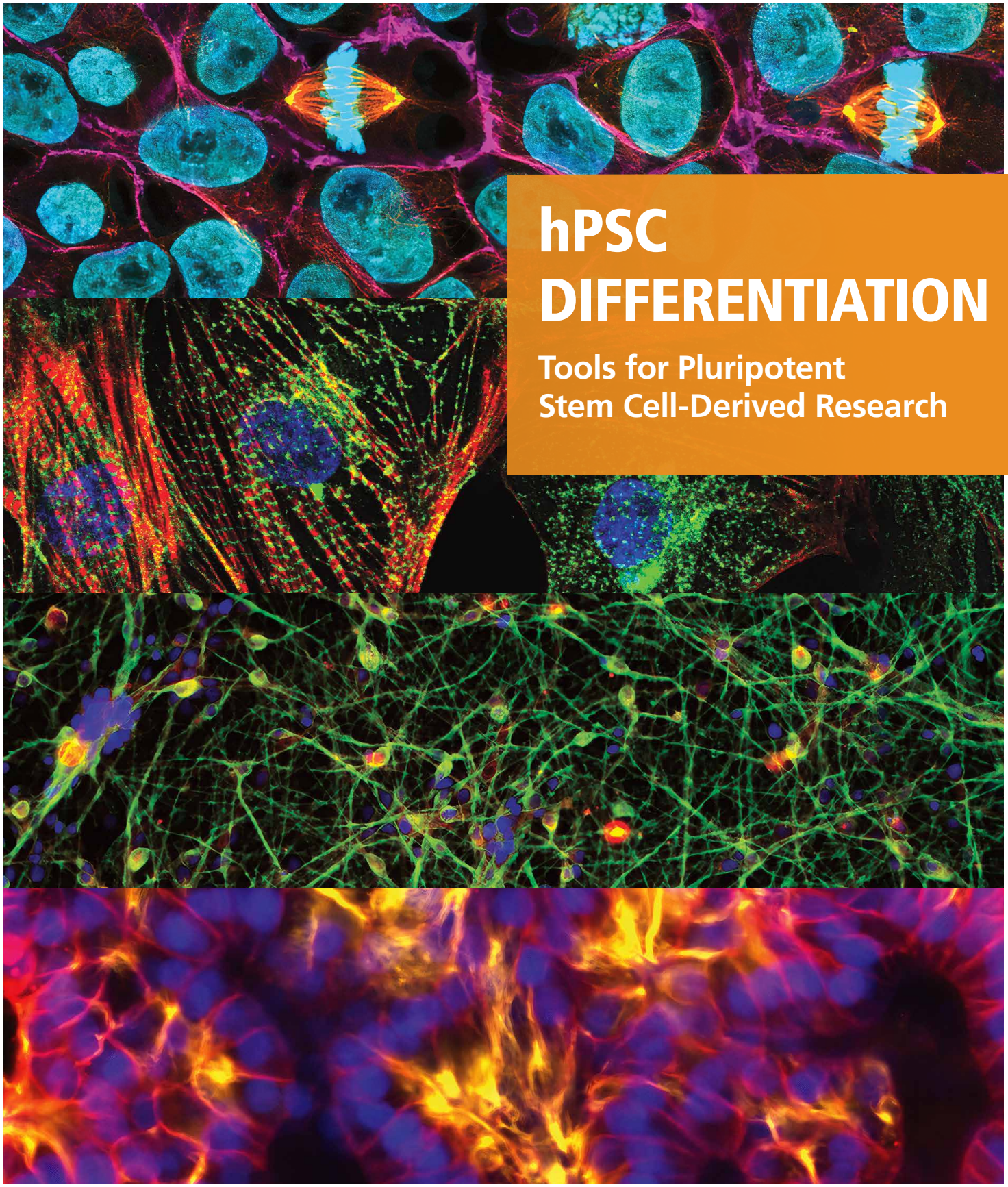

hPSC DIFFERENTIATION

Tools for Pluripotent Stem Cell-Derived Research

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

PDF

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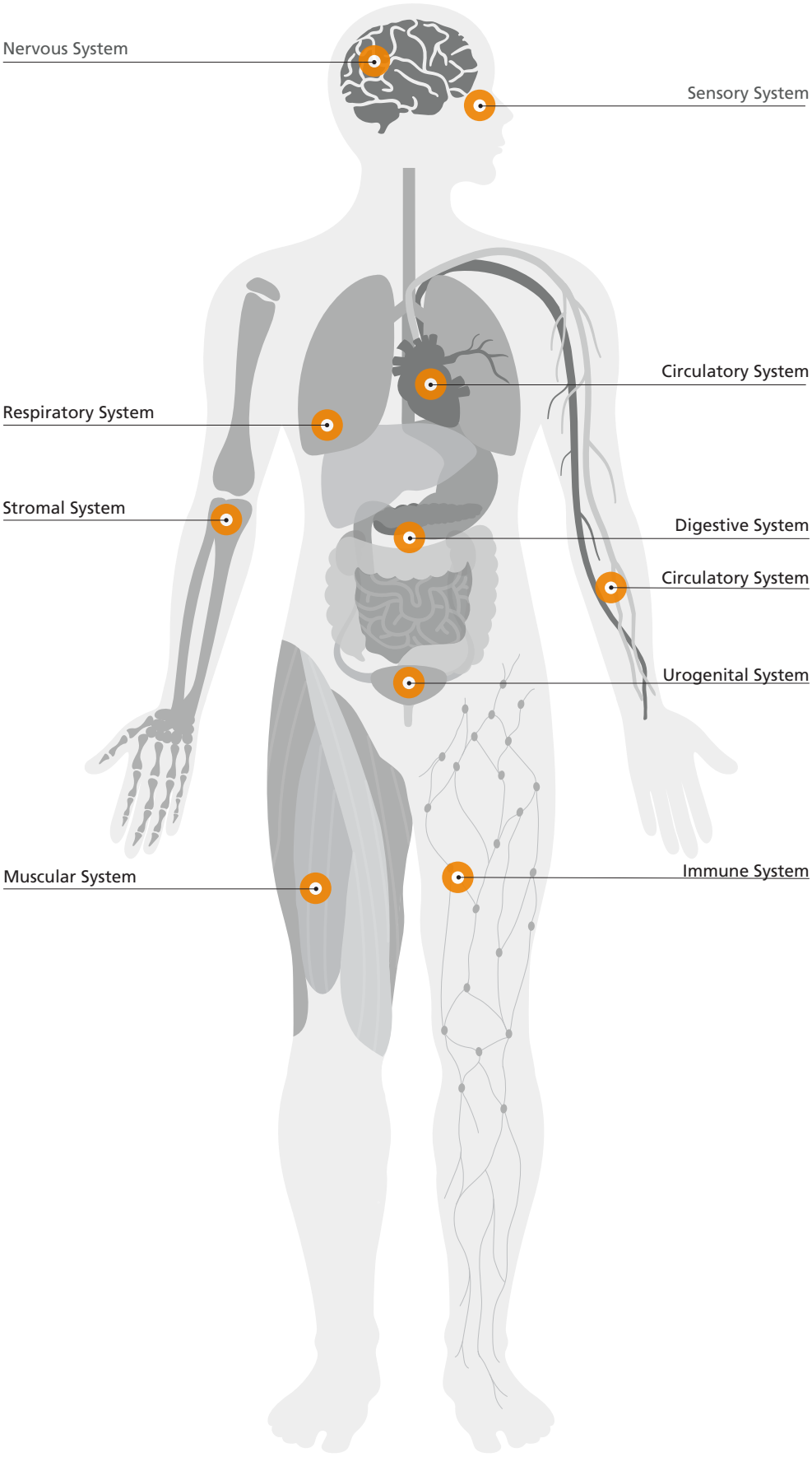

Nervous System
Sensory System
Circulatory System
Respiratory System
Stromal System
Digestive System
Circulatory System
Urogenital System
Muscular System
Immune System

STEMdiff™ Pluripotent Stem Cell Differentiation Media

Consistent human pluripotent stem cell (hPSC) differentiation is pivotal to high-quality results. Without standardized hPSC culture conditions, even the most detailed and rigorously followed stem cell differentiation protocols may still lead to inconsistent differentiation.[1,2] Use STEMdiff™ —a line of culture medium kits specifically optimized for hPSC differentiation to reproducibly differentiate multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines to 2D cell types and 3D organoid models originating from all three embryonic germ layers. Each easy-to-use kit comes with detailed, user-friendly protocols to standardize your differentiation protocols. 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.

Explore the following pages for tools to support nervous system, circulatory system, respiratory system, digestive system, immune system, sensory system, muscular system, stromal system, urogenital system, and customizable cell and organoid differentiation.

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

Learn more at www.STEMdiff.com

hPSC Maintenance: Start Your Cells Right

In order to maintain undifferentiated ES and iPS cells that are capable of self-renewal, specific culture conditions and best practices are critical to success in all downstream research applications. Use our defined, feeder-free TeSR™ family of media, xeno-free cell attachment substrates, and chemically defined passaging reagents to culture hPSCs and minimize variation in your research. hPSC characterization tools are also available to assist with best practices for quality control and research transparency. For long-term storage, our suite of cryopreservation media is designed to maintain high viability and maximize hPSC recovery after thawing.

Reproducible research with human induced pluripotent stem cells (iPSCs) depends on thoroughly characterized and quality-controlled cell banks. Start your research confidently with a reliable source of high-quality iPSCs by using the SCTi003-A control line. If you are creating your own lines, STEMCELL offers reprogramming solutions to support various donor cell types. Looking to speed up your research and start with differentiation? iPSCdirect™ is a ready-to-use cell product that eliminates the need for developing, maintaining, and characterizing hPSC banks, as well as maintenance of long-term iPSC cultures

Select the Right Tools for Maintenance and More

Browse tools for hPSC reprogramming, maintenance, genome editing, characterization, and cryopreservation.

Successful Differentiation Begins with High-Quality hPSCs

Start your hPSC workflows with confidence. Learn more about our hPSC lines and hPSC-derived cells.

Nervous System

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; #200-0621). 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), 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. These cells are karyotypically stable, demonstrate trilineage differentiation potential, express undifferentiated cell markers, and were 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

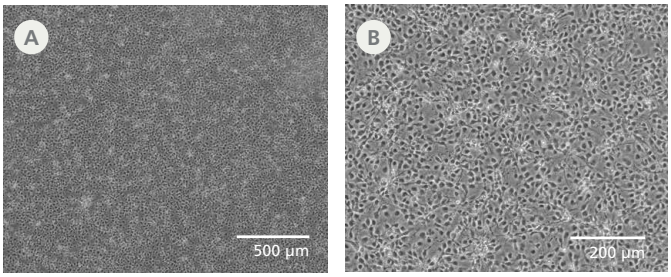


Figure 1. Human iPSC-Derived Neural Progenitor Cells Exhibit High-Quality Morphology Characteristic of Multipotent Central Nervous System Progenitor Cells

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

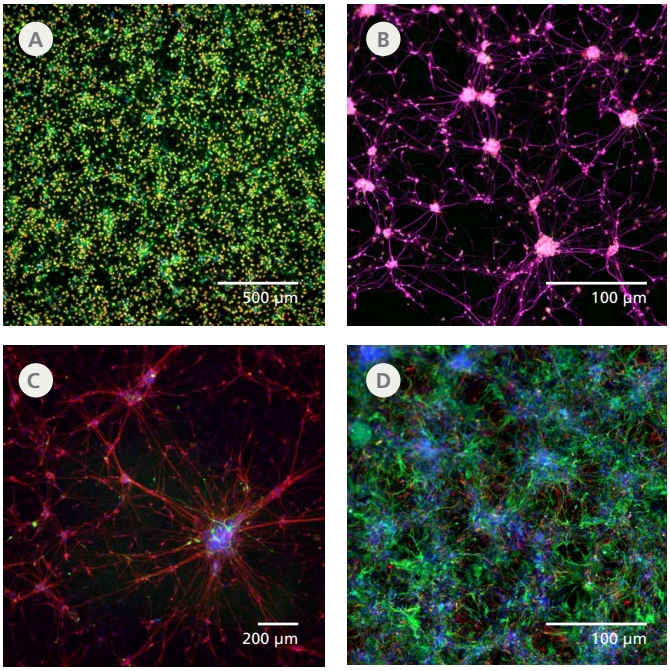


Figure 2. Human iPSC-Derived Neural Progenitor Cells Can Effectively Differentiate into Forebrain Neurons, Midbrain Neurons, and Astrocytes

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).

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.[3]

Published protocols using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (Catalog #05792; based on the published B27 formulation[4]) and N2 supplement (Catalog #05793),[5] 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.[3]

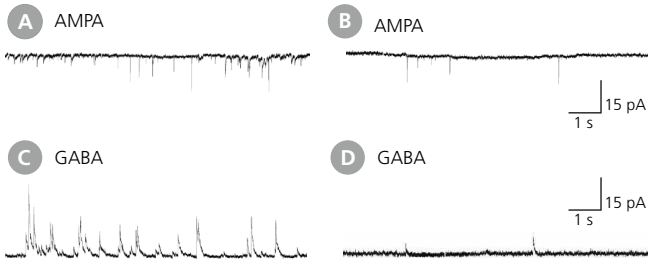


Figure 3. hPSC-Derived Neurons Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity by 44 Days

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.[6] Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications.

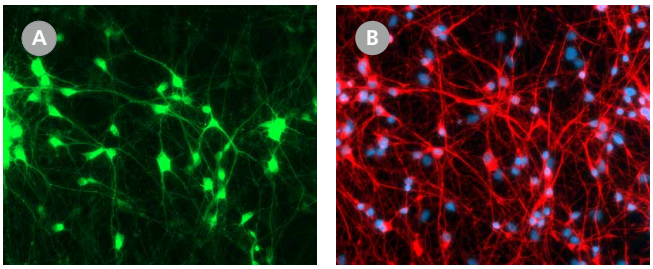


Figure 4. NeuroFluor™ NeuO Selectively Labels hPSC-Derived Neurons

(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

2D Neural Models

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 5D) 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.

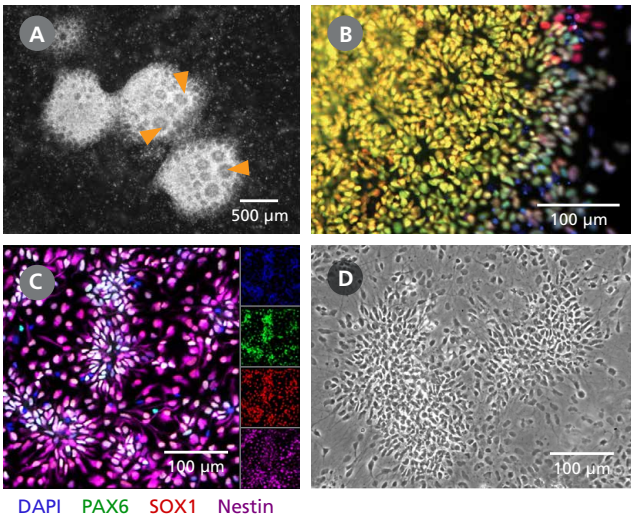


Figure 5. Neural Induction Using the STEMdiff™ SMADi Neural Induction Kit and STEMdiff™ Neural Progenitor Medium Generates Neural Rosettes and Enriches for CNS-type Neural Progenitor Cells

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 Neuron 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.

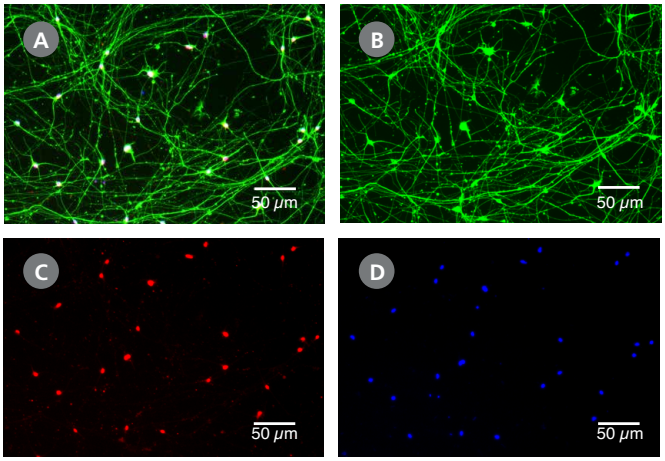


Figure 6. Downstream Differentiation of Neural Progenitor Cells to Neurons Is Possible Using the STEMdiff™ Differentiation and Maturation Kits

(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 Neuron 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 7).

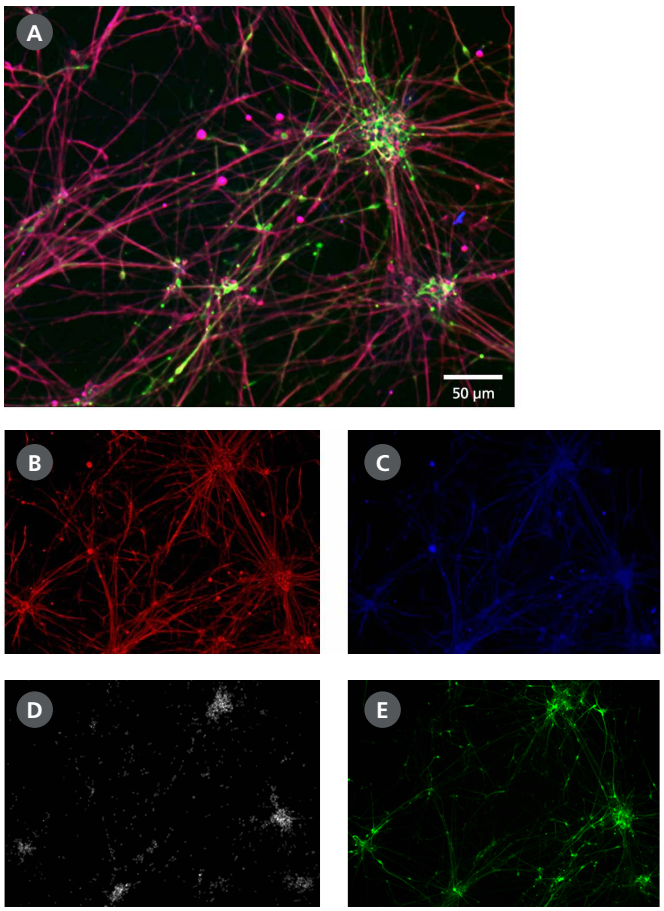


Figure 7. Midbrain-Type Neurons Express Tyrosine Hydroxylase and Dopamine Transporters (DAT) After Differentiation and Maturation in STEMdiff™ Midbrain Neuron Kits

(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 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.

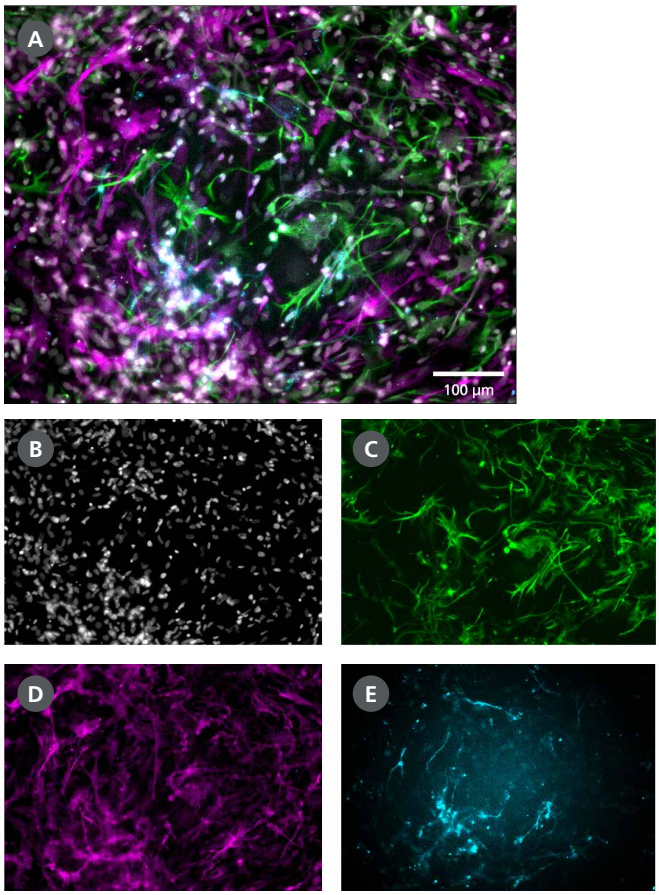


Figure 8. Cortical-Type Astrocytes Are Generated After Culture in STEMdiff™ Astrocyte Differentiation and Maturation Kits

(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

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

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

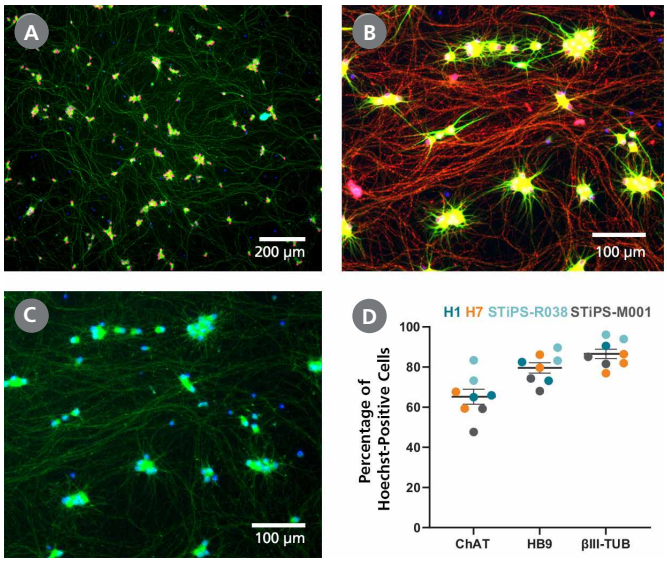


Figure 9. hPSC-Derived Motor Neurons Can Be Further Matured in STEMdiff™ Motor Neuron Maturation Medium

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.

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.

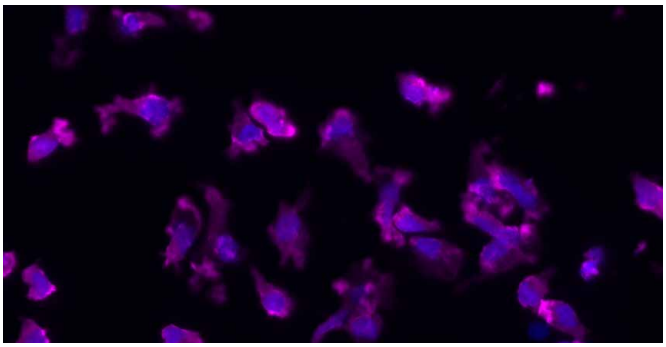


Figure 10. Microglia Generated with STEMdiff™ Microglia Culture System Show Expected Morphology and Markers

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,[7] 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).

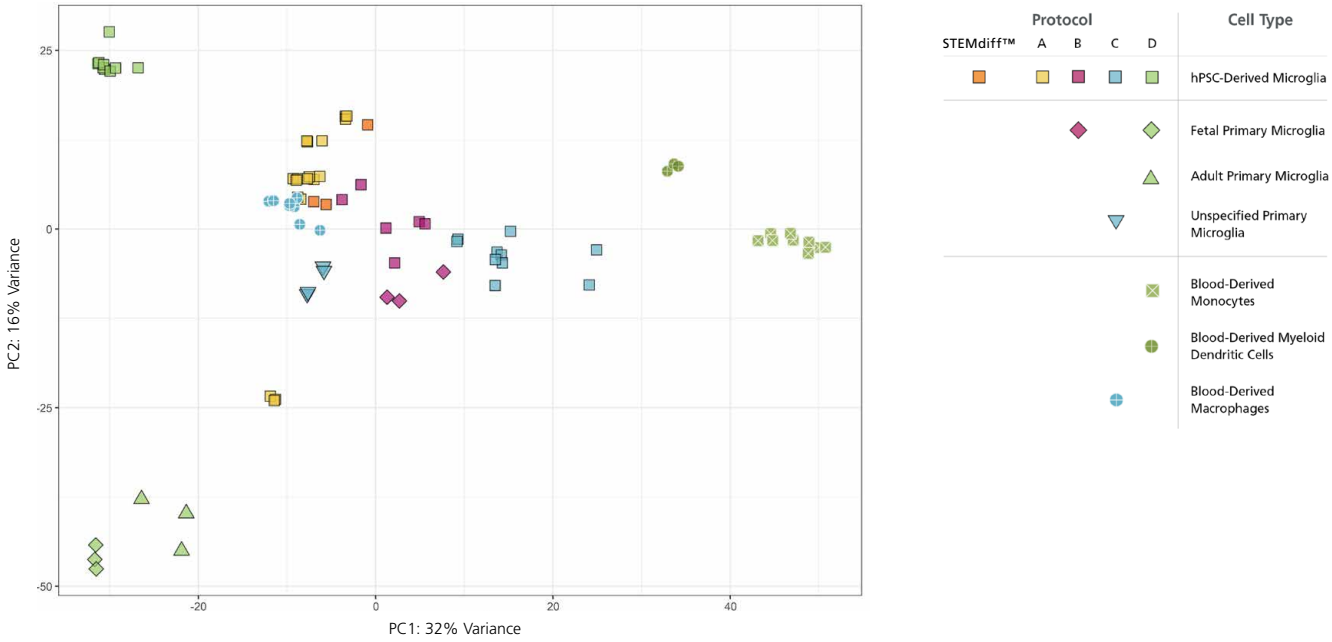


Figure 11. Microglia Generated with STEMdiff™ Microglia Culture System Are Typical PSC-Derived Microglial Cells

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

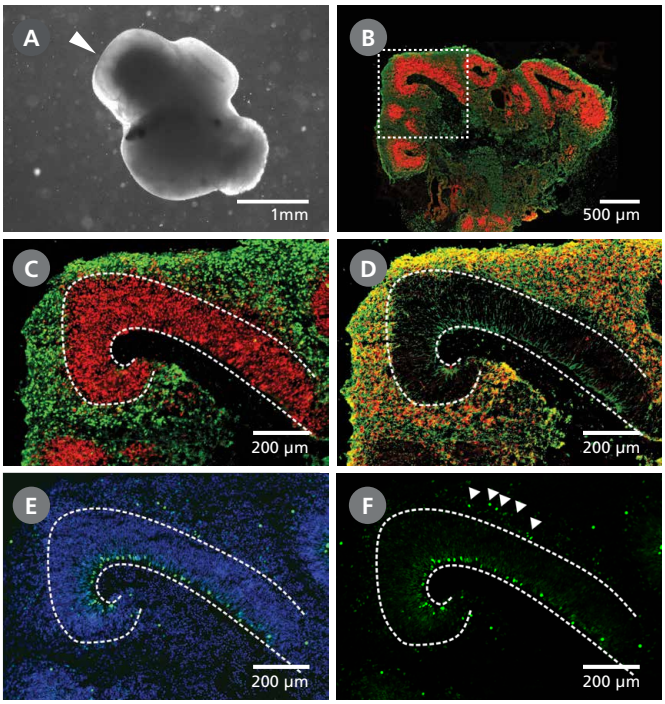
PROTOCOL

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

For differentiation to neural crest cells or sensory neurons, please see page 29.

3D Neural Models
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[8]
- Generate new or modified organoid models with this highly compatible platform

Learn more at www.stemcell.com/COKit

Figure 12. 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).

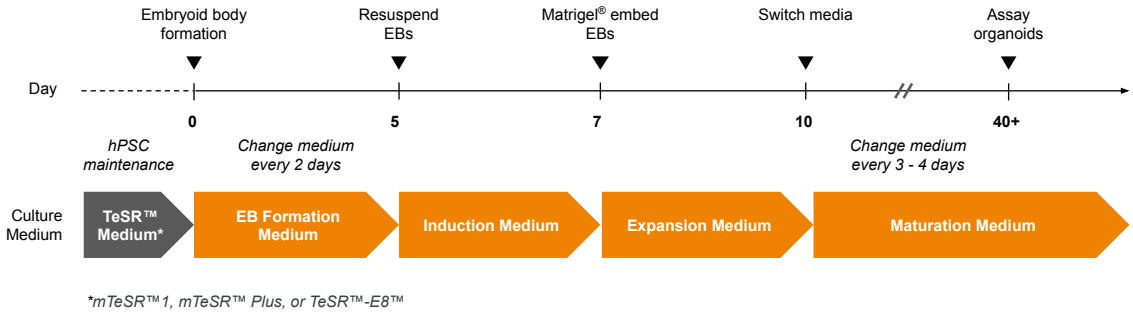


Figure 13. Schematic for Generating Unpatterned Neural Organoids Using the STEMdiff™ Cerebral Organoid Kit

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.[8]

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

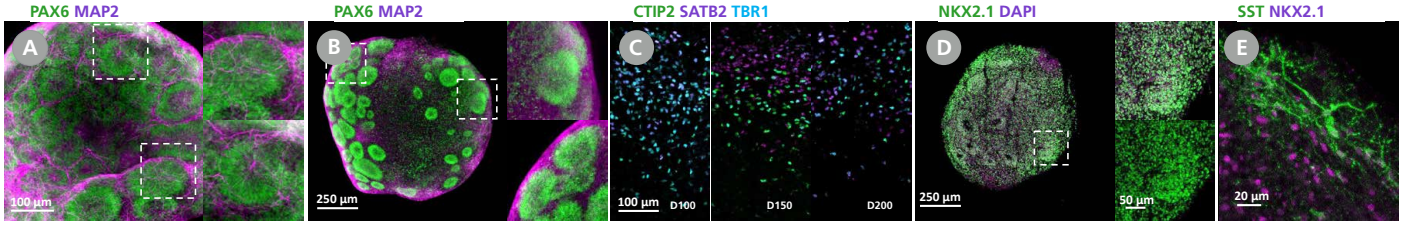


Figure 14. Dorsal Forebrain Organoids Exhibit Cortical Layering, and Both Dorsal and Ventral Organoids Express Markers Characteristic of Their Respective Brain Regions

(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.

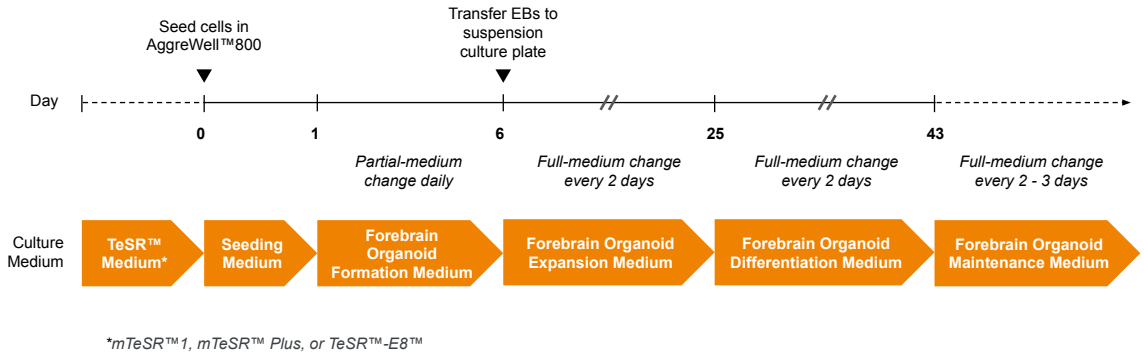


Figure 15. Schematic for the STEMdiff™ Dorsal and Ventral Forebrain Organoid Differentiation Kits

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.[9]

STEMdiff™ Midbrain Organoid Kit

Reliably generate midbrain organoids with the efficient and matrix-free STEMdiff™ Midbrain Organoid Differentiation Kit (Catalog #100-1096). When paired with AggreWell™800 (Catalog #34811) microwell culture plates, this serum-free cell culture media can prevent organoid fusion and support the generation of over 500 organoids per kit for higher-powered statistical replicates and more detailed longitudinal study.

Midbrain organoids can be combined with organoids generated using the STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit (Catalog #08620) to generate cortico-striatal AssemBloid™ cultures. Organoids can be maintained with STEMdiff™ Neural Organoid Maintenance Kit (Catalog #08571) to support long-term culture survival (> 50 days) for predictive assays, high-throughput phenotypic screening, and neurotoxicity assays.

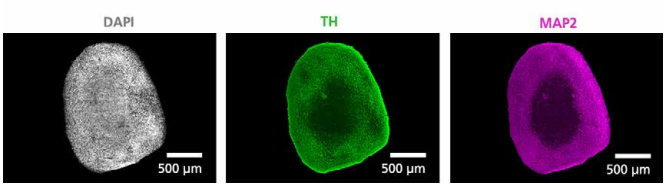


Figure 16. STEMdiff™ Midbrain Organoids Express Catecholaminergic Protein Tyrosine Hydroxylase

Midbrain organoids were generated using STEMdiff™ Midbrain Organoid Differentiation Kit. Organoids were further matured to Day 50 with STEMdiff™ Midbrain Organoid Maturation Kit. Midbrain organoids express the neuronal marker MAP2 and the catecholaminergic neuron specific marker tyrosine hydroxylase (TH).

Learn more at www.stemcell.com/midbrain-org

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).

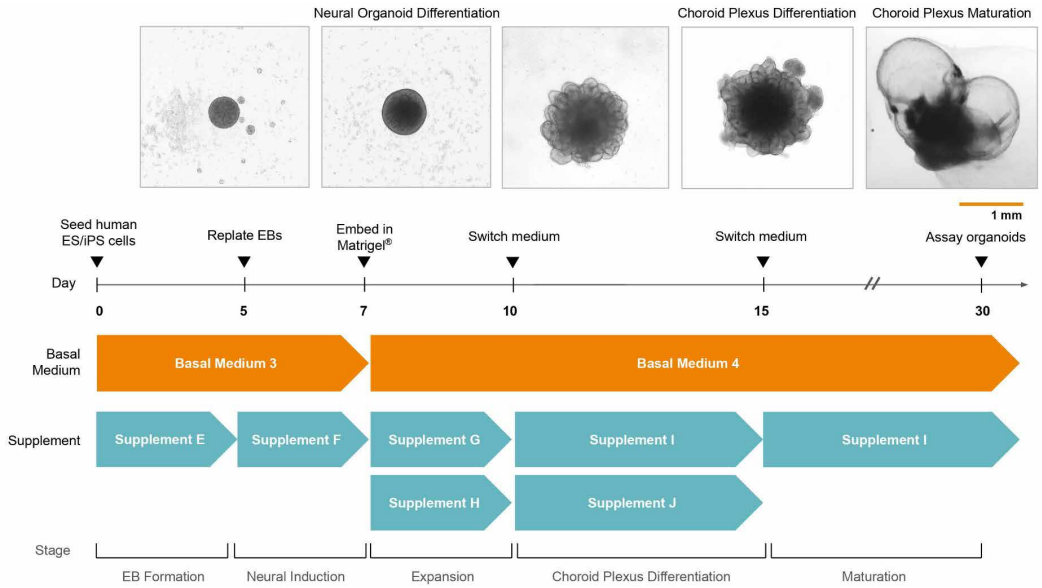


Figure 17. Schematic for the STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits

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.[10]

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

Circulatory System

Circulating Cells

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.

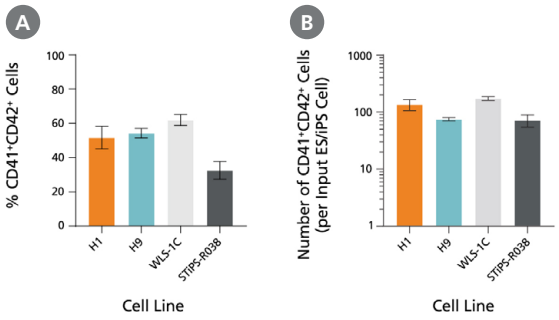


Figure 18. hPSC-Derived HPCs Efficiently Expand and Differentiate to CD41a+CD42b+ Megakaryocytes

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.

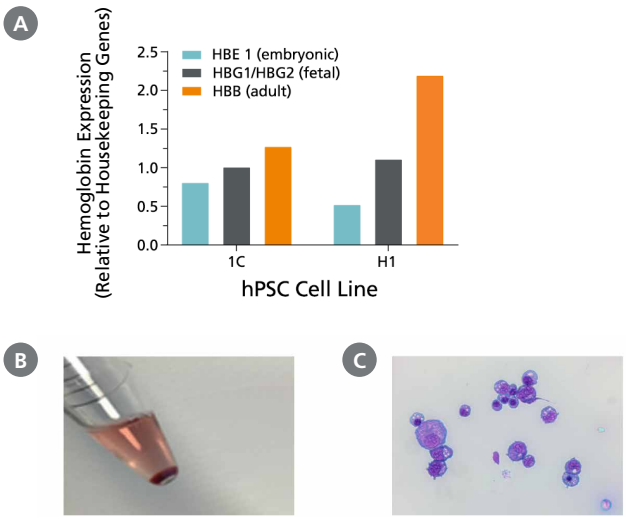


Figure 19. hES and hiPS Cell-Derived Erythroid Cells Are Hemoglobinized and Display Typical Erythroid Morphology

(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™ Hematopoietic Kit

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.

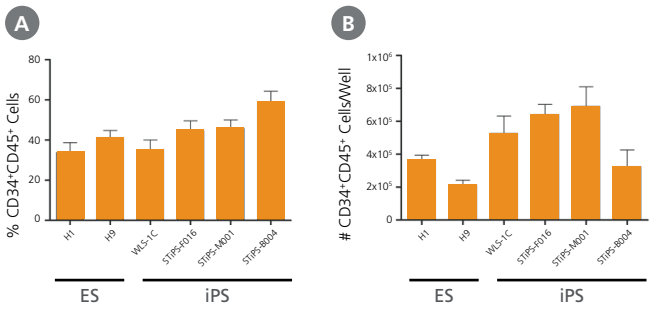


Figure 20. Efficient and Robust Generation of $CD34^+CD4^+$ HPCs

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; $n \geq 3$.

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

Vessels

STEMdiff™ Endothelial 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).

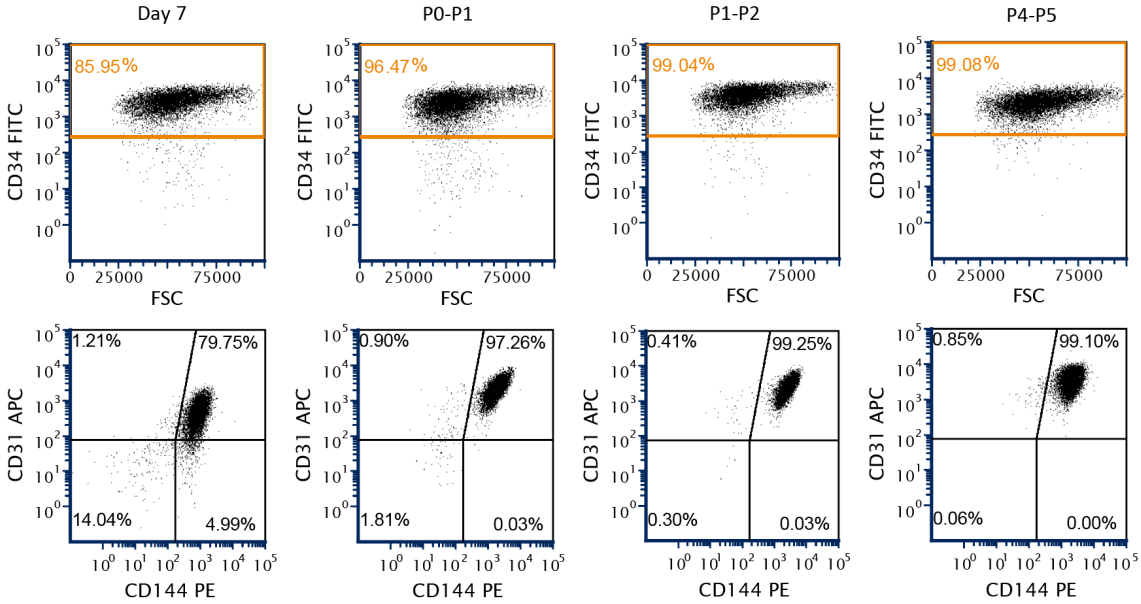


Figure 21. A Representative Flow Cytometric Analysis of Endothelial Marker Expression in hPSC-Derived Endothelial Cells

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.

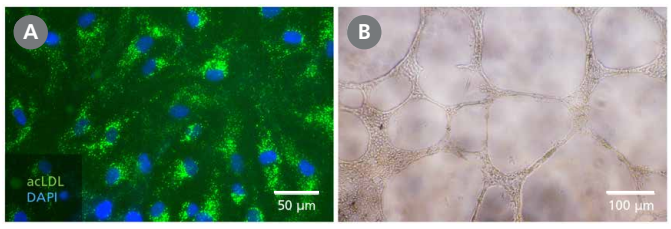


Figure 22. STEMdiff™ Endothelial Differentiation Kit Generates Functional hPSC-Derived Endothelial Cells

(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*.

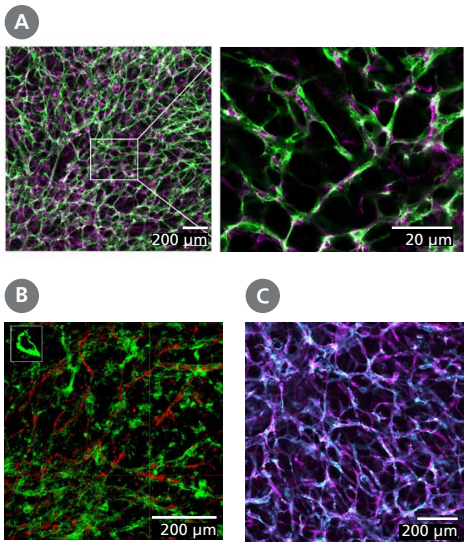


Figure 23. Vascular Networks Mature into Stable Blood Vessels When Cultured Within the Extracellular Matrix in STEMdiff™ Blood Vessel Maturation Medium

(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.

Heart

STEMdiff™ Ventricular Cardiomyocyte System

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 24). 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.

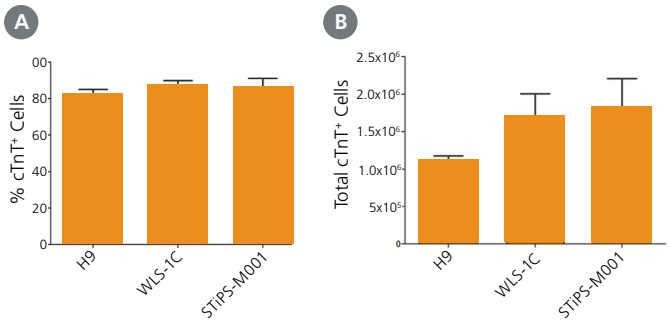


Figure 24. Efficient and Robust Generation of cTnT-Positive Ventricular Cardiomyocytes

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™ Cardiomyocyte Expansion Kit

Expand early-stage human pluripotent stem cell (hPSC)-derived cardiomyocytes consistently using the serum-free STEMdiff™ Cardiomyocyte Expansion Kit (Catalog #100-1109). This kit generates a large number of functional and highly pure hPSC-derived cardiomyocytes and is compatible with ventricular or atrial cardiomyocytes generated with STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Catalog #05010) or STEMdiff™ Atrial Cardiomyocyte Differentiation Kit (Catalog #100-0215), respectively.

The STEMdiff™ Cardiomyocyte Expansion kit allows you reach cardiomyocyte populations in the billions with a single round of cardiomyocyte differentiation. Early-stage hPSC-derived cardiomyocytes are expanded directly instead of the traditional method of PSC expansion, followed by differentiation. Using this kit, expanded early-stage hPSC-derived cardiomyocytes retain a stable electrical profile and have cTnT percentages over 90% at passage 5. The expanded hPSC-derived cardiomyocytes are ready for high-throughput drug testing, tissue engineering, and regenerative medicine research.

By using this first-to-market kit to efficiently expand cardiomyocytes, you can save time and resources.

Other STEMdiff™ Cardiomyocyte Products:	Catalog #
STEMdiff™ Cardiomyocyte Expansion Kit	100-1109
STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit	05010
STEMdiff™ Atrial Cardiomyocyte Differentiation Kit	100-0215
STEMdiff™ Cardiomyocyte Dissociation Kit	05025
STEMdiff™ Cardiomyocyte Support Medium	05027
STEMdiff™ Cardiomyocyte Freezing Medium	05030
STEMdiff™ Cardiomyocyte Maintenance Kit	05020

Respiratory System

2D Pulmonary Models

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.

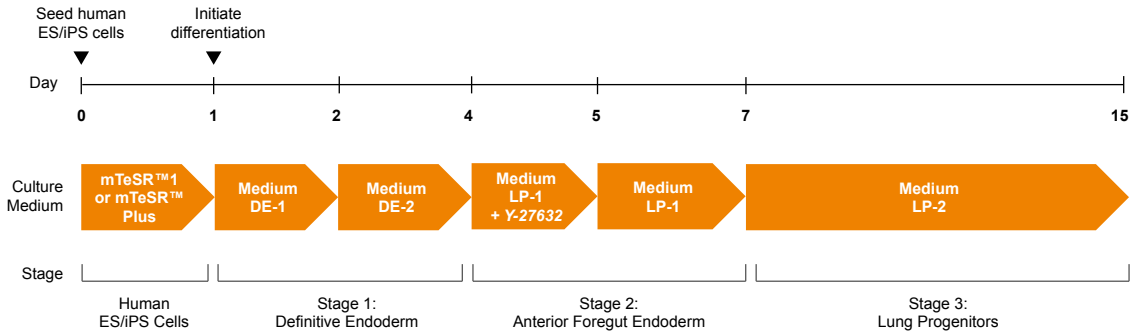


Figure 25. Schematic for Generating Lung Progenitor Cells from Human ES/iPS Cells Using STEMdiff™ Lung Progenitor Kit

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.

3D Pulmonary Models

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.

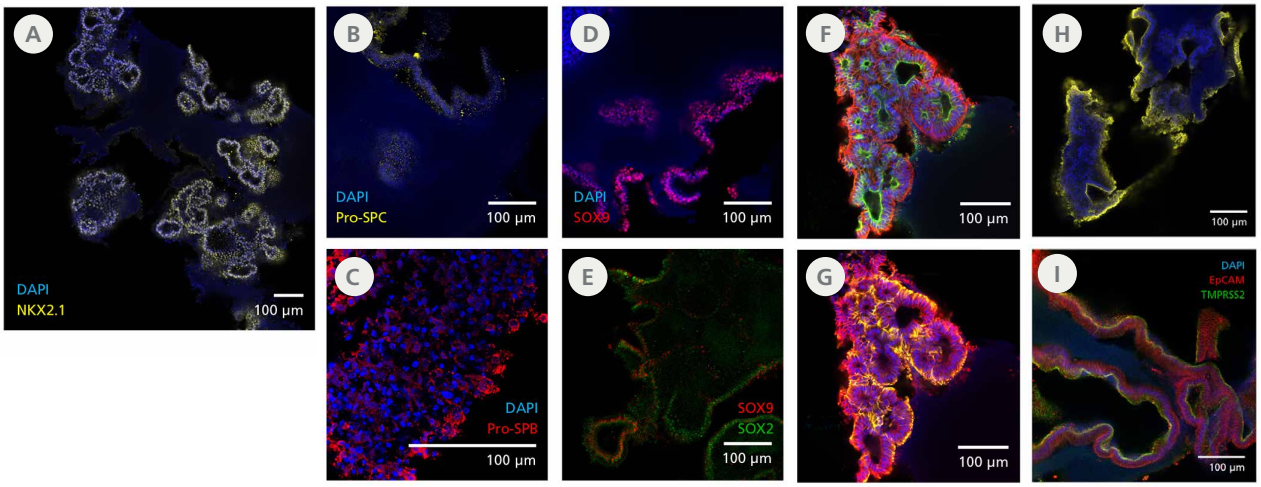


Figure 26. Branching Lung Organoids Cultured in STEMdiff™ Branching Lung Organoid Kit Feature Key Protein Markers and Exhibit Branching Morphogenesis

(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

Digestive System

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[11] and pancreatic[12] progenitor cells for drug development, toxicity testing, research for development of cell-based therapies, or studying developmental pathways.

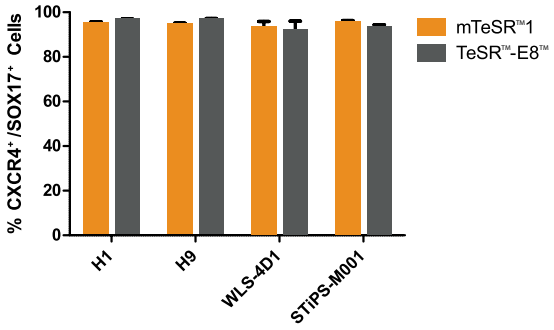


Figure 27. Definitive Endoderm Differentiation Is Efficient Across Multiple Human ES and iPS Cell Lines, Regardless of hPSC Maintenance Medium

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.

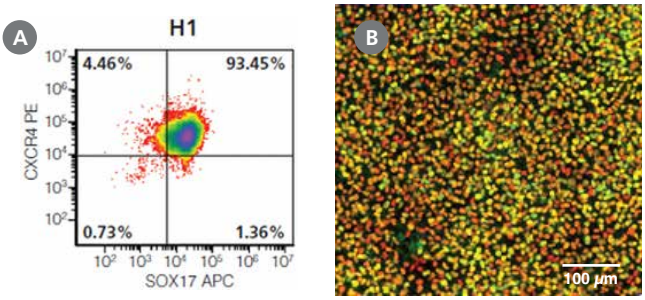


Figure 28. hPSCs Differentiated with STEMdiff™ Definitive Endoderm Kit Are Highly Enriched for Expression of Key Definitive Endoderm Markers

(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

Intestine

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.

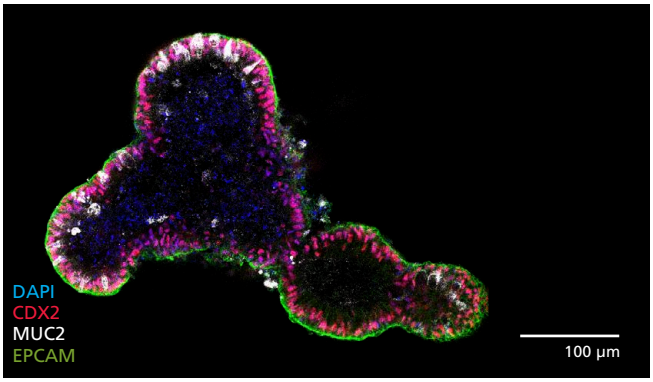


Figure 29. hPSC-Derived Intestinal Organoids Incorporate Features of the Intestinal Epithelium and Mesenchyme

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

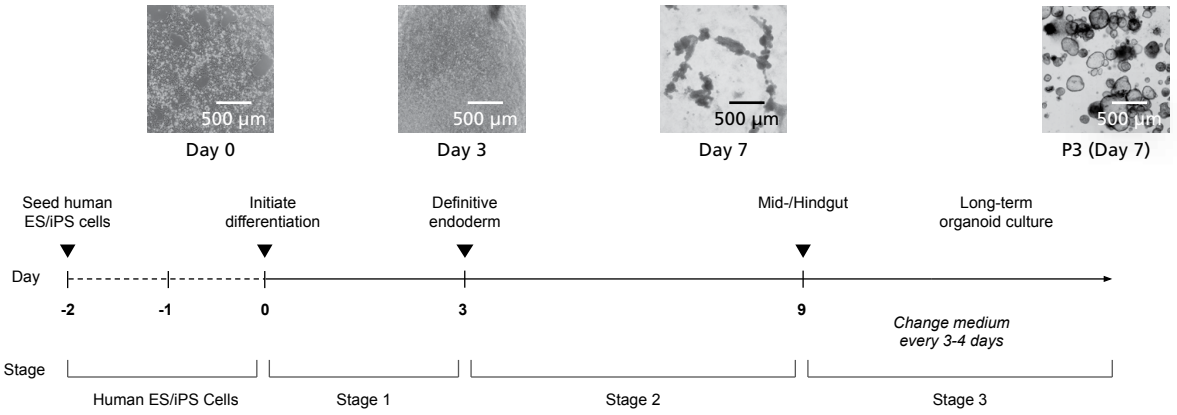


Figure 30. Schematic for Differentiating from hPSCs to Human Intestinal Organoids with the STEMdiff™ Intestinal Organoid Kit

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

Stomach

STEMdiff™ Gastric Organoid 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

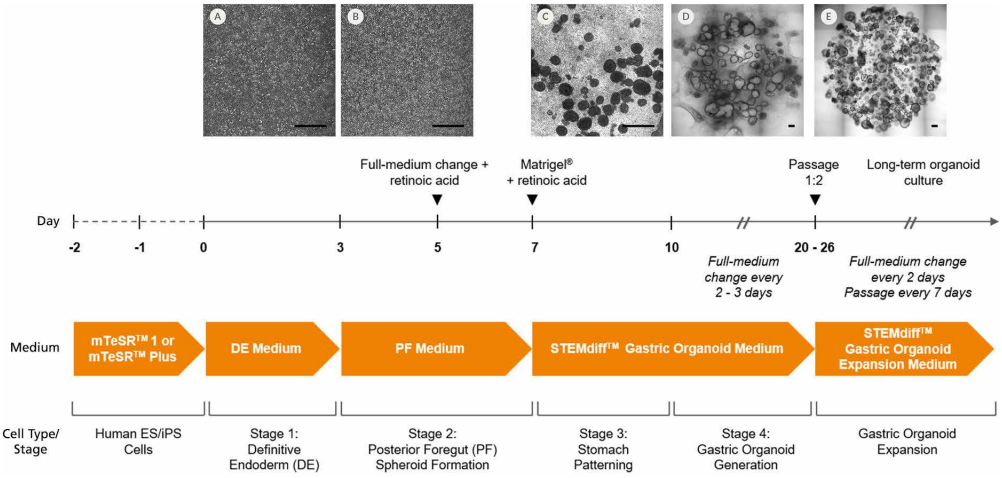


Figure 31. Schematic for Generation of Human Gastric Organoid Cultures Using STEMdiff™ Gastric Organoid Differentiation Kit

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 attached overnight. Two-dimensional monolayer cultures were maintained with daily medium changes until near-confluence (85 - 90%) was achieved. (A) On Day 0, differentiation was initiated with STEMdiff™ Definitive Endoderm (DE) Medium (Stage 1), with daily medium changes. (B) On Day 3, DE Medium was 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, floating posterior foregut spheroids were harvested from the supernatant and embedded into Corning® Matrigel®. Between Days 7 and 10, 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 in STEMdiff™ Gastric Organoid Medium until expression of gastric markers were 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.

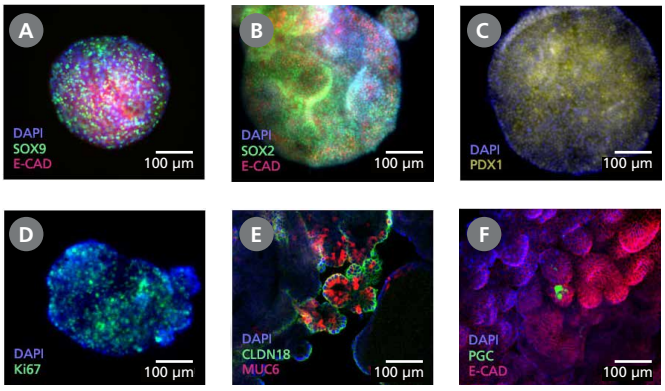


Figure 32. Immunohistochemistry Confirms Expression of Gastric-Specific Markers in Human Gastric Organoids Cultured in Gastric Organoid Expansion Medium

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

Pancreas

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 33 and 34). 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 b-cell maturation, disease modeling, and studying pancreatic cancer.

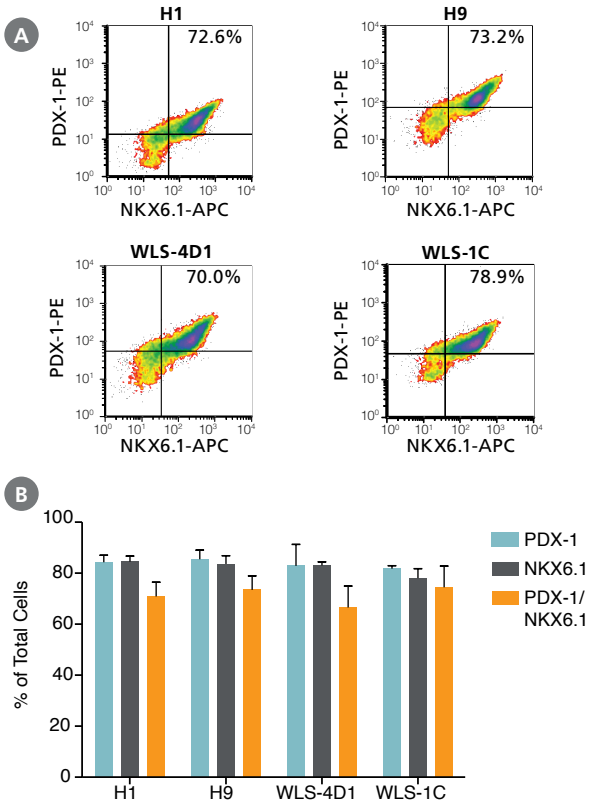


Figure 33. STEMdiff™ Pancreatic Progenitor Kit Efficiently Generates PDX-1, NKX6.1-Positive Progenitors Across Multiple hPSC Lines

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.[13]

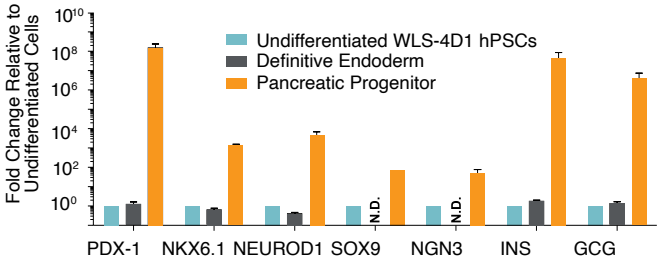


Figure 34. Gene Expression Profile Indicates Transition to Pancreatic Progenitor Cell

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.[14] N.D.: Not Determined.

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

Liver

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.

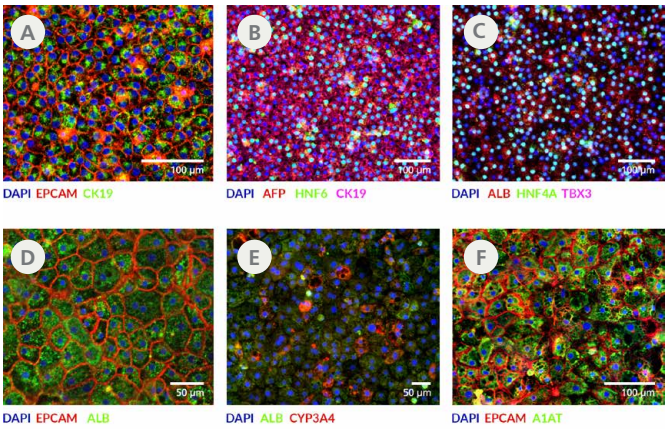


Figure 35. hPSC-Derived Hepatic Progenitor Cells and Hepatocyte-Like Cells Express Hepatic Markers As Confirmed by Immunocytochemistry Analysis

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

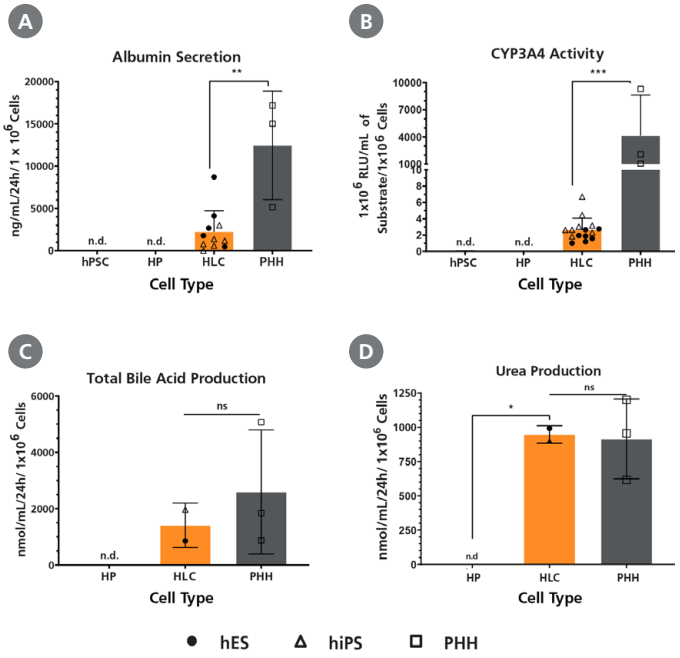


Figure 36. hPSC-Derived HLCs Exhibit Key Liver Functionalities

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

Immune System

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

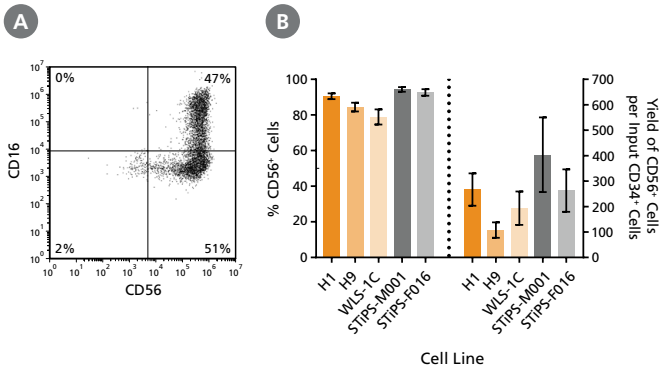


Figure 37. hPSCs Differentiate into CD56⁺ NK Cells After 40 Days of Culture

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).

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

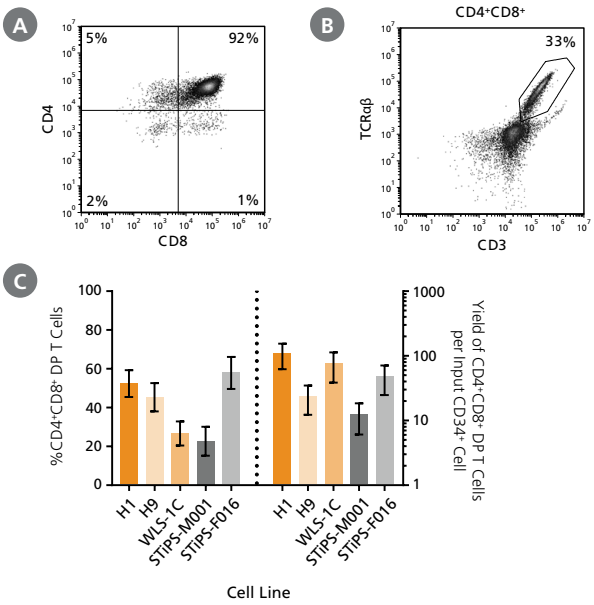


Figure 38. CD4⁺CD8⁺ DP T Cells Can Be Generated from Human hPSCs After a Total of 40 Days of Culture with the STEMdiff™ T Cell Kit

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).

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.

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

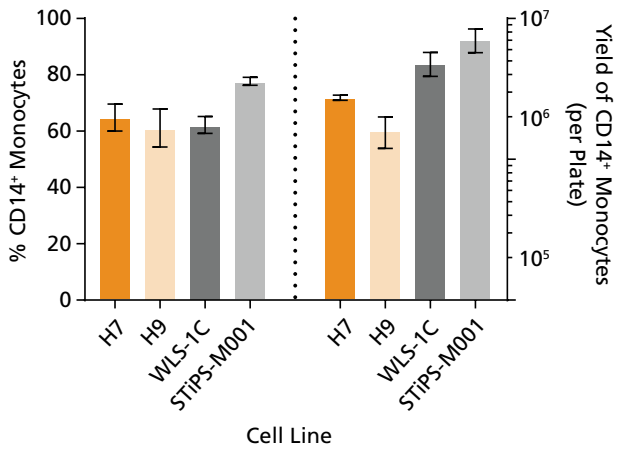


Figure 39. STEMdiff™ Monocyte Kit Enables Robust and Efficient Generation of CD14+ Monocytes

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

For differentiation to microglia, please see page 11.

Sensory System

STEMdiff™ Neural Crest 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.

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 40F), and to the adipogenic lineage using MesenCult™ Adipogenic Differentiation Kit (Catalog #05412).

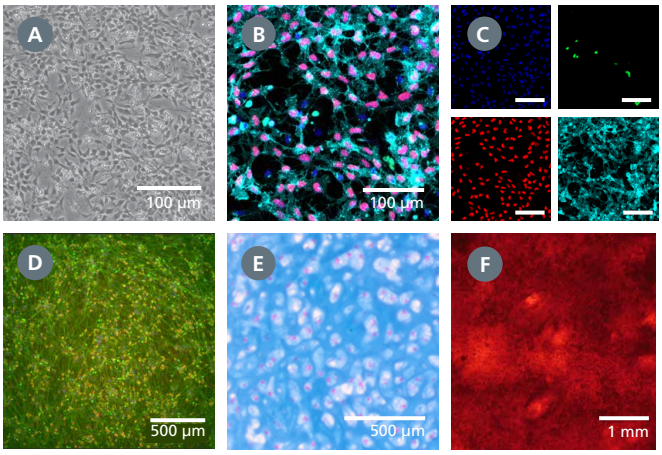


Figure 40. STEMdiff™ Neural Crest Differentiation Kit Generates a Highly Pure Population of Multipotent NCCs

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 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.

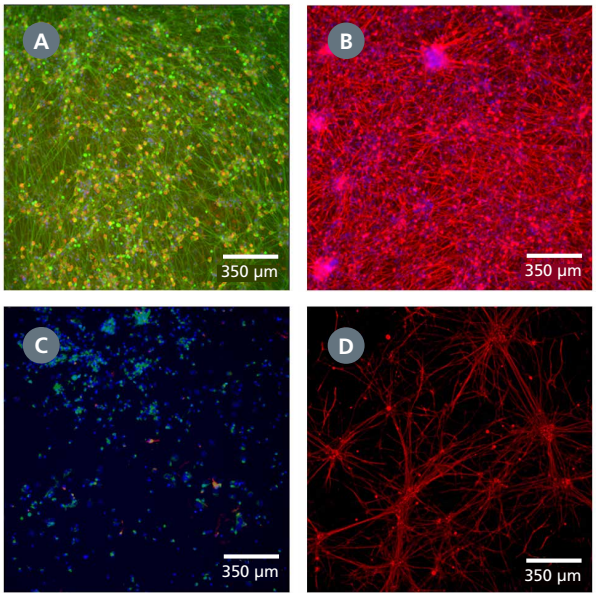


Figure 41. Sensory Neurons of the Peripheral Nervous System Can Be Generated Using STEMdiff™ Sensory Neuron Kits

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

Muscular System

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.

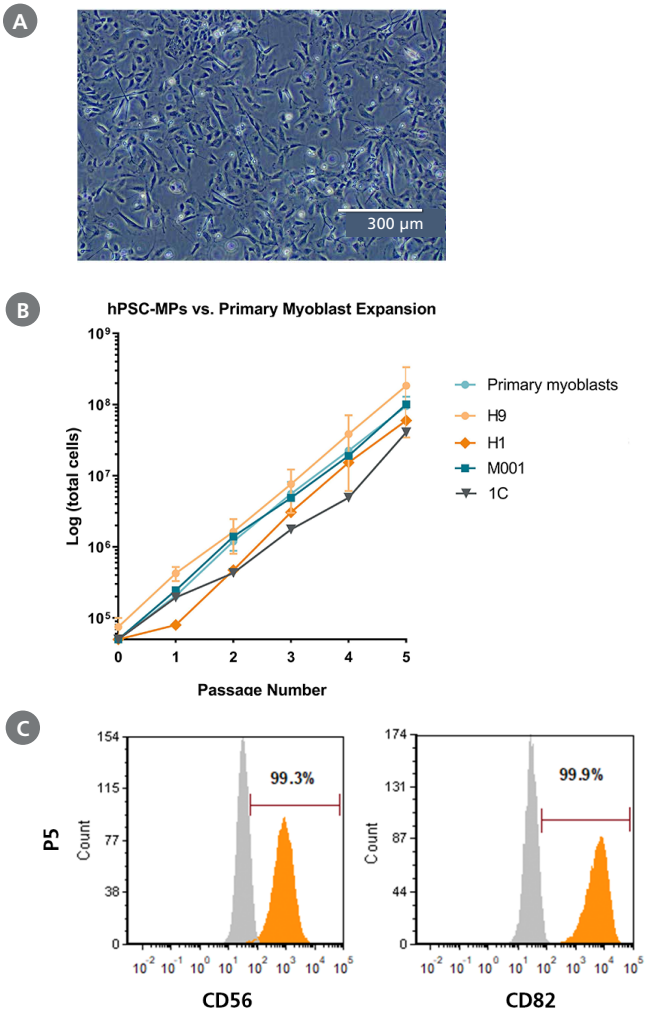


Figure 42. STEMdiff™ Myogenic Progenitor Kit Generates Expandable hPSC-Derived Myogenic Progenitors

(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.

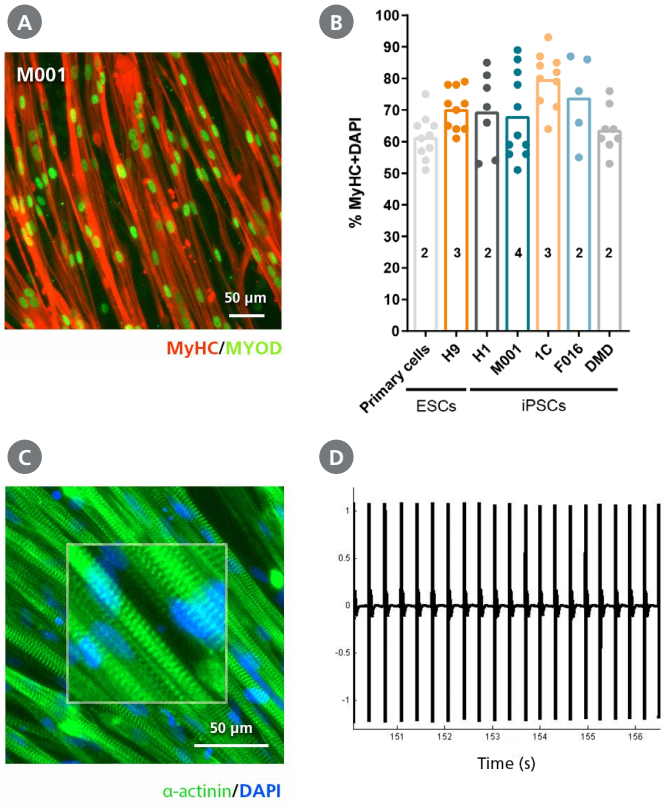


Figure 43. hPSC-Derived Myotubes Generated Using the STEMdiff™ Myogenic Progenitor Kit Are Efficiently Differentiated and Functionally Contractile

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

Stromal System

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 44).

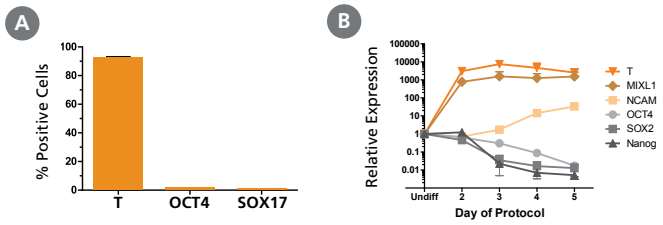


Figure 44. STEMdiff™ MIM Efficiently Generates a Homogenous Population of Early Mesoderm Cells

(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 45). The derived MPCs are characterized by strong expression of cell-surface markers CD73, CD90, and CD105, and lack expression of CD45.

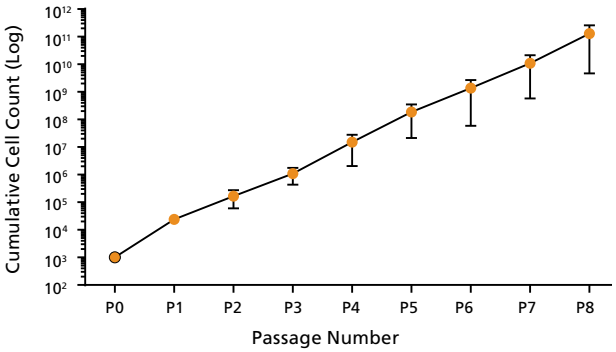


Figure 45. hPSC-Derived MPCs Generated Using the STEMdiff™ Mesenchymal Progenitor Kit Exhibit a High Rate of Cell Expansion in MesenCult™-ACF Plus Medium

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

Urogenital System

STEMdiff™ Kidney Organoid Kit

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.[15,16] 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.[17,18]

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.

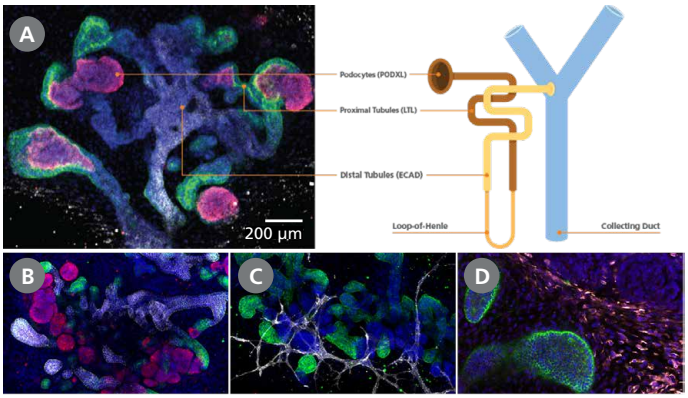


Figure 47. Kidney Organoids Display Distinct Domains of the Developing Nephron

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

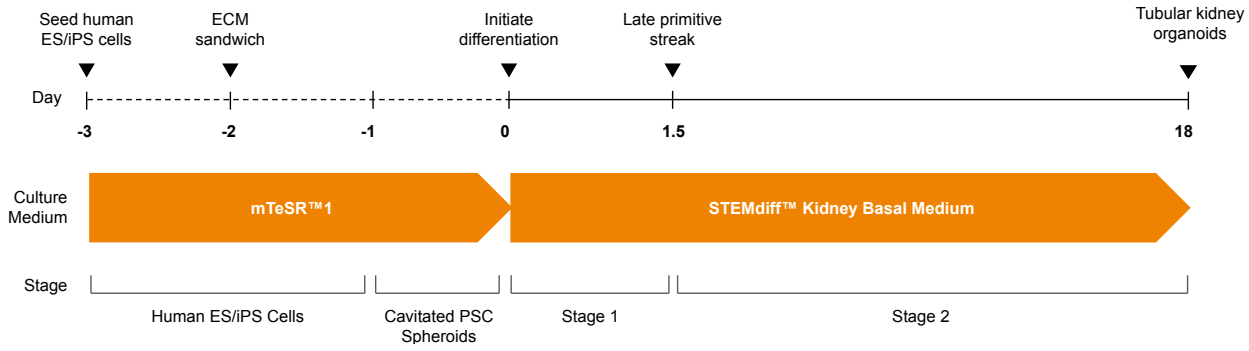


Figure 46. Schematic for Differentiation from hPSCs to Human Kidney Organoids with the STEMdiff™ Kidney Organoid Kit

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.

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.[19] 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 36). Appropriate induction factors must be added before use.

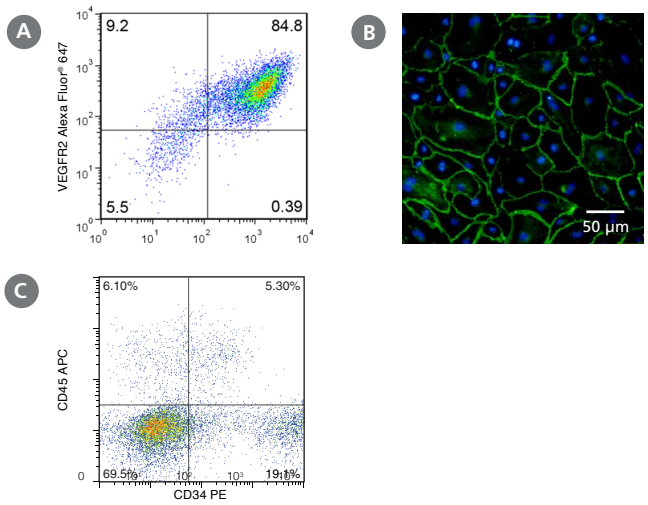


Figure 48. STEMdiff™ APEL™ Media Can Be Used for Customized Differentiation to Various Mesodermal Cell Lineages

(A) Endothelial differentiation of STiPS-F001 human iPS cells using STEMdiff™ APEL™ medium*, based on methods by Tan et al.[20] (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.[19] and Chadwick et al.[21] 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

Cell Quality Characterization

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.

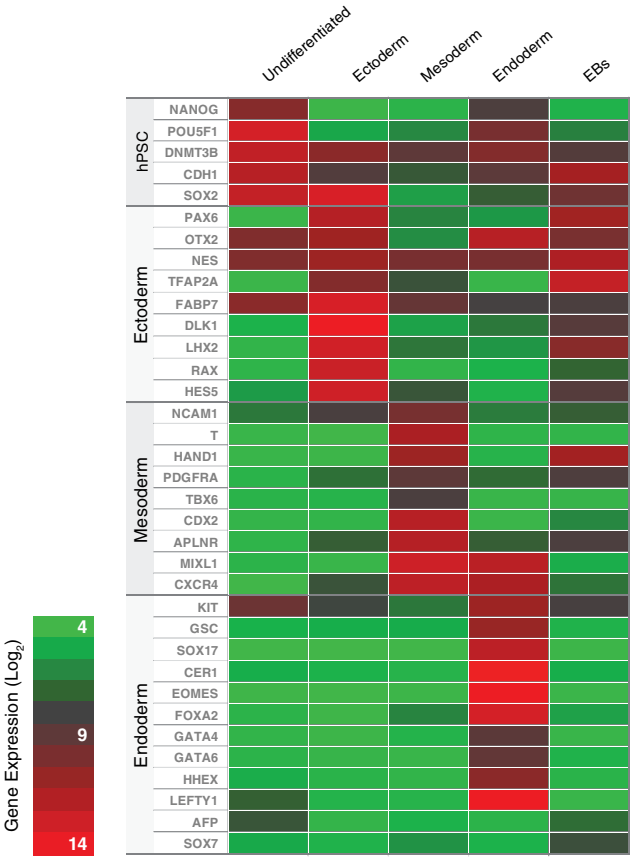


Figure 49. Molecular Analysis of Cultures Differentiated with the STEMdiff™ Trilineage Differentiation Kit Shows Strong Separation of Lineage-Specific Markers

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.

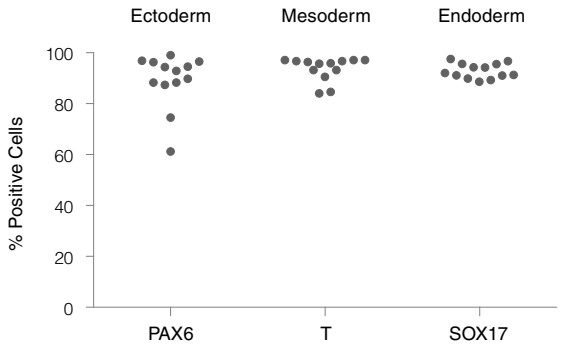


Figure 50. The STEMdiff™ Trilineage Differentiation Kit Promotes Efficient Differentiation to All Three Germ Layers

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

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 #
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
Purmorphamine	Hedgehog pathway activator Activates Smoothened	Differentiation	72202
DAPT	Notch pathway inhibitor Inhibits g-secretase	Differentiation	72082
Prostaglandin E2	Prostanoid pathway activator Activates prostaglandin receptors EP1, EP2, EP3 and EP4	Differentiation	72192
Dibutyryl-cAMP	cAMP pathway activator Activates cAMP-dependent protein kinases	Differentiation	73882
SB202190	p38 MAPK inhibitor	Maintenance, Differentiation	72632
IWR-1-endo	WNT pathway inhibitor AXIN2 stabilizer	Maintenance, Differentiation	72562
All-Trans Retinoic Acid	Retinoid pathway activator Activates retinoic acid receptor (RAR)	Differentiation	72262
BIO	WNT pathway activator Inhibits GSK3	Reprogramming, Maintenance, Differentiation	72032

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[22] result in EBs that are heterogeneous in size and shape (Figure 51A), leading to inefficient and uncontrolled differentiation.[23]

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 51B) and to ensure reproducibility of differentiation experiments.[24]

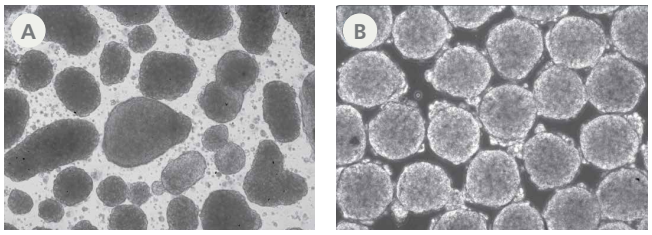


Figure 51. AggreWell™ Plates Are Used to Generate Uniform EBs

(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.

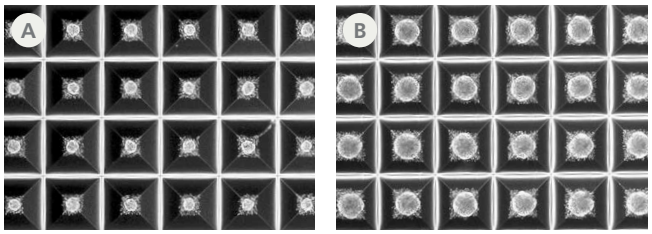


Figure 52. The Size of EBs Can Be Controlled in AggreWell™

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.

Learn more at www.stemcell.com/GloCell

Mitochondrial Kit & Dyes

Mitochondria maintain crucial energy balance and play important roles in regulating normal cell function, activity, as well as cellular senescence. Fluorescent-based dyes and kits for mitochondrial sample preparation are emerging as useful tools for elucidating mitochondrial activity in physiological and pathological conditions. Explore the following tools to study mitochondrial activity and cellular metabolism after culturing cells in our core media products.

Product	Catalog #
Mitochondrial Isolation Kit	100-0990
Mitochondrial Superoxide Dye	100-0991
TMRE (Perchlorate)	100-0992
JC-1 (Iodide)	100-0993
Rhod-2 AM (Bromide)	100-0994
Mitochondrial Tracking Dye, Deep Red	100-0995
Mitochondrial Tracking Dye, Blue	100-0996

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

Learn Firsthand from Our Experienced Scientists

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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Access our free, self-paced, online training courses

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 at www.stemcell.com/psc-training

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hPSC DIFFERENTIATION

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