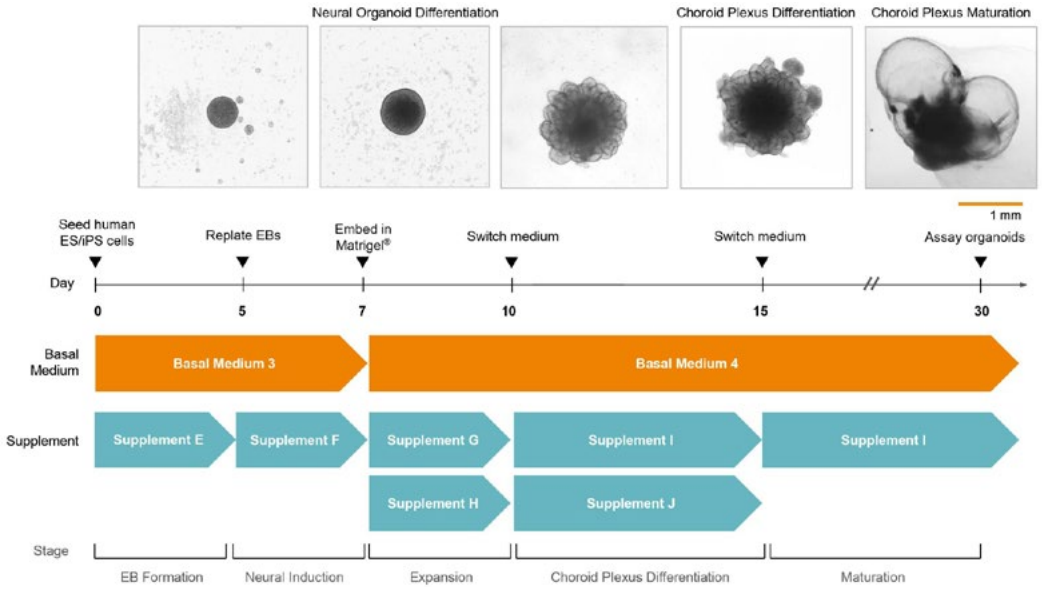


Figure 73. Schematic for the STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits

Choroid plexus organoids can be generated from human embryonic stem (ES) or induced pluripotent stem (iPS) cells in 30 days. The protocol begins with embryoid body (EB) formation, followed by expansion of neuroepithelia and patterning to choroid plexus-like epithelium. After a period of epithelial maturation, including extensive bubbling, the organoids develop cystic structures surrounded by an ependymal epithelial layer and filled with a fluid resembling cerebrospinal fluid (CSF). Adapted from protocols published by Pellegrini et al.[24]

Learn more at www.stemcell.com/choroid-plexus-organoid

BrainPhys™ Neuronal Medium

Culture Active Neurons Under Physiological Conditions

Efficiently generate neurons from hPSC-derived NPCs using BrainPhys™ Neuronal Medium (Catalog #05790) and supplements. Using BrainPhys™ Neuronal Medium as the basal medium for hPSC-derived NPC differentiation and neuronal maturation will generate a more neurophysiologically active culture that better represents the human brain environment.[25]

Published protocols using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (Catalog #05792; based on the published B27 formulation[26]) and N2 supplement (Catalog #05793),[27] as well as various cytokines and small molecules, are available for the generation of many neuronal subtypes.

BrainPhys™ Neuronal Medium may also be used to culture induced neurons derived through lineage conversion of somatic cells (i.e. without transitioning through an hPSC intermediate) or through forced Ngn2 expression in hPSCs.[25]

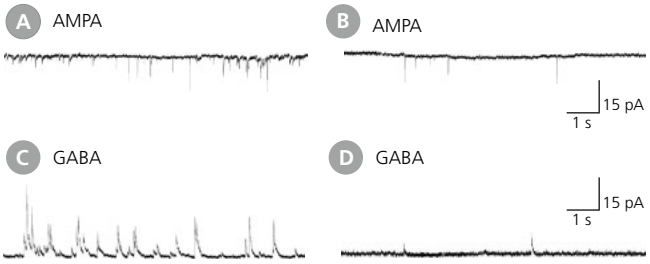


Figure 74. hPSC-Derived Neurons Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity by 44 Days

NPCs were generated from H9 cells using STEMdiff™ Neural Induction Medium in an EB-based protocol. Next, NPCs were cultured for 44 days in vitro in (A,C) BrainPhys™ Neuronal Medium, supplemented with 2% NeuroCult™ SM1 Supplement, 1% N2 Supplement-A, 20 ng/mL GDNF, 20 ng/mL BDNF, 1 mM db-cAMP, and 200 nM ascorbic acid to initiate neuronal differentiation, or (B,D) in DMEM/F12 under the same supplementation conditions. (A,C) Neurons matured in BrainPhys™ Neuronal Medium showed spontaneous excitatory (AMPA-mediated; A) and inhibitory (GABA-mediated; C) synaptic events as measured by patch clamp analysis. The frequency and amplitude of spontaneous synaptic events is consistently greater in neuronal cultures matured in BrainPhys™ Neuronal Medium, compared to neurons plated and matured in DMEM/F12 (B,D). Traces are representative. hPSC-derived neurons have been successfully matured in BrainPhys™ Neuronal Medium for up to 126 days in vitro.

Learn more at www.BrainPhys.com

Why Use BrainPhys™ Neuronal Medium?

- Create more physiological culture conditions with a medium that mimics the brain's extracellular environment
- Improve neuronal function and yield a higher proportion of synaptically active neurons
- Perform functional assays without changing media and shocking cells
- Support long-term culture of ES/iPS cell- and CNS-derived neurons
- Ensure consistent results with a medium that passes rigorous raw material screening and quality control to ensure minimal lot-to-lot variability

NeuroFluor™ NeuO

Selectively Label Live Neurons

NeuroFluor™ NeuO (Catalog #01801) is a membrane-permeable fluorescent probe that selectively labels primary and pluripotent stem cell-derived neurons in live cultures.[28] Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications.

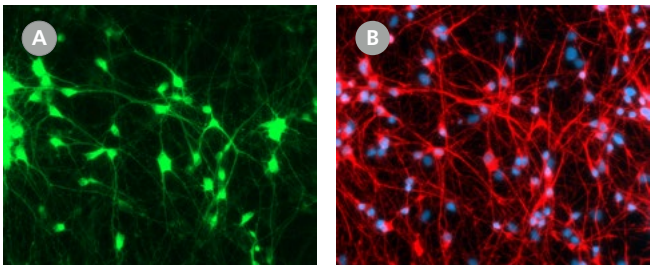


Figure 75. NeuroFluor™ NeuO Selectively Labels hPSC-Derived Neurons

(A) Neuronal precursors generated from hPSC-derived (XCL-1) NPCs were cultured in STEMdiff™ Neuron Maturation Medium. After 18 days of culture, hPSC-derived neurons were labeled with NeuroFluor™ NeuO (green). (B) The same culture was later fixed and immunostained for class III β-tubulin (red). Nuclei are counterstained with DAPI. The images show that NeuroFluor™ NeuO specifically labels class III β-tubulin-positive neurons.

Learn more at www.stemcell.com/NeuO-imaging

STEMdiff™ Neural Crest Differentiation Kit

Generate Pure Populations of Neural Crest Cells

The STEMdiff™ Neural Crest Differentiation Kit (Catalog #08610) consists of a serum-free basal medium and supplement for highly efficient and reproducible differentiation of hPSCs into neural crest cells (NCCs).

Further expansion of the NCC population is possible for up to 3 passages using the STEMdiff™ Neural Crest Differentiation Kit or MesenCult™-ACF Plus Medium (Catalog #05445), depending on the desired downstream application.

The NCCs produced using this kit are multipotent and can be further differentiated to cell types of both the neural and ecto-mesenchymal lineages. Sensory neurons expressing peripherin and BRN3A may be generated using STEMdiff™ Sensory Neuron Differentiation (Catalog #100-0341) and Maturation (Catalog #100-0684) Kits (Figure 76D), with potential applications for pain research.

Passaging NCCs into MesenCult™-ACF Plus Medium allows for differentiation to the chondrogenic lineage using the MesenCult™-ACF Chondrogenic Differentiation Kit (Catalog #05455) (Figure 76E), to the osteogenic lineage using MesenCult™ Osteogenic Differentiation Kit (Catalog #05465) (Figure 76F), and to the adipogenic lineage using MesenCult™ Adipogenic Differentiation Kit (Catalog #05412).

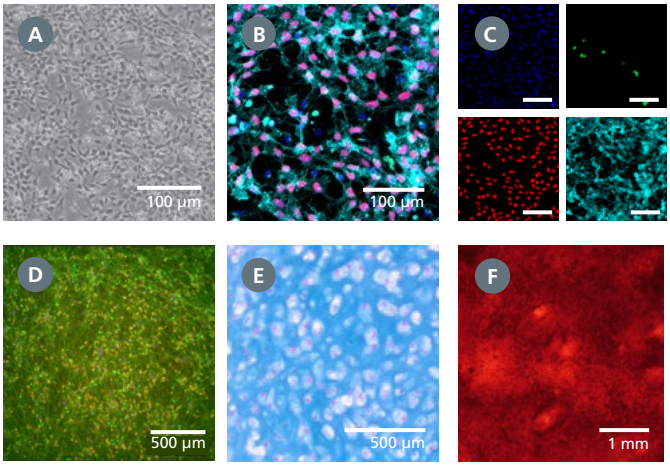


Figure 76. STEMdiff™ Neural Crest Differentiation Kit Generates a Highly Pure Population of Multipotent NCCs

After 6 days in culture, neural crest cells (A) display typical morphology, (B) express relevant markers (SOX10+, red; CD271+, light blue, DAPI, dark blue), and outnumber central nervous system (CNS)-type progenitors (PAX6+, green), assayed 2 days after a Day 6 passage. (C) Individual immunofluorescence channels for (B). (D) Culturing NCCs using STEMdiff™ Sensory Neuron Kits generates peripheral neurons (PRPH, green; BRN3a, red; DAPI, blue). (E) Passaging NCCs into MesenCult™-ACF Plus Medium and then into the MesenCult™-ACF Chondrogenic Differentiation Kit generates a chondrocyte pellet (Alcian Blue, Nuclear Fast Red) with deposition of cartilage around the cells. (F) Passaging NCCs into MesenCult™-ACF Plus Medium and then into the MesenCult™ Osteogenic Differentiation Kit (Human) generates an osteoblast culture with high levels of alizarin red-positive mineral deposition. Scale bar = (A-C) 100 µm, (D-E) 500 µm, (F) 1 mm.

Learn more at www.stemcell.com/NCKit

STEMdiff™ Sensory Neuron Differentiation and Maturation Kits

Peripheral neurons expressing PRPH and BRN3A can be generated using the serum-free STEMdiff™ Sensory Neuron Differentiation Kit (Catalog #100-0341) and STEMdiff™ Sensory Neuron Maturation Kit (Catalog #100-0684). With BrainPhys™ (Catalog #05790) providing physiological glucose levels and osmolarity, the neurons exhibit activity in response to sensory ligands and temperature changes.

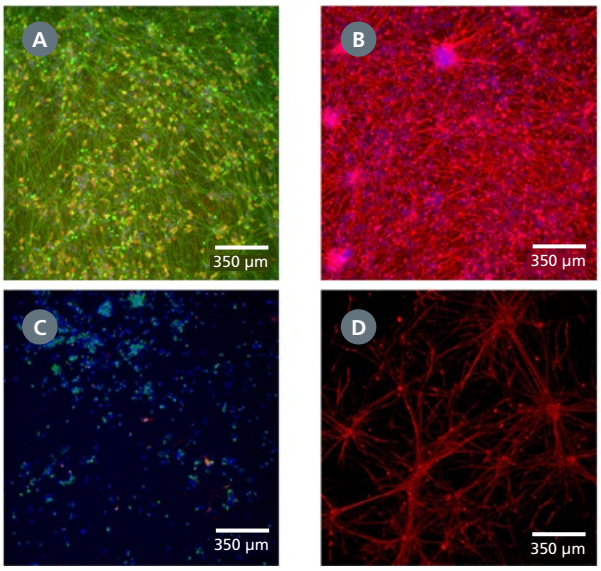


Figure 77. Sensory Neurons of the Peripheral Nervous System Can Be Generated Using STEMdiff™ Sensory Neuron Kits

NCCs generated from hPSCs in mTeSR™ Plus using the STEMdiff™ Neural Crest Differentiation Kit for 6 days were differentiated and matured to sensory neurons (SNs) using the STEMdiff™ Sensory Neuron Differentiation and Maturation Kits for 6 days each. (A) The resulting cultures contain a population of cells expressing SN markers peripherin (green) and BRN3A (red) along with (B) neuronal marker class III β-tubulin (TUJ1, red). (C) Midbrain neuron controls generated with STEMdiff™ Midbrain Neuron Differentiation and Maturation Kits do not have detectable peripherin (green) or BRN3A (red) expression, although they express (D) neuronal marker class III β-tubulin (TUJ1, red). Nuclei are labeled with DAPI (blue).

Learn more at www.stemcell.com/stemdiff-sensory-neuron

STEMdiff™ Microglia Culture System

Differentiate to Microglia from hPSCs

The STEMdiff™ Microglia Differentiation (Catalog #100-0019) and Maturation (Catalog #100-0020) Kits consist of a serum-free basal medium and supplements for highly efficient and reproducible generation of microglia from hPSCs via a hematopoietic progenitor cell (HPC) intermediate.

These kits are optimized for use on HPCs generated with the STEMdiff™ Hematopoietic Kit (Catalog #05310), taking 28 days to generate functional microglia.

Microglia produced using the STEMdiff™ Microglia Culture System are versatile tools for studying human neurological development, neuroimmune responses, and disease, in particular for modeling neuroinflammation and neurodegeneration. Cells can also be applied in both 2D and 3D co-culture with other neuronal cell types.

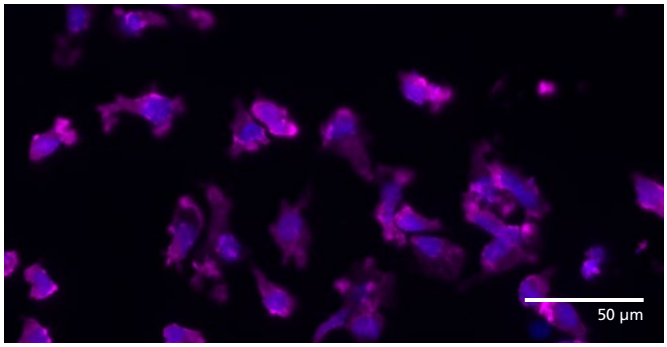


Figure 78. Microglia Generated with STEMdiff™ Microglia Culture System Show Expected Morphology and Markers

Microglia (STiPS-M001 cell line) cultured for 24 days in STEMdiff™ Microglia Differentiation Medium followed by 4 days in STEMdiff™ Microglia Maturation Medium express IBA1 (magenta; DAPI, blue). Based on the protocol from the laboratory of Mathew Blurton-Jones,[29] the resulting cells are a highly pure population of microglia (at least 80% CD45/CD11b-positive, and at least 50% TREM2-positive cells) with no more than 20% morphologically distinct monocytes or macrophages. The microglia also express other expected markers, such as TMEM119, and APOE (data not shown).

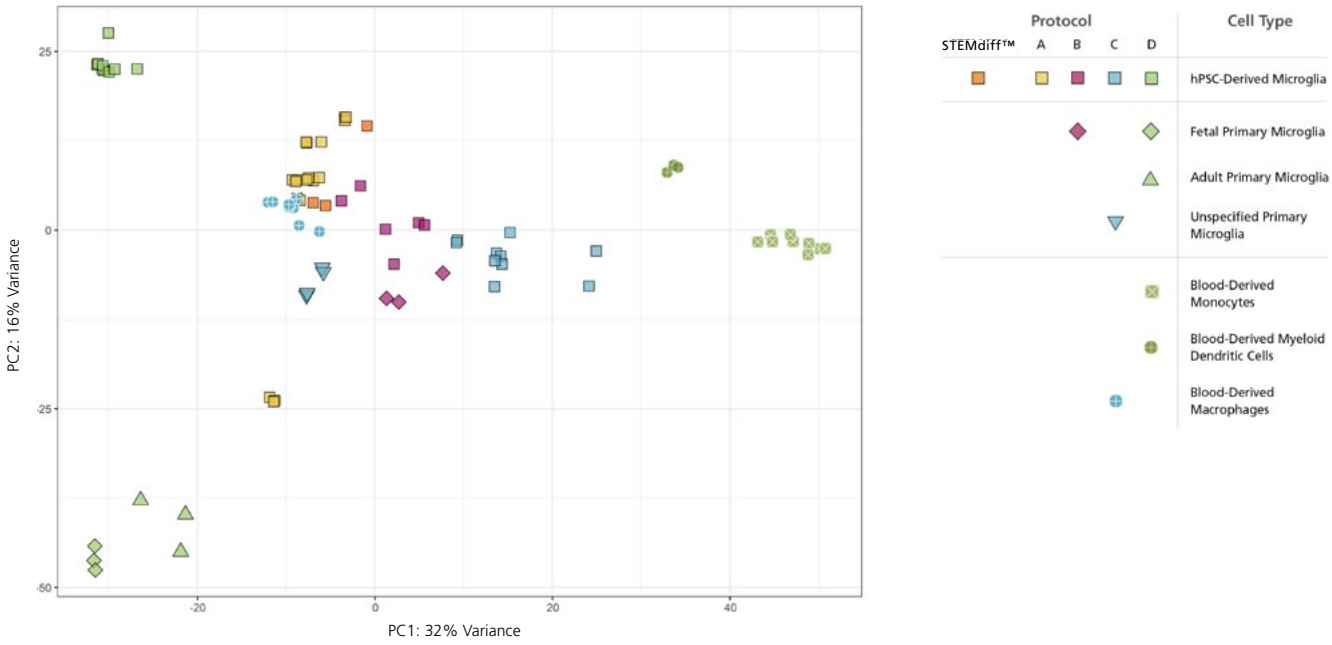


Figure 79. Microglia Generated with STEMdiff™ Microglia Culture System Are Typical PSC-Derived Microglial Cells

RNA-seq datasets of hPSC-derived and primary microglia and other immune cell types were extracted from 4 different publications (Protocols A-D). Principal component analysis (PCA) was performed on these data along with RNA-seq data from microglia generated with the STEMdiff™ Microglia Culture System. The hPSC-derived microglia from STEMdiff™ Microglia Culture System plot most closely to those from Protocols A and B.

Learn more at www.stemcell.com/microglia

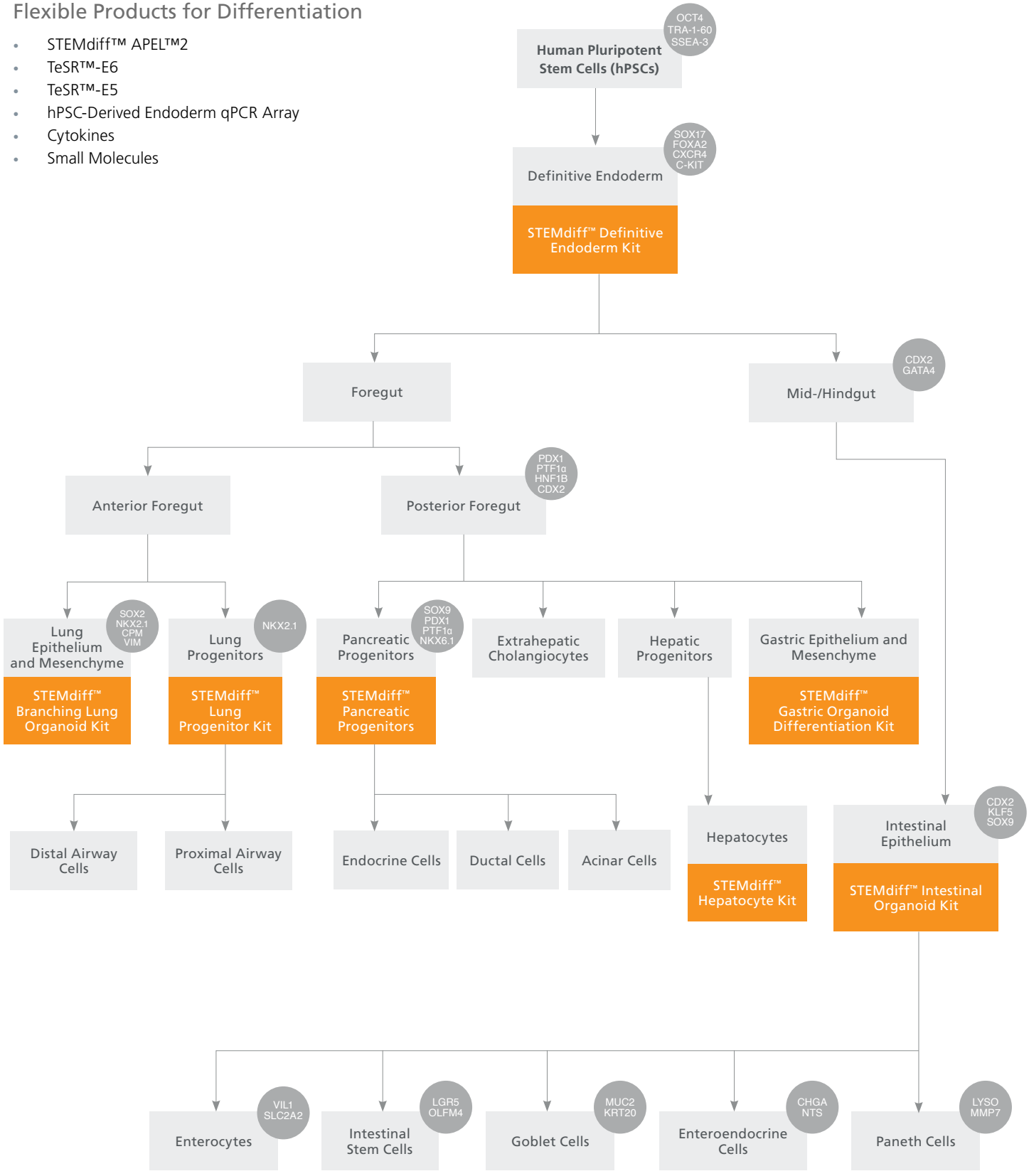
PROTOCOL

How to Tri-Culture hPSC-Derived Forebrain Neurons, Astrocytes, and Microglia

Endoderm Differentiation Pathways

Flexible Products for Differentiation

- STEMdiff™ APEL™2
- TeSR™-E6
- TeSR™-E5
- hPSC-Derived Endoderm qPCR Array
- Cytokines
- Small Molecules



STEMdiff™ Definitive Endoderm Kit

Quickly and Easily Differentiate Definitive Endoderm

The STEMdiff™ Definitive Endoderm Kit (Catalog #05110) is a serum-free, animal component-free system that enables differentiation of hPSCs to multipotent definitive endoderm cells using a short and simple protocol. This product is available in formulations optimized for use with hPSCs cultured in mTeSR™ Plus (Catalog #100-0276), mTeSR™1 (Catalog #85850), or TeSR™-E8™ (Catalog #05990). Definitive endoderm cells generated with this kit can be further differentiated to multiple downstream endodermal cell types, including hepatic[30] and pancreatic[31] progenitor cells for drug development, toxicity testing, research for development of cell-based therapies, or studying developmental pathways.

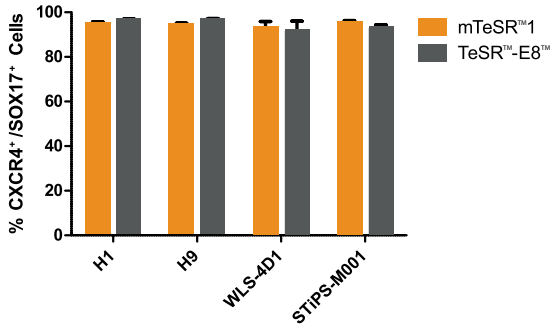


Figure 80. Definitive Endoderm Differentiation Is Efficient Across Multiple Human ES and iPS Cell Lines, Regardless of hPSC Maintenance Medium

Quantitative analysis of definitive endoderm formation in multiple human ES (H1 and H9) and iPS (WLS-4D1 and STiPS-M001) cell lines, as measured by co-expression of CXCR4 and SOX17. Cells maintained in mTeSR™1 medium were differentiated using STEMdiff™ Definitive Endoderm Kit, and cells maintained in TeSR™-E8™ were differentiated using STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized). Data are expressed as the mean percentage of cells expressing both markers. Error bars indicate SEM; n = 4 to 18 per cell line.

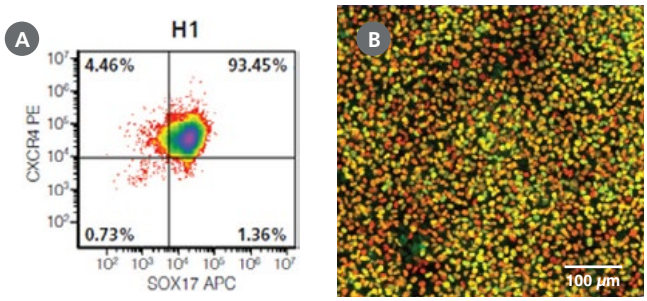


Figure 81. hPSCs Differentiated with STEMdiff™ Definitive Endoderm Kit Are Highly Enriched for Expression of Key Definitive Endoderm Markers

(A) Representative density plot showing CXCR4 and SOX17 expression in mTeSR™1-cultured H1 ES cells, following 5 days of differentiation (B) Representative image of FOXA2 (green) and SOX17 (red) in WLS-4D1 iPS cells following 4 days of differentiation. Yellow indicates cells co-expressing FOXA2 and SOX17.

Learn more at www.stemcell.com/STEMdiff-DE

hPSC-Derived Endoderm qPCR Array

The hPSC-Derived Endoderm qPCR Array (Catalog #07531) provides a validated 90-gene assay to characterize definitive endodermal progenitor cells and their differentiated progeny, including pancreatic, hepatic, and intestinal cells. Housekeeping controls and a synthetic DNA positive control are included. Data analysis is streamlined with our flexible online app (**www.stemcell.com/qPCRanalysis**).

Learn more at www.stemcell.com/DE-array

STEMdiff™ Intestinal Organoid Kit

Differentiate Human ES and iPS Cell Lines to Intestinal Organoids

hPSC-derived organoids provide direct relevance to human tissues while retaining the genotype and phenotype of donor cells.

The STEMdiff™ Intestinal Organoid Kit (Catalog #05140) enables the culture of intestinal organoids from embryonic stem (ES) or induced pluripotent stem (iPS) cells within 30 days. These organoids incorporate the key cell types and features of the developing intestinal epithelium, including the incorporation of some mesenchymal components. Intestinal organoids can be expanded and maintained in culture through passaging, or cryopreserved for future experiments.

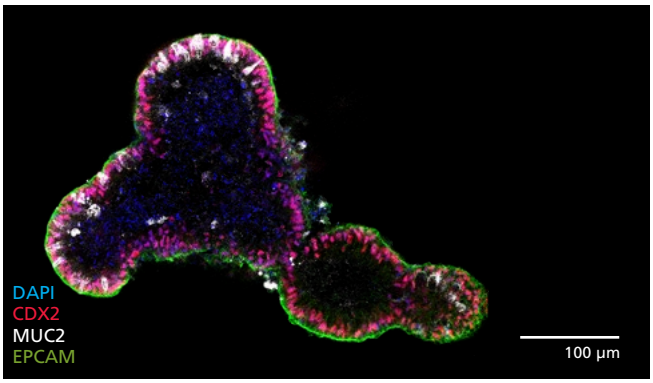


Figure 82. hPSC-Derived Intestinal Organoids Incorporate Features of the Intestinal Epithelium and Mesenchyme

Organoids grown using STEMdiff™ Intestinal Organoid Kit display markers of the intestinal epithelium (EPCAM, CDX2, MUC2). Organoids also exhibit markers for intestinal mesenchyme and intestinal progenitor cells.

Why Use the STEMdiff™ Intestinal Organoid Kit?

- Generate small intestinal organoid cultures that model the developing intestinal epithelium and associated mesenchyme
- Differentiate human ES and iPS cell lines from multiple sources or donors with high efficiency
- Maintain intestinal organoids through long-term passaging while allowing cryopreservation for experimental flexibility
- Reduce experimental variability by removing serum-containing components

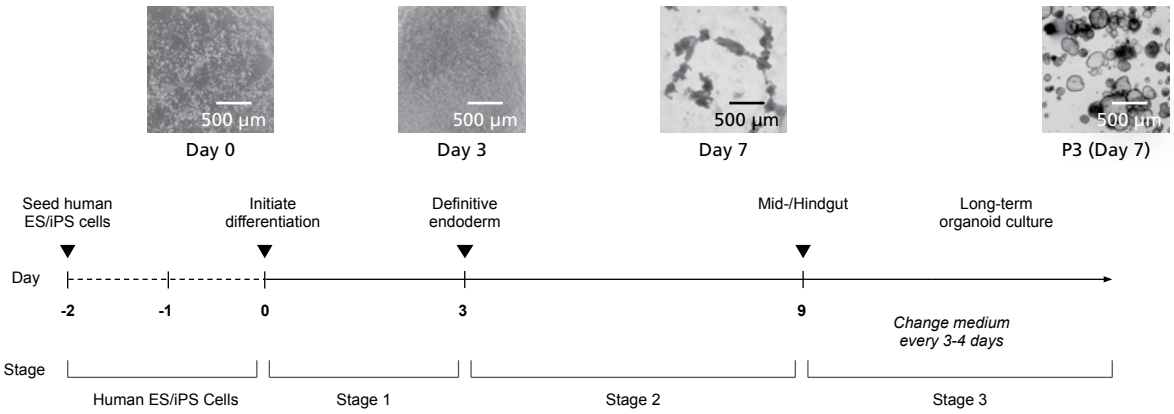


Figure 83. Schematic for Differentiating from hPSCs to Human Intestinal Organoids with the STEMdiff™ Intestinal Organoid Kit

hPSC cultures progress through a three-stage differentiation process to generate human intestinal organoids. By Day 3 of the protocol, cultures exhibit characteristics typical of definitive endoderm and mid-/hindgut differentiation is initiated. During mid-/hindgut differentiation (Days 5 - 7), cells form mid-/hindgut spheroids that are released from the cell monolayer into the culture medium. These spheroids are collected, embedded in extracellular matrix, and cultured in STEMdiff™ Intestinal Organoid Growth Medium to mature into intestinal organoids. Days in parentheses indicate days post-embedding in a given passage.

Learn more at www.stemcell.com/STEMdiff-HIO

STEMdiff™ Gastric Organoid Differentiation Kit

Culture medium kit for differentiation of human gastric organoids

Reliably generate hPSC-derived gastric organoids for studying gastric development, inflammation, regeneration, microbial interaction, or for disease modeling. With the STEMdiff™ Gastric Organoid Differentiation Kit (Catalog #100-0475), you can grow and expand organoids across hPSC lines with high efficiency and reproducibility, to form a convenient model system with direct relevance to the developing stomach.

Why Use the STEMdiff™ Gastric Organoid Differentiation Kit?

- Generate gastric organoids that provide a human-specific model system for studying the developing gastric epithelium and associated mesenchyme
- Differentiate human ES and iPS cell lines from multiple sources or donors with high efficiency
- Maintain gastric organoids through long-term passaging while allowing cryopreservation for experimental flexibility
- Reduce experimental variability by removing serum-containing components

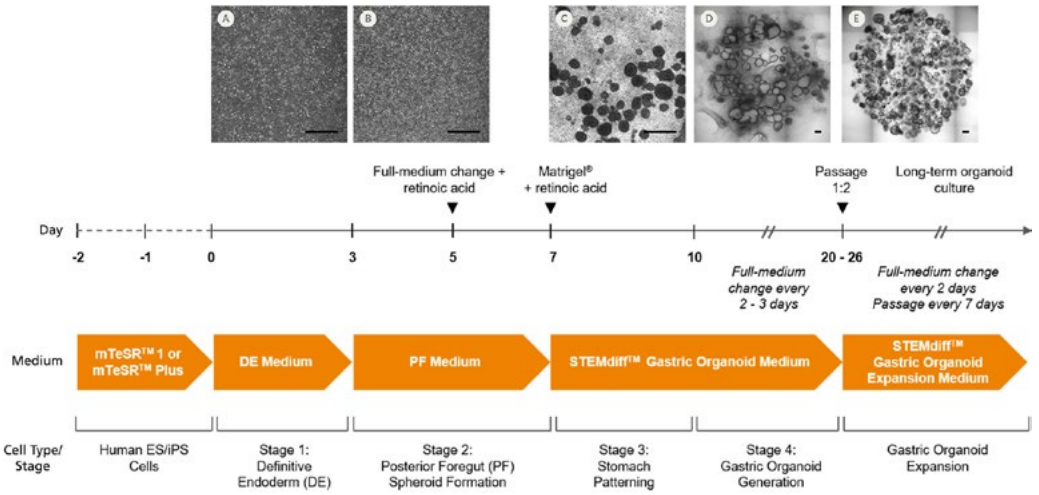


Figure 84. Schematic for Generation of Human Gastric Organoid Cultures Using STEMdiff™ Gastric Organoid Differentiation Kit

hPSCs were seeded as small aggregates (50 - 200 µm) at low density (4000 aggregates/well) in mTeSR™1 or mTeSR™ Plus on Corning® Matrigel®-coated 24-well plates and allowed to attach overnight. Two-dimensional (2D) monolayer cultures were maintained with daily mTeSR™1 medium changes until a near-confluent monolayer (85 - 90%) was achieved. (A) On Day 0, differentiation was initiated by replacing the medium with STEMdiff™ Definitive Endoderm (DE) Medium (Stage 1), then daily medium changes were performed. (B) On Day 3, DE Medium was removed and replaced with STEMdiff™ Gastric Posterior Foregut (PF) Medium (Stage 2). On Day 5, retinoic acid (RA) was added to PF Medium. (C) On Day 7 of differentiation, floating posterior foregut spheroids were harvested from the supernatant and embedded into Corning® Matrigel®. Between Days 7 and 10, embedded PF spheroids were cultured in STEMdiff™ Gastric Organoid Medium + RA (Stage 3). Between Days 10 and 26, spheroids were matured to gastric organoids surrounded by mesenchyme in STEMdiff™ Gastric Organoid Medium. (D) Between Days 20 and 26, gastric organoids were passaged for full differentiation in STEMdiff™ Gastric Organoid Medium until expression of gastric markers was observed (~Day 34) and/or (E) expanded in STEMdiff™ Gastric Organoid Expansion Medium to be used for downstream applications or cryopreserved for future experiments. Scale bars = 500 µm.

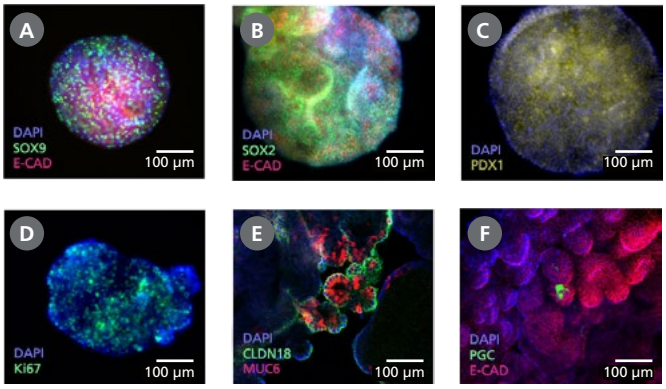


Figure 85. Immunohistochemistry Confirms Expression of Gastric-Specific Markers in Human Gastric Organoids Cultured in Gastric Organoid Expansion Medium

Representative organoids in Expansion Medium at passage 5 expressed progenitor markers (A) SOX9, (B) SOX2, and (C) PDX1; (A,B & F) epithelial marker E-CAD; (D) marker of proliferation Ki67; and (E) gastric tight junction marker CLDN18. (E) Presence of gland cells was detected by expression of MUC6 in the gland regions of the organoids. (F) Detection of scattered expression of PGC indicates differentiation of chief cells (n = 2 - 5).

Learn more at www.stemcell.com/stemdiff-gastric

STEMdiff™ Pancreatic Progenitor Kit

Produce Pancreatic Progenitor Cells from hPSCs

The STEMdiff™ Pancreatic Progenitor Kit (Catalog #05120) is a serum-free medium that supports efficient and reproducible generation of pancreatic progenitor cells from hPSCs. The kit directs efficient differentiation from multiple hPSC lines through definitive endoderm, primitive gut tube, and posterior foregut endoderm before transitioning to pancreatic progenitor cells. The differentiated cells are characterized by the expression of key transcription factors, including PDX-1, NKX6.1, and NEUROD1, and by the upregulation of insulin and glucagon (Figures 86 and 87). The resulting pancreatic progenitor cells can be further differentiated to both exocrine and endocrine cell fates, making them useful research tools for studying diabetes and β-cell maturation, disease modeling, and studying pancreatic cancer.

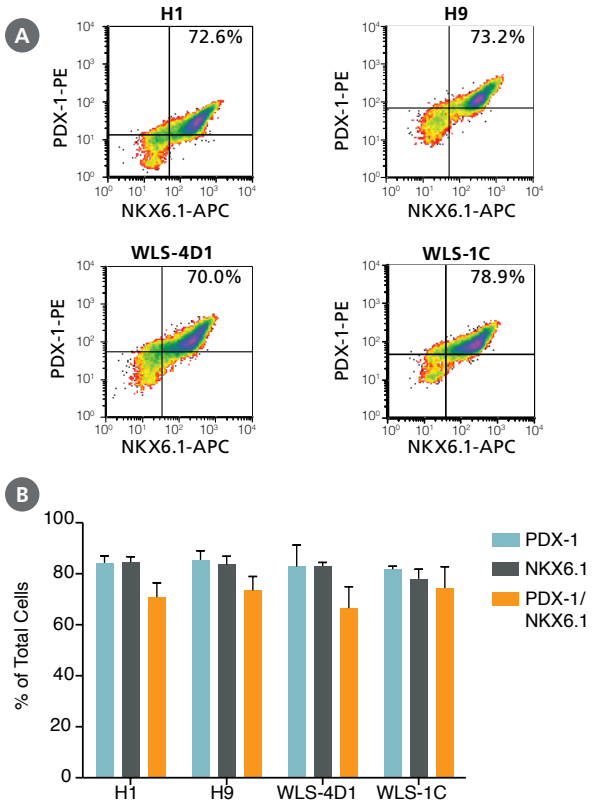


Figure 86. STEMdiff™ Pancreatic Progenitor Kit Efficiently Generates PDX-1, NKX6.1-Positive Progenitors Across Multiple hPSC Lines

PDX-1 and NKX6.1 expression measured in pancreatic progenitor cells derived from four different hPSC lines (H1, H9, WLS-4D1, and WLS-1C). (A) Representative flow cytometry plots for PDX-1 and NKX6.1 expression at the end of Stage 4. (B) Cumulative quantitative data for PDX-1 and NKX6.1 co-expression at the end of Stage 4 of differentiation (mean ± SD; n = 3 - 5 per cell line). The average efficiency of differentiation ranges from 66.5% to 74.5% depending on the cell line. The efficiency of conversion from definitive endoderm to pancreatic progenitor ranges from 77.3% to 96.3%. In addition, nearly all NKX6.1+ cells co-express PDX-1 as observed in the developing human pancreas.[31]

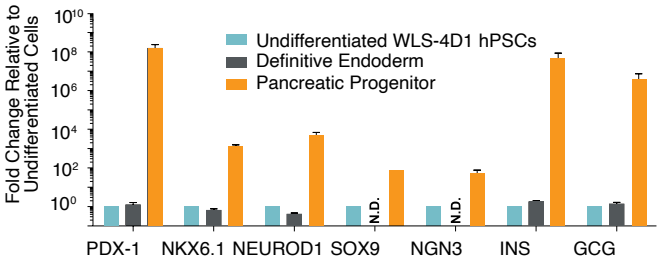


Figure 87. Gene Expression Profile Indicates Transition to Pancreatic Progenitor Cell

Gene expression profile of key transcription factors or hormones (INS: insulin, GCG: glucagon) expressed in pancreatic progenitor cells (mean ± SEM; n = 3 - 7 experiments on WLS-4D1 cells). Expression was first normalized to 18S ribosomal RNA and then to the expression level found in undifferentiated cells. Gene expression is shown for WLS-4D1 cells at the end of Stage 1 (Definitive Endoderm) and at the end of Stage 4 (Pancreatic Progenitor). Expression pattern is consistent with published data.[33] N.D.: Not Determined.

Learn more at
www.stemcell.com/STEMdiff-Pancreatic

STEMdiff™ Hepatocyte Kit

Differentiate Human PSCs to Hepatocyte-Like Cells

Generate a reliable supply of hepatocyte-like cells (HLCs) for your experiments by reproducibly differentiating hPSCs into HLCs. The serum-free formulation minimizes experimental variability by limiting the presence of undefined components, thus enabling you to robustly differentiate HLC cultures from a variety of hPSC lines. HLCs generated using the STEMdiff™ Hepatocyte Kit (Catalog #100-0520) are suitable for a variety of applications in liver research, disease modeling, and hepatotoxicity testing, and can be further expanded into 3D liver organoids for long-term maintenance, further differentiation, and cryopreservation.

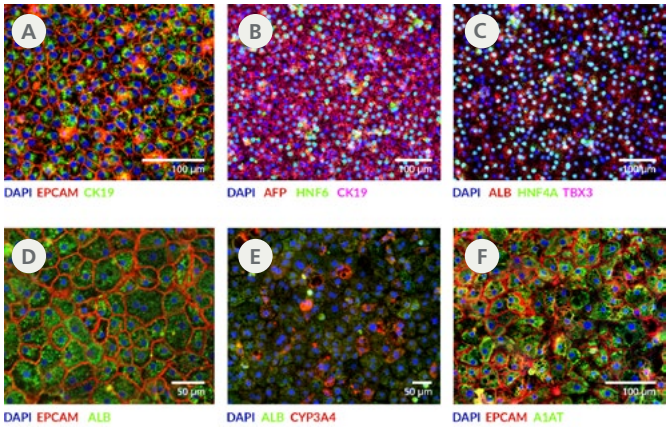


Figure 88. hPSC-Derived Hepatic Progenitor Cells and Hepatocyte-Like Cells Express Hepatic Markers As Confirmed by Immunocytochemistry Analysis

Cells cultured to Day 10 (HPs) and Day 21 (HLCs) were fixed with 4% paraformaldehyde and permeabilized before being stained with primary and secondary antibodies. (A-C) HPs expressed the epithelial marker EPCAM, ductal marker CK19, fetal serum protein AFP, the hepatic transcription factors HNF6 and HNF4a, and the stage-specific transcription factor TBX3. (C) By Day 10, some of the HPs also began to express the mature serum protein albumin. (D-F) Most HLCs expressed the mature hepatic markers ALB, CYP3A4, and A1AT by Day 21. HPs = Hepatic progenitors; HLCs = Hepatocyte-like cells; CK19 = Cytokeratin 19; AFP = Alpha fetoprotein; ALB = Albumin.

Why Use the STEMdiff™ Hepatocyte Kit?

- Generate mature hepatocyte-like cells (HLCs) that express key hepatic markers and demonstrate liver-specific activities
- Start from a variety of undifferentiated hPSC lines to efficiently establish HLC cultures
- Obtain HLCs that can be further expanded and differentiated in 3D organoid cultures using the HepatiCult™ Organoid Kit (Catalog #100-0386)
- Assess drug hepatotoxicity in HLC cultures, which have higher sensitivity than the immortalized cell line HepG2

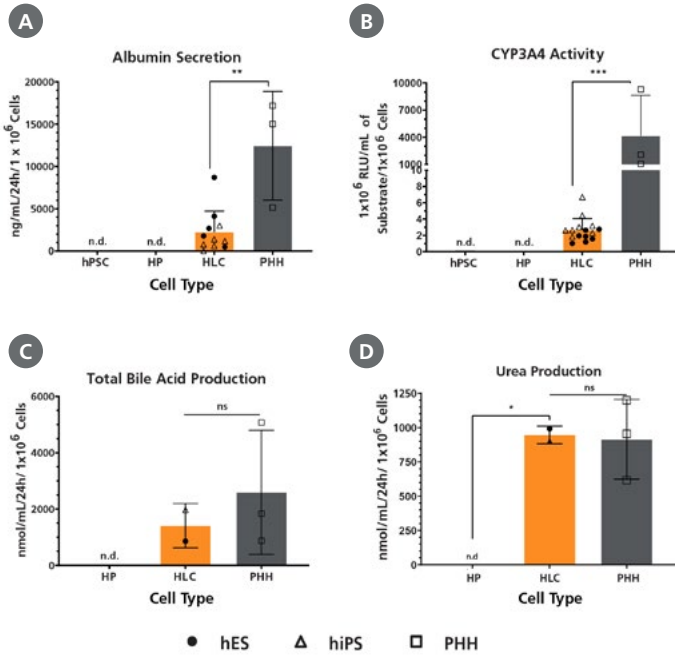


Figure 89. hPSC-Derived HLCs Exhibit Key Liver Functionalities

Upon maturation of HPs to HLCs, the cells acquired the ability to (A) synthesize and secrete serum protein albumin (n = 11), as detected by ELISA (Abcam Catalog #ab108788), (B) and exhibited CYP3A4 enzymatic activity (n = 15), as assessed using the P450-GloTM CYP3A4 Assay (Promega Catalog #V9002). (C) Day 21 HLCs were also capable of producing bile acids (n = 2) (D) and synthesizing and secreting urea (n = 2) at levels comparable to primary human hepatocytes (PHH; n = 3), as detected by colorimetric assays (Abcam Catalog #ab239702, ab83362, respectively). Error bars = SD. Ordinary one-way ANOVA used for statistical testing (*** represents an adjusted p-value of 0.0007, ** represents an adjusted p-value of 0.0011, * represents an adjusted p-value of 0.0179, ns = not significant). HPs = Hepatic progenitors; HLCs = Hepatocyte-like cells; PHH = Primary human hepatocyte.

Learn more at www.stemcell.com/STEMdiff-Hepatocyte

STEMdiff™ Lung Progenitor Kit

Generate hPSC-Derived Lung Progenitor Cells

The STEMdiff™ Lung Progenitor Kit (Catalog #100-0230) is a serum-free culture medium system for efficient and reproducible generation of lung progenitor cells from human ES and iPS cells. Differentiated cells will express NKX2.1, a key marker of lung progenitor cells. The resulting cells can be further matured toward proximal or distal airway cells, using published protocols, for the study of lung diseases and lung development.

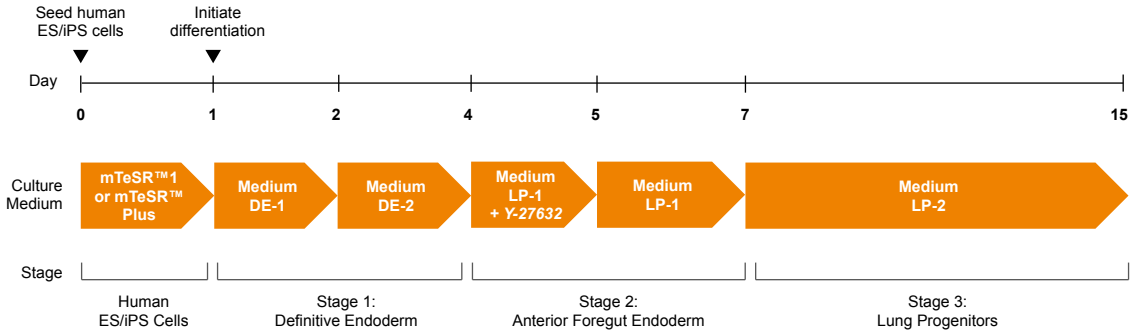


Figure 90. Schematic for Generating Lung Progenitor Cells from Human ES/iPS Cells Using STEMdiff™ Lung Progenitor Kit

hPSC cultures progress through a simple three-stage process to generate lung progenitor cells. hPSC clumps are first seeded in mTeSR™1. On Day 1, differentiation is initiated with Medium DE-1. Subsequently, on Day 2 and 3, the medium is changed to Medium DE-2 for definitive endoderm patterning. On Day 4, to initiate anterior foregut endoderm patterning, the endoderm monolayer is passaged in Medium LP-1 and Y-27632. Finally, at Day 7, the cells are differentiated into the lung progenitor stage with Medium LP-2. All media mentioned (DE-1, DE-2, LP-1, and LP-2) are included in the STEMdiff™ Lung Progenitor Kit.

STEMdiff™ Branching Lung Organoid Kit

Generate hPSC-Derived Branching Lung Organoids

The STEMdiff™ Branching Lung Organoid Kit (Catalog #100-0195) supports the efficient and reproducible generation of branching lung organoids from human pluripotent stem cells (hPSCs) through four stages of differentiation: 1) definitive endoderm, 2) anterior foregut endoderm, 3) lung bud organoids, and 4) branching lung organoids. The resulting organoids develop proximal and distal-like branching airway epithelial structures expressing EPCAM, NKX2.1, SOX2, SOX9, MUC1, and P63. Extended periods of organoid culture results in increased expression levels of mature lung cell markers such as SFTPC, SFTPB, and ABCA3.

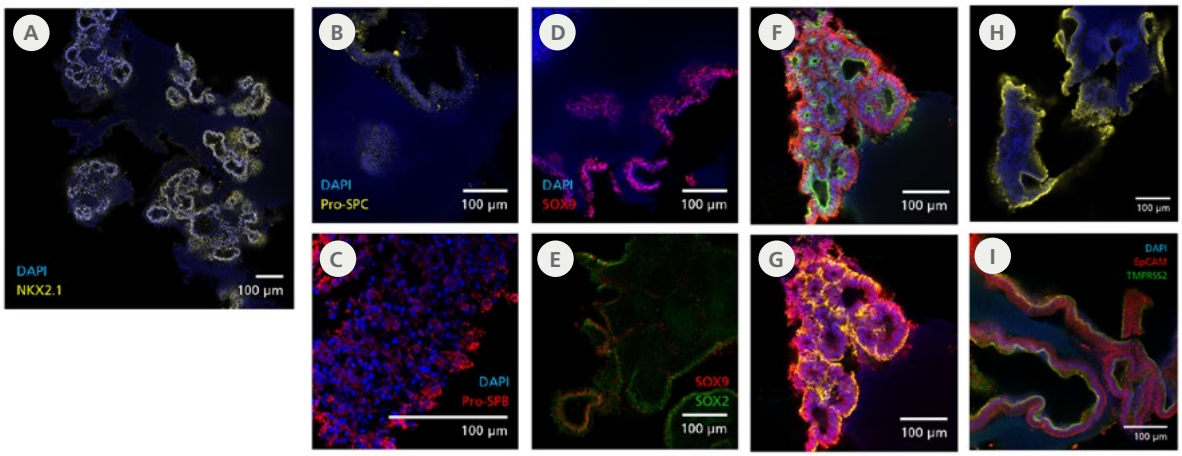


Figure 91. Branching Lung Organoids Cultured in STEMdiff™ Branching Lung Organoid Kit Feature Key Protein Markers and Exhibit Branching Morphogenesis

(A) Branching lung organoids express lung progenitor marker NKX2.1 throughout their branching structures and (B, C) demonstrate the presence of alveolar type II-like cells with pro-surfactant protein B and C expressions. (D, E) These organoids undergo proximodistal differentiation demonstrated by the differential expression of SOX2 and SOX9. (F) MUC1 can be found luminally expressed while the (G) organoids are surrounded by VIM-expressing mesenchyme. (H, I) Branching lung organoids generated with the STEMdiff™ Branching Lung Organoid Kit also express proteins associated with SARS-CoV-2 entry, ACE2, and TMPRSS2. Protein expression was visualized by immunohistochemistry and confocal microscopy of branching lung organoids on Day 63.

Learn more at www.stemcell.com/STEMdiff-Respiratory-Research

Flexible User-Directed Differentiation

STEMdiff™ APEL™2

STEMdiff™ APEL™2 Medium (Catalog #05270) is a fully defined, serum-free, and animal component-free medium for differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cells. It is based on the APEL formulation published by Ng et al.[34] and lacks undefined components, such as protein-free hybridoma medium. This medium can be used in adherent or embryoid body (EB)-based protocols, such as with AggreWell™ plates (see page 56). Appropriate induction factors must be added before use.

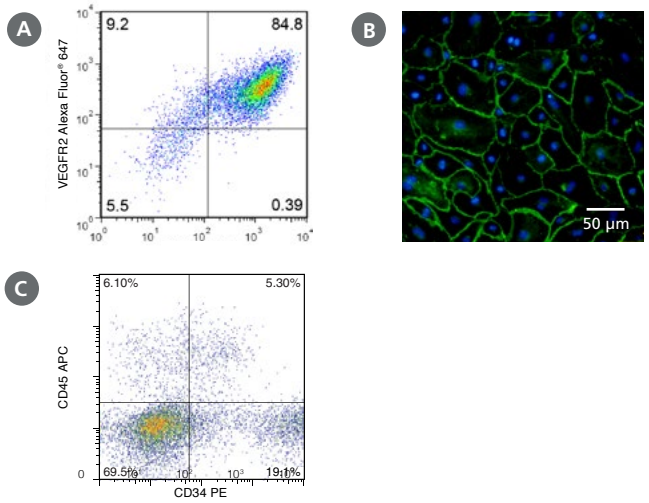


Figure 92. STEMdiff™ APEL™ Media Can Be Used for Customized Differentiation to Various Mesodermal Cell Lineages

(A) Endothelial differentiation of STiPS-F001 human iPS cells using STEMdiff™ APEL™ medium*, based on methods by Tan et al.[35] (B) Immunocytochemistry image of CD31 (green; nuclei shown in blue) in endothelial cells differentiated from H1 cells using STEMdiff™ APEL™ medium. Image courtesy of the Cao Tong lab, University of Singapore. (C) Hematopoietic differentiation of H9 cells, based on methods by Ng et al.[34] and Chadwick et al.[36] with the following changes: (1) STEMdiff™ APEL™ medium was used as the basal medium; (2) prior to differentiation, cells were maintained in mTeSR™1 on Matrigel®; (3) differentiation was performed in adherent cell culture on a Matrigel®-coated surface, instead of using an EB-based method.

*STEMdiff™ APEL™ has been updated to STEMdiff™ APEL™2, which lacks undefined components such as protein-free hybridoma medium.

Learn more at www.stemcell.com/APEL2

Why Use STEMdiff™ APEL™2?

- Ensure defined growth with this animal origin-free (AOF) formulation
- Tailor your differentiation protocols to your specific cells using this robust and published basal medium
- Differentiate to a variety of cell lineages, including hematopoietic, endothelial, and epithelial
- Benefit from versatility with adherent- or EB-based protocols

TeSR™-E5 and TeSR™-E6 Media

TeSR™-E5 (Catalog #05916) and TeSR™-E6 (Catalog #05946) are defined, serum-, and xeno-free media that are based on the formulation of TeSR™-E8™, but do not contain transforming growth factor b (TGFb) or basic fibroblast growth factor (bFGF). Additionally, TeSR™-E5 does not contain insulin. These formulations may be used as basal media for differentiation of human ES and iPS cells, or other applications where removal of the above cytokines and insulin is desirable.

Learn more at www.STEMdiff.com/#custom

Accessory Products

Small Molecules

Small molecules are increasingly being used as critical tools to understand stem cell biology. Whether used to affect reprogramming, self-renewal, or differentiation, the right small molecule can transform a research project. Choose from a wide variety of small molecules that are being widely used in high impact research to target the key pathways in stem cell biology.

For a complete listing and more details on the small molecules available, and to see how they are being used in high-impact studies, visit **www.stemcell.com/smallmolecules**.

Most Popular Small Molecules

MOLECULE	PATHWAY/ TARGET	APPLICATIONS	CATALOG #
Y-27632	RHO/ROCK pathway inhibitor Inhibits ROCK1, ROCK2	Maintenance	72302
CHIR99021	WNT pathway activator Inhibits GSK3	Reprogramming, Maintenance, Differentiation	72052
IWP-2	WNT pathway inhibitor Inhibits Porcupine	Differentiation	72122
LDN193189	BMP pathway inhibitor Inhibits ALK1, ALK2, ALK3, ALK6	Differentiation	72147
SB431542	Activin/BMP/TGF-β pathway inhibitor Inhibits ALK4, ALK5, ALK7	Reprogramming, Differentiation	72232
Thiazovivin	RHO/ROCK pathway inhibitor Inhibits ROCK	Reprogramming, Maintenance	72252
PD0325901	MEK/ERK pathway inhibitor Inhibits MEK	Reprogramming, Maintenance	72182
Purmorphamine	Hedgehog pathway activator Activates Smoothened	Differentiation	72202
DAPT	Notch pathway inhibitor Inhibits γ-secretase	Differentiation	72082
Prostaglandin E2	Prostanoid pathway activator Activates prostaglandin receptors EP1, EP2, EP3 and EP4	Differentiation	72192
A 83-01	Activin/NODAL/TGF-β pathway inhibitor Inhibits ALK5, ALK4, ALK7	Reprogramming, Maintenance	72022
SU5402	MEK/ERK pathway inhibitor Inhibits VEGFR2, FGFR1, PDGFRβ	Maintenance	73912

Cytokines

Cytokines are commonly used tools in lineage-specific differentiation protocols, as well as for self-renewal of hPSCs. For a complete listing of cytokines available, including animal component-free (ACF) versions, please visit **www.stemcell.com/cytokines**.

Most Popular Cytokines

PRODUCT	CATALOG #	
	Non-ACF	ACF*
Activin A[1]	78001	78132
B18R Protein	78075	-
bFGF	78003	78134
BMP-2	78004	78135
BMP-4	78211	-
DKK-1	78208.1	-
EGF[1]	78006	78136
EGFR	78171.1	-
Flt3/Flk-2 Ligand	78009	78137
Heregulin-beta 1	79071	-
IGF-I	-	78142
LIF	78055	78149
Noggin	78060	-
SCF	78062	78155
TGF-β1[1]	78067	-
VEGF-165	78073	78159
VEGF-121	78127	-
PDGF-DD	78222	-

*All ACF cytokines are human recombinant proteins produced in E. coli and are guaranteed free of animal or human components.
[1]International Units (IU) data available. Visit **www.stemcell.com/IU-data**.

AggreWell™ Plates

Reproducible Production of Uniform Embryoid Bodies

Many hPSC differentiation protocols begin with the formation of three-dimensional aggregates of cells called embryoid bodies (EBs). Conventional EB formation methods[37] result in EBs that are heterogeneous in size and shape (Figure 93A), leading to inefficient and uncontrolled differentiation.[38]

AggreWell™ plates provide an easy and standardized approach to the production of EBs. Each well contains microwells of defined size, making it easy to produce large numbers of highly uniform EBs (Figure 93B) and to ensure reproducibility of differentiation experiments.[39]

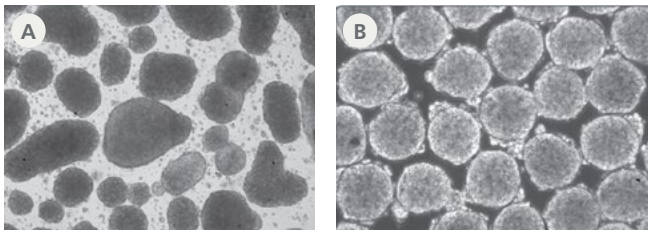


Figure 93. AggreWell™ Plates Are Used to Generate Uniform EBs

(A) Human EBs formed using conventional methods are heterogeneous in size and shape. (B) Human EBs formed using AggreWell™ plates are uniform in size and consistently spherical in shape. Shown are EBs generated with 2000 cells using AggreWell™400.

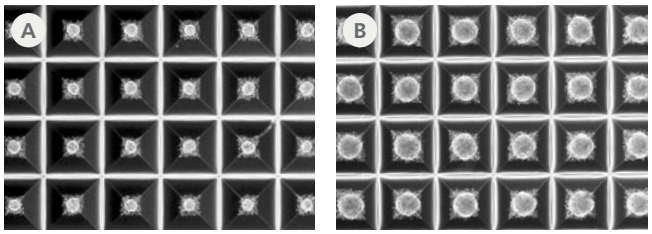


Figure 94. The Size of EBs Can Be Controlled in AggreWell™

Starting from a single cell suspension, hPSCs form EBs after 24 hours in AggreWell™. The size of the EB can be easily modified by adjusting the seeding density. Shown are EBs seeded at (A) 250 cells per microwell and (B) 1000 cells per microwell in AgreWell(TM)400.

Learn more at www.stemcell.com/AggreWell

AggreWell™ is available in 2 sizes of microwells: 400 µm (AggreWell™400) or 800 µm (AggreWell™800).

PRODUCT	MICROWELL SIZE	CELL RANGE	PLATE FORMAT	NUMBER OF EMBRYOID BODIES	CATALOG #
AggreWell™400	400 µm	50 - 3000 cells per EB	24-well plate	~ 1200 per well	34411/34415
			6-well plate	~ 5900 per well	34421/34425
AggreWell™800	800 µm	3000 - 20,000 cells per EB	24-well plate	~ 300 per well	34811/34815
			6-well plate	~1500 per well	34821/34825

Anti-Adherence Rinsing Solution (Catalog #07010) is required for optimal performance.

Antibodies

For Human Pluripotent Stem Cells and Differentiated Cells

Be confident in your experimental results, save valuable research time, and ensure experimental reproducibility by choosing antibodies from STEMCELL Technologies. Our high-quality primary and secondary antibodies are verified to work with our pluripotent stem cell reagents in specific applications, ensuring that your downstream cell analysis, including phenotyping and purity assessments, works consistently.

Popular Antibodies for hPSC Research

TARGET ANTIGEN	CLONE	ISOTYPE	CATALOG #
OCT4 (OCT3)	3A2A20	Mouse IgG2b	60093
OCT4 (OCT3)	40	Mouse IgG1	60059
SSEA-1 (CD15)	MC-480	Mouse IgM	60060
SSEA-3	MC-631	Rat IgM	60061
SSEA-4	MC-813-70	Mouse IgG3	60062
SSEA-5	8e11	Mouse IgG1	60063
TRA-1-60	TRA-1-60R	Mouse IgM	60064
TRA-1-81	TRA-1-81	Mouse IgM	60065
TRA-2-49	TRA-2-49/6E	Mouse IgG1	60066
TRA-2-54	TRA-2-54/2J	Mouse IgG1	60067

For a complete listing of antibodies and conjugates available, visit **www.stemcell.com/antibodies**.

GloCell™ Fixable Viability Dyes

For Live/Dead Cell Staining

GloCell™ Fixable Viability Dyes are fluorescent amine-labeling dyes for live/dead staining of mammalian cells, allowing clear exclusion of dead cells from flow cytometry data. These dyes are resistant to washing and fixation and are compatible with intracellular antibody staining protocols. Stained cells can also be cryopreserved without loss of fluorescence intensity.

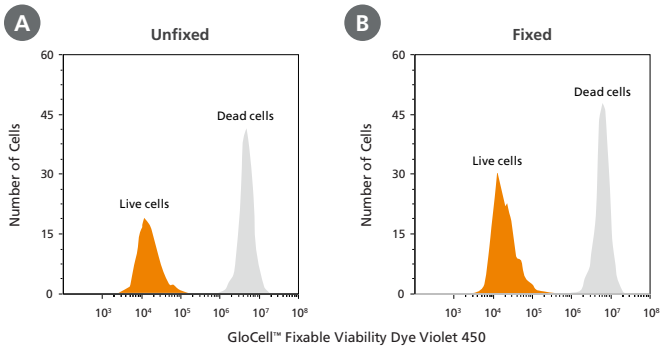


Figure 95. Fluorescence Signals in Unfixed and Fixed Cells Stained with GloCell™ Fixable Viability Dye Violet 450 Are Preserved

A mixture of live and dead (heat-shocked at 95°C for 30 minutes) WLS-1C human iPS cells were stained with GloCell™ Fixable Viability Dye Violet 450 (Catalog #75009) with or without fixation in 4% paraformaldehyde. After staining, (A) unfixed and (B) fixed cells were immediately analyzed by flow cytometry.

Learn more at www.stemcell.com/GloCell

Annexin V Dyes & Caspase 3/7 Assay Reagents

For Detection of Early-Stage Cell Apoptosis

Annexin V is a characteristic cell death marker that can be used to specifically detect early apoptotic mammalian cells. The Annexin V Apoptosis Detection Kit can be used for the combined detection of early-stage cell apoptosis using Annexin V and late-stage cell apoptosis or necrosis using both Annexin V and 7-Aminoactinomycin D (7-AAD).

Caspase 3/7 is widely accepted as a reliable indicator of apoptosis, since caspase 3 activation is a necessary step to initiate the apoptotic cascade in a broad spectrum of cell types.

STEMCELL's caspase 3/7 products can be used to detect caspase 3/7 activity in apoptotic cells, are robust in detecting caspase 3/7 activity, and can be easily adapted to be used as high throughput assays for flow cytometry and microplates.

Lab Training Courses and Programs

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Performing an unfamiliar laboratory technique can be challenging. Protocols are often lengthy and complicated, and inexperience may contribute to user or experimental errors. Increase your chances of success and perform your experiments with confidence by enrolling in one of our training programs before you begin.

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Learn the techniques and protocols to move your research forward. Recorded lectures, step-by-step instructional videos, and libraries of curated resources will help guide you through entire workflows. Topics include hPSC maintenance and cell quality, neural induction,and expansion of hPSCs in 3D suspension culture. From passaging to cryopreservation, learn fundamental laboratory skills from the comfort of your own home.

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Access instructor-led training from your home or lab

Join from anywhere in the world to watch real-time video demonstrations, participate in online workshops, and learn from our team of scientific experts in our interactive, virtual training courses. Learn techniques and protocols to successfully derive and maintain high-quality human iPS cells from somatic cells and differentiate them toward specialized cell types in a virtual and interactive setting.

STEMCELL also offers in-person training to support the culture of hPSCs and their differentiation toward cerebral organoids, intestinal organoids, cardiomyocytes, or hematopoietic progenitors.

Learn more about all of our PSC training courses at **www.stemcell.com/psc-training**

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