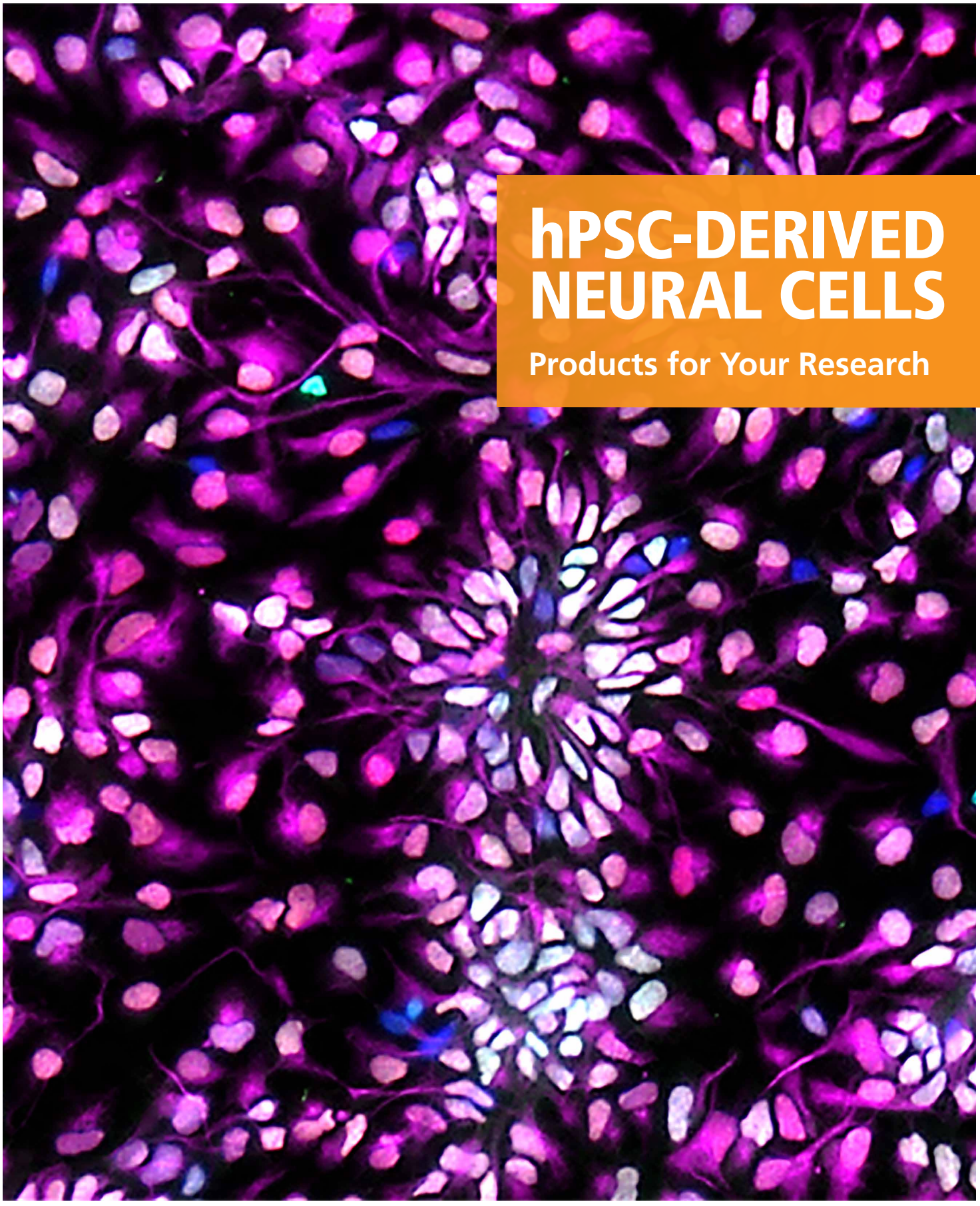


hPSC-DERIVED NEURAL CELLS

Products for Your Research

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

TABLE OF CONTENTS

hPSC-Derived Neural Cell Research

Tools for hPSC-Based Neurological Modeling

Neural cells derived from human pluripotent stem cells (hPSCs), including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, provide a physiologically relevant model for drug discovery, cell therapy validation, and neurological disease research. The ability to generate patient-specific differentiated cells, including neural stem cells and neuronal and glial subtypes, bridges the gap between studies using animal models, and clinical research. To facilitate research in this growing field, STEMCELL Technologies has developed the STEMdiff™ Neural System; a suite of products specifically designed for the generation, expansion, differentiation, characterization, and cryopreservation of hPSC-derived neural progenitor cells (NPCs). For a flexible approach to differentiation, BrainPhys™ Neuronal Medium can be combined with NeuroCult™ SM1 Neuronal Supplement, N2 Supplement-A or B, small molecules, and cytokines in a wide range of neuronal protocols. BrainPhys™ Neuronal Medium is designed to better support in vitro function, and will generate cultures containing a higher proportion of synaptically active neurons.

Choose the ideal neural induction workflow to generate neural progenitor cells from human pluripotent stem cells with this practical guide.

TECH TIP

Designing Your Neural Induction and Differentiation Workflow

www.stemcell.com/Neural-Induction-Workflow

Whichever stage of research you're in, explore our complete portfolio of products to support your neural culture workflow with this infographic.

NEURAL PRODUCT WORKFLOWS

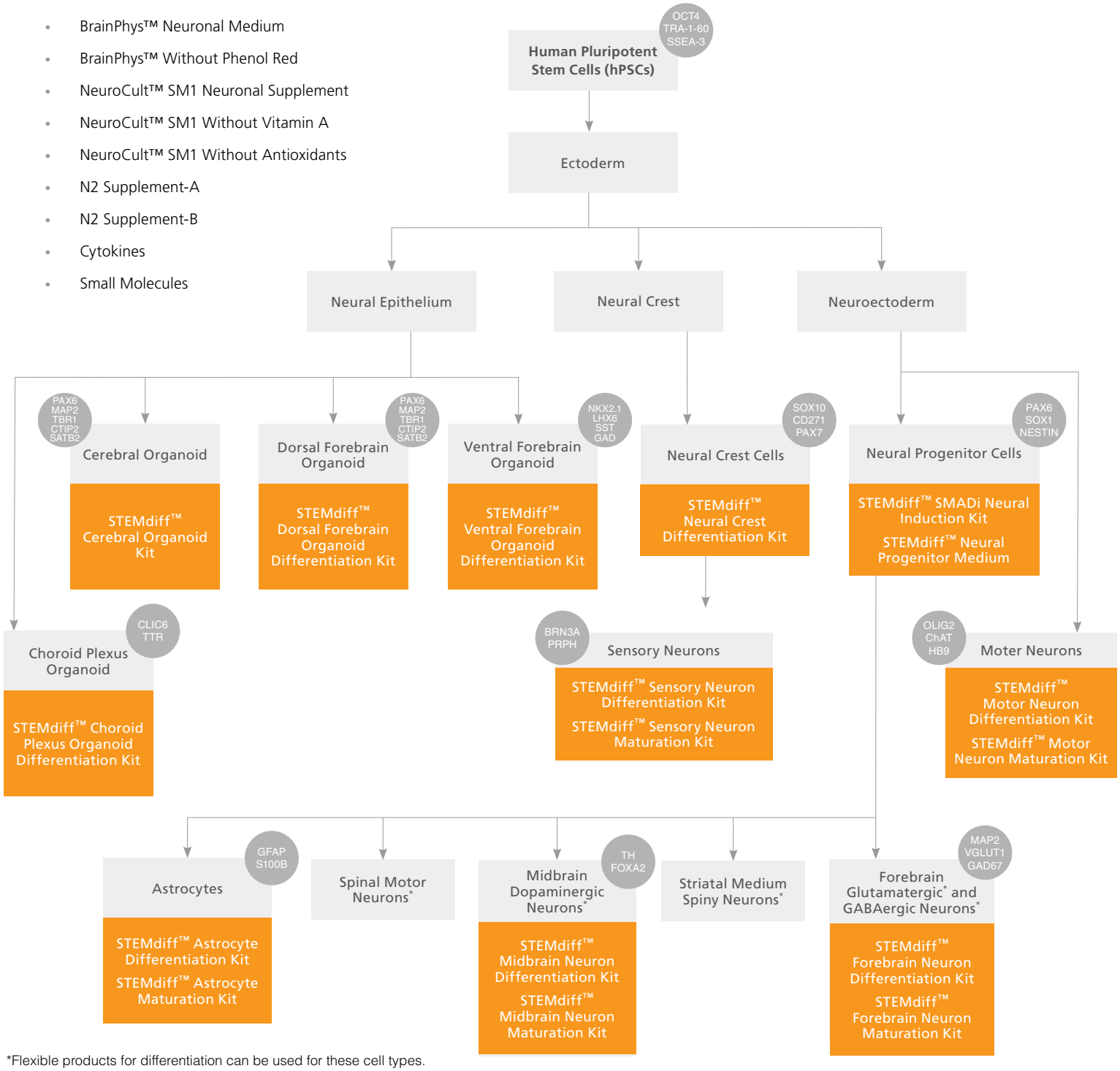
Portfolio Infographic

www.stemcell.com/Neural-Product-Workflow

Ectoderm Differentiation Pathways

Flexible Products for Differentiation

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



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

Other Products for Differentiation

- STEMdiff™ Microglia Differentiation Kit

STEMdiff™ Neural System

Differentiate hPSCs to Neural Progenitor Cells, Neurons, and Glia

The STEMdiff™ SMADi Neural Induction Kit combines STEMdiff™ Neural Induction Medium 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 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 and STEMdiff™ Neural Progenitor Freezing Medium, respectively.

NPCs cultured in STEMdiff™ Neural Progenitor Medium display typical NPC morphology (Figure 1D) 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.

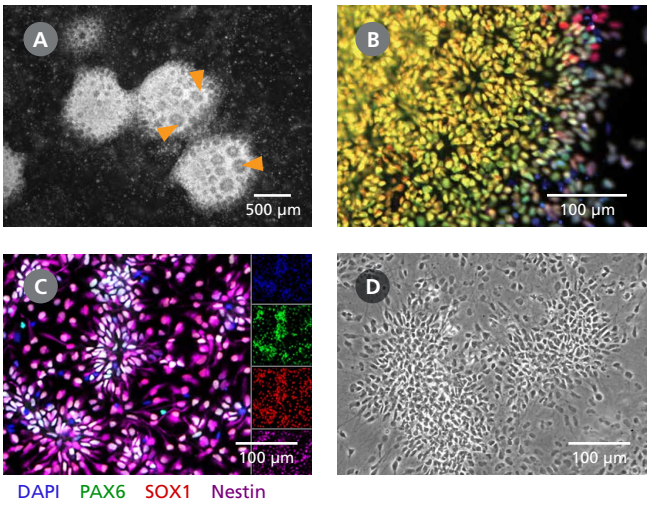


Figure 1. Neural Induction Using the STEMdiff™ SMADi Neural Induction Kit and STEMdiff™ Neural Progenitor Medium Generates Neural Rosettes and Enriches for CNS-type Neural Progenitor Cells

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).

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

Why Use the STEMdiff™ Neural System?

DEVELOPMENTALLY RELEVANT. Follow the in vivo developmental program with a small molecule-based system, with no introduction of foreign genetic material.

VERSATILE. Configure your NPC protocol with or without dual SMADi inhibition, and use embryoid body or monolayer protocols for workflow flexibility.

SCALABLE. Save time and effort by expanding NPCs several fold without loss of differentiation potential, and cryopreserve NPCs for additional flexibility.

COMPATIBLE. Seamlessly transition from TeSR™ hPSC maintenance media to STEMdiff™ neural induction.

STEMdiff™ Forebrain Differentiation and Maturation Kits

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

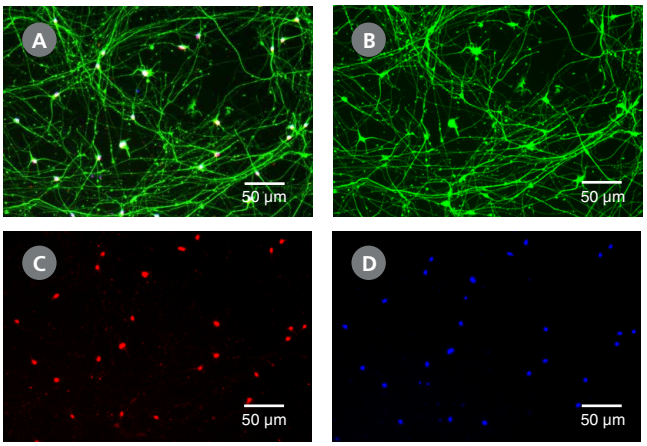


Figure 2. Downstream Differentiation of Neural Progenitor Cells to Neurons Is Possible Using the STEMdiff™ Differentiation and Maturation Kits

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

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

STEMdiff™ Midbrain Differentiation and Maturation Kits

Dopaminergic neurons can be generated using the serum-free STEMdiff™ Midbrain Neuron Differentiation Kit and STEMdiff™ Midbrain Neuron Maturation Kit. The midbrain-patterned cell population produced contains FOXA2$^+$, LMX1A$^+$ neuronal precursors, yielding neurons that can be maintained long-term in culture (Figure 3).

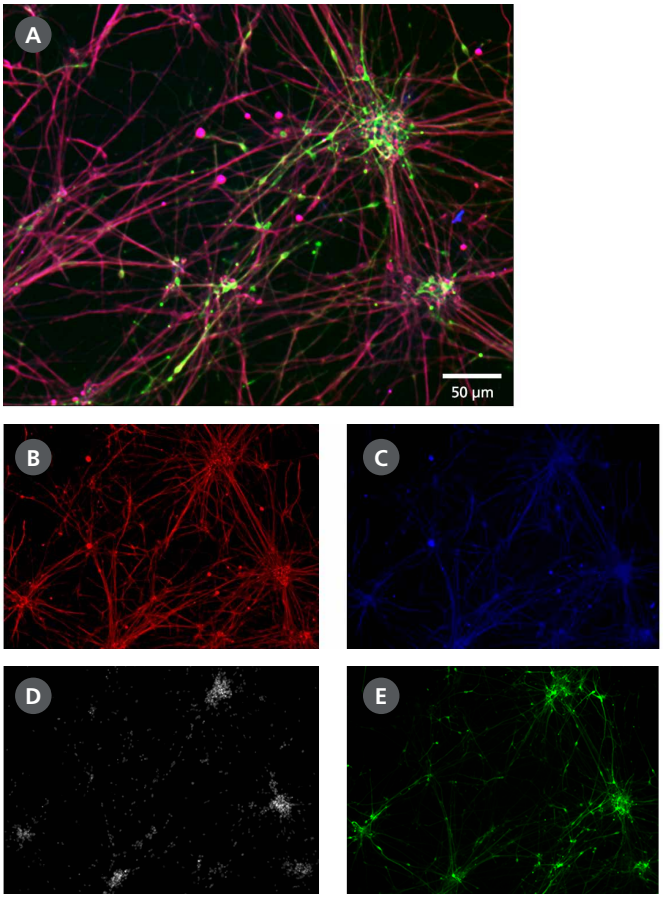


Figure 3. Midbrain-Type Neurons Express Tyrosine Hydroxylase and Dopamine Transporters (DAT) After Differentiation and Maturation in STEMdiff™ Midbrain Neuron Kits

(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 followed by 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 (blue), and (E) more than 15% tyrosine hydroxylase-positive cells (green). (D) Nuclei are labeled with DAPI (white).

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

STEMdiff™ Astrocyte Differentiation and Maturation Kits

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

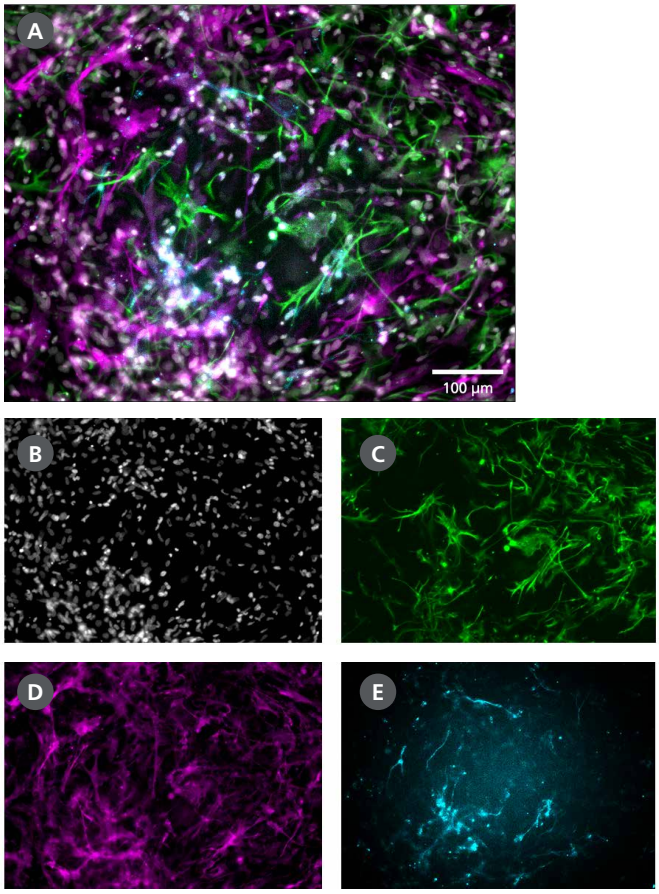


Figure 4. Cortical-Type Astrocytes Are Generated After Culture in STEMdiff™ Astrocyte Differentiation and Maturation Kits

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

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

STEMdiff™ Motor Neuron Differentiation and Maturation Kits

Generate pure in vitro populations of motor neurons from hPSCs in only 14 days using the STEMdiff™ Motor Neuron Differentiation Kit. These motor neurons can be further matured with the STEMdiff™ Motor Neuron Maturation Kit. The resultant motor neuron populations exhibit high-level expression of expected motor neuron markers across multiple cell lines, including ChAT (Figure 5D). Motor neurons derived using these kits are suitable for neuromuscular co-culture with muscle cells generated using MyoCult™ Differentiation Kit.

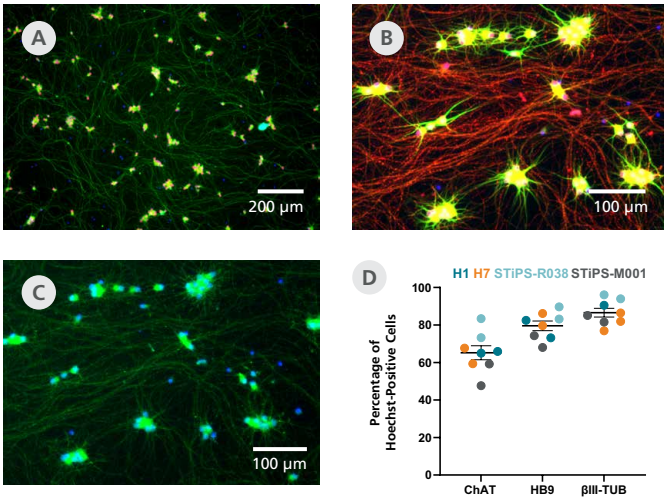


Figure 5. hPSC-Derived Motor Neurons Can Be Further Matured in STEMdiff™ Motor Neuron Maturation Medium

Motor neuron progenitors derived from a variety of cell lines were matured to motor neurons using the STEMdiff™ Motor Neuron Maturation Kit. (A) Mature motor neurons were generated after hPSCs were cultured with the STEMdiff™ Motor Neuron Differentiation Kit for 14 days and then the STEMdiff™ Motor Neuron Maturation Kit for an additional 14 days. The resulting 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 and 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) and βIII-TUB$^+$ (86.56% ± 2.331%, mean ± SEM) motor neurons. Numbers are % positive of total Hoechst-positive cells. hPSCs = human pluripotent stem cells

Why Use the STEMdiff™ Motor Neuron Kits?

RAPID. Generate motor neuron cells from human induced pluripotent stem cells in only 14 days.

EFFICIENT. Obtain pure populations of >60% OLIG2$^+$ motor neurons.

PHYSIOLOGICAL. Produce physiologically relevant results with integrated BrainPhys™ media supporting neuronal activity and maturation.

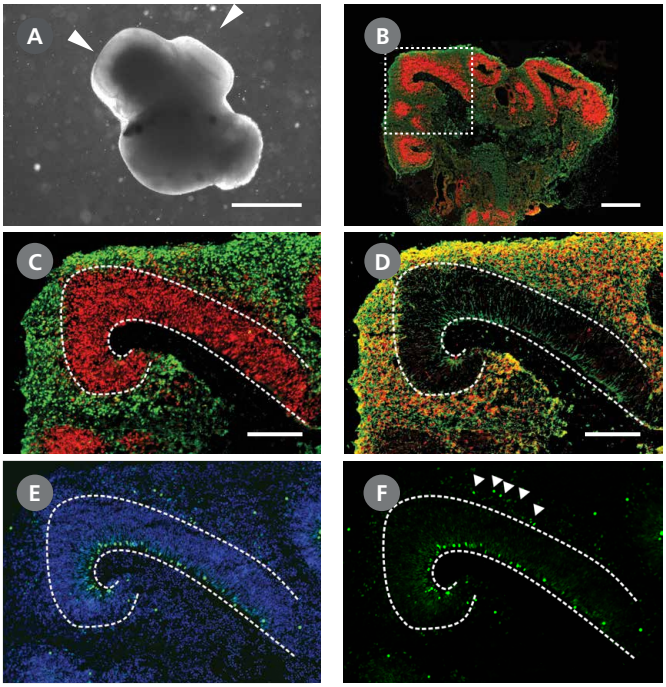
COMPATIBLE. Establish neuromuscular co-culture for in vitro modeling by combining with myotubes generated using the MyoCult™ Differentiation Kit or with microglia generated with the STEMdiff™ Microglia Kit.

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

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 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.



Why Use the STEMdiff™ Cerebral Organoid Kit?

UNPATTERNED. Allow differentiation to occur spontaneously to generate multiple brain regions within the same organoid.

FLEXIBLE. Culture under matrix droplet embedding or liquid matrix conditions.

OPTIMIZED. Enjoy increased organoid formation efficiency with a formulation based on a popular published protocol.[1]

COMPATIBLE. Use as a platform for generating new or modified organoid models.

Learn more at www.stemcell.com/COKit

Figure 6. 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). Scale bars = (A) 1 mm, (B) 500 µm, and (C-F) 200 µm.

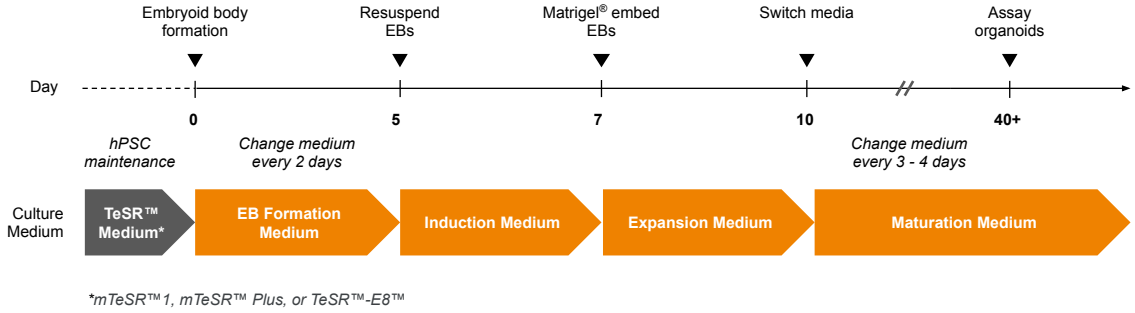


Figure 7. Schematic for Generating Unpatterned Neural Organoids Using the STEMdiff™ Cerebral Organoid Kit

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.[1]

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 and Ventral Forebrain Organoid Differentiation Kits are serum-free cell culture media that work with AggreWell™-generated embryoid bodies (EBs) 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.

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

PATTERNED. Direct differentiation to brain regions of interest with small molecule patterning factors, based on a popular published protocol.[2]

REPRODUCIBLE. Take advantage of reproducible structural morphology between lines and individual organoids to detect subtle disease phenotypes.

SCALABLE. Use with AggreWell™800 to generate over 500 organoids per kit for screening or more detailed longitudinal study.

MATRIX-FREE. Eliminate embedding steps and reduce handling while preventing organoid fusion.

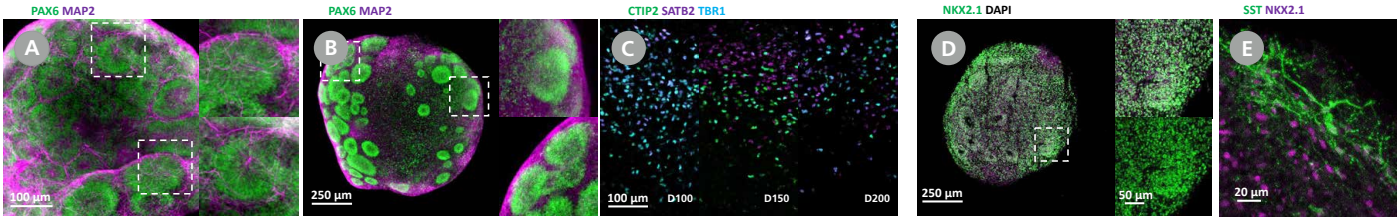


Figure 8. Dorsal Forebrain Organoids Exhibit Cortical Layering, and Both Dorsal and Ventral Organoids Express Markers Characteristic of Their Respective Brain Regions

(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.

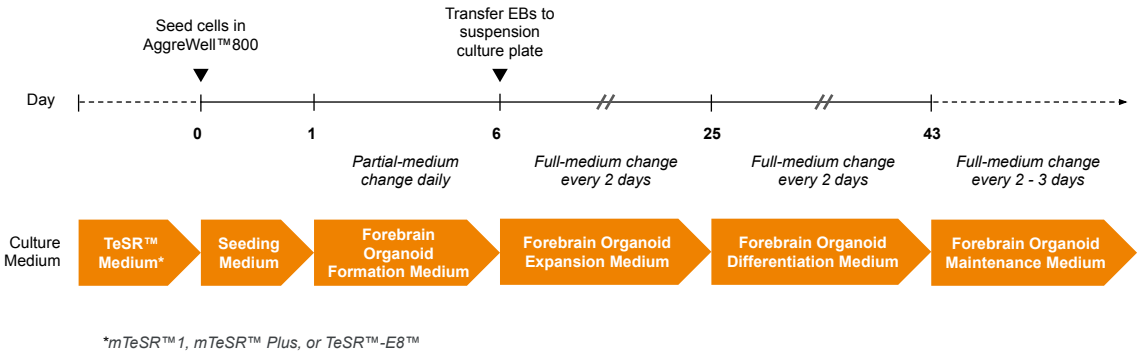


Figure 9. Schematic for the STEMdiff™ Dorsal and Ventral Forebrain Organoid Differentiation Kits

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.[2]

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 STEMdiff™ Choroid Plexus Differentiation Kit 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 STEMdiff™ Choroid Plexus Organoid Maturation Kit.

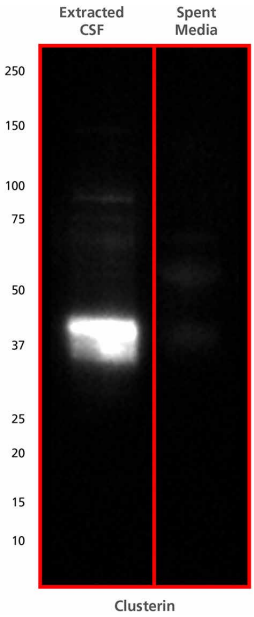


Figure 10. Fluid Extracted from Cysts in Choroid Plexus Organoids Is Enriched with Clusterin Protein, a Marker of Cerebrospinal Fluid (CSF)

Day 40 choroid plexus organoids were generated from hPSCs using STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits. CSF-like fluid was extracted from cysts contained in Day 40 choroid plexus organoids using a 28G syringe. A western blot was performed on the extracted fluid to detect Clusterin and shows a band between the 37 and 50 kDa molecular weight marker. Clusterin is a soluble secreted chaperone protein and biomarker relevant to Alzheimer's disease[3] found in high abundance in CSF.

Why Use the STEMdiff™ Choroid Plexus Organoid Kits?

RELEVANT. Adopt a human CNS barrier model for screening and modeling applications with the precise control of an in vitro system.

SIMPLE. Eliminate the need for complex co-cultures or Transwell® systems in your screening pipeline.

PURE. Extract CSF-like fluid to identify human CNS-specific biomarkers in a 100% blood-free system, without requiring a lumbar puncture.

SCALABLE. Obtain 50X more CSF-like fluid from a single kit than obtained from a single mouse, and eliminate the donor-to-donor variability of pooled clinical samples.

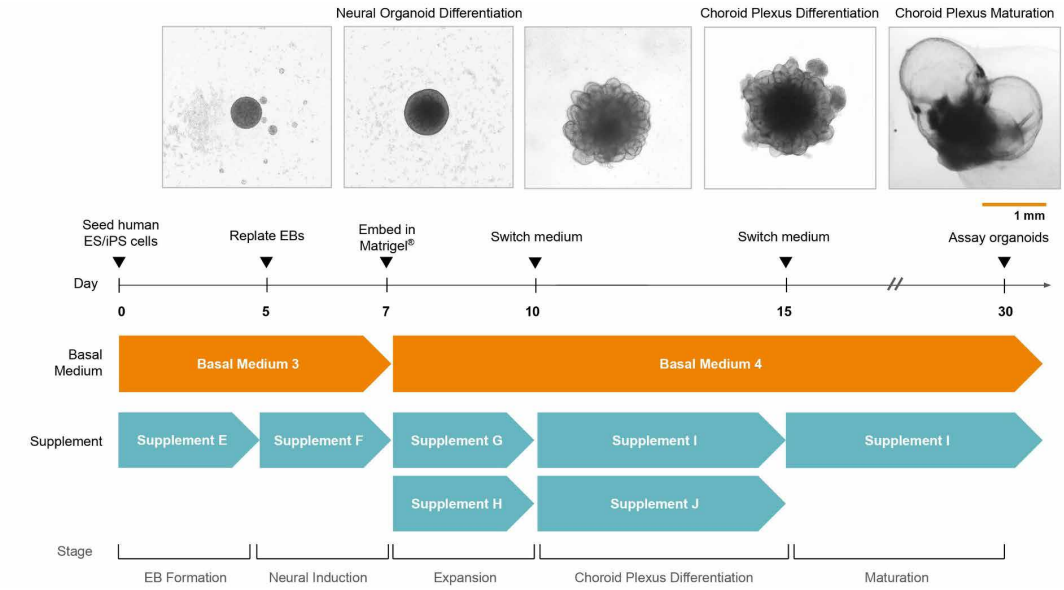


Figure 11. Schematic for the STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits

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.[4]

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

BrainPhys™ Neuronal Medium

Culture Active Neurons Under Physiological Conditions

Neurons can be generated efficiently from hPSC-derived NPCs using BrainPhys™ Neuronal Medium 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.[5]

Published protocols using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (based on the published B27 formulation[6]) and N2 supplement,[7] 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.[5]

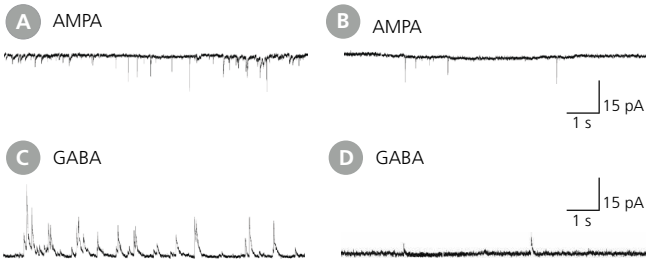


Figure 12. hPSC-Derived Neurons Matured in BrainPhys™ Neuronal Medium Show Improved Excitatory and Inhibitory Synaptic Activity by 44 Days

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?

PHYSIOLOGICAL. Use a medium more representative of the brain's extracellular environment.

ACTIVE. Improve neuronal function and generate a higher proportion of synaptically active neurons.

STREAMLINED. Perform functional assays without replacing media.

VERSATILE. Achieve long-term culture of hPSC- and CNS-derived neurons.

NeuroFluor™ NeuO

Selectively Label Live Neurons

NeuroFluor™ NeuO is a membrane-permeable fluorescent probe that selectively labels primary and pluripotent stem cell-derived neurons in live cultures.[8] Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications.

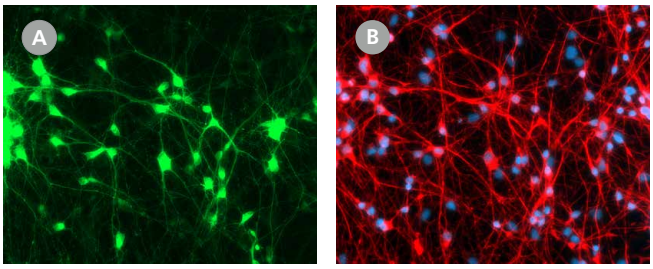


Figure 13. NeuroFluor™ NeuO Selectively Labels hPSC-Derived Neurons

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

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

STEMdiff™ Neural Crest Differentiation Kit

Generate Pure Populations of Neural Crest Cells

The STEMdiff™ Neural Crest Differentiation Kit 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, depending on the desired downstream application.

The NCCs produced using this kit are multipotent and can be further differentiated to cell types of both the neural and ecto-mesenchymal lineages. Sensory neurons expressing peripherin and BRN3A may be generated using STEMdiff™ Sensory Neuron Differentiation and Maturation Kits (Figure 14D), with potential applications for pain research.

Passaging NCCs into MesenCult™-ACF Plus Medium allows for differentiation to the chondrogenic lineage using the MesenCult™-ACF Chondrogenic Differentiation Kit (Figure 14E), to the osteogenic lineage using MesenCult™ Osteogenic Differentiation Kit (Figure 14F), and to the adipogenic lineage using MesenCult™ Adipogenic Differentiation Kit.

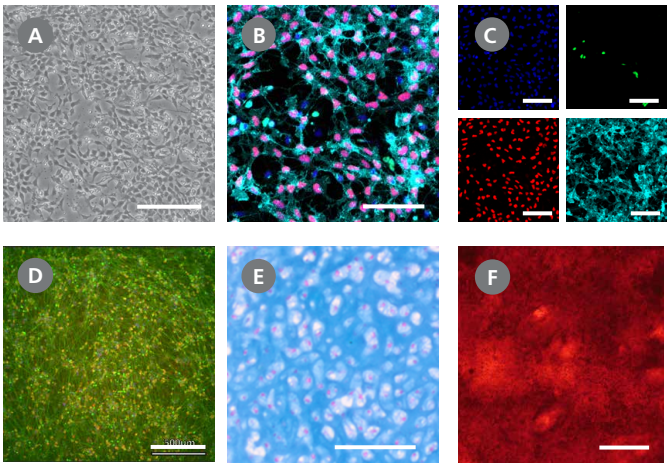


Figure 14. STEMdiff™ Neural Crest Differentiation Kit Generates a Highly Pure Population of Multipotent NCCs

After 6 days in culture, neural crest cells display (A) typical morphology, (B) express relevant markers (SOX10$^+$, red; CD271$^+$, light blue, DAPI, dark blue), and outnumber 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. CNS = central nervous system; NCCs = neural crest cells.

Learn more at www.stemcell.com/NCKit

Why Use the STEMdiff™ Neural Crest System?

RAPID. Generate neural crest cells with an easy-to-use monolayer protocol in only 6 days.

EFFICIENT. Obtain greater than 70% purity of bankable SOX10$^+$CD271$^+$ neural crest cells.

MULTIPOTENT. Produce cells capable of downstream differentiation, including to peripheral neurons, chondrocytes, or osteoblasts.

STEMdiff™ Sensory Neuron Differentiation and Maturation Kits

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

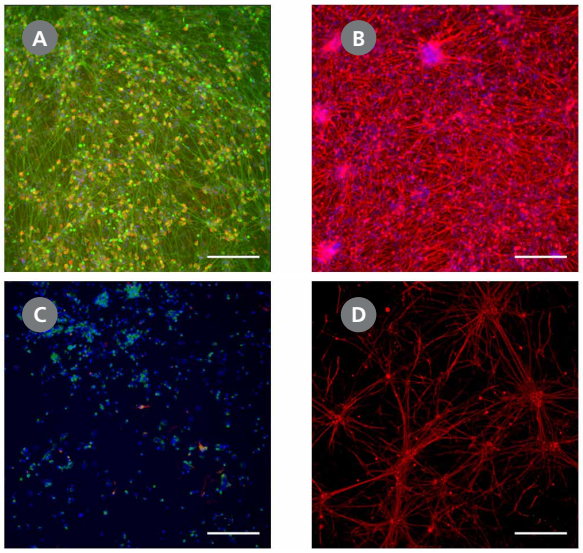


Figure 15. Sensory Neurons of the Peripheral Nervous System Can Be Generated Using STEMdiff™ Sensory Neuron Kits

NCCs generated from hPSCs in mTeSR™ Plus using the STEMdiff™ Neural Crest Differentiation Kit for 6 days were differentiated and matured to 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). Scale bar = 350 µm. NCCs = neural crest cells; hPSCs = human pluripotent stem cells; SNs = sensory neurons.

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

STEMdiff™ Microglia Culture System

Differentiate to Microglia from hPSCs

The STEMdiff™ Microglia Differentiation and Maturation 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, 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.

Why Use the STEMdiff™ Microglia Differentiation and Maturation Kits?

EFFICIENT. Differentiate greater than 80% of source HPCs into microglia, with few contaminating macrophages or monocytes.

OPTIMIZED. Use with HPCs generated using STEMdiff™ Hematopoietic Kit.

FUNCTIONAL. Produce microglia capable of phagocytosis and activation.

CONVENIENT. Employ an easy-to-use culture system that can replace workflows based on various published differentiation protocols.

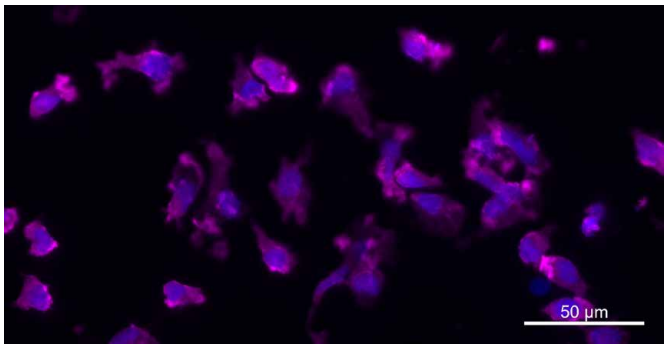


Figure 16. Microglia Generated with STEMdiff™ Microglia Culture System Show Expected Morphology and Markers

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,[9] 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).

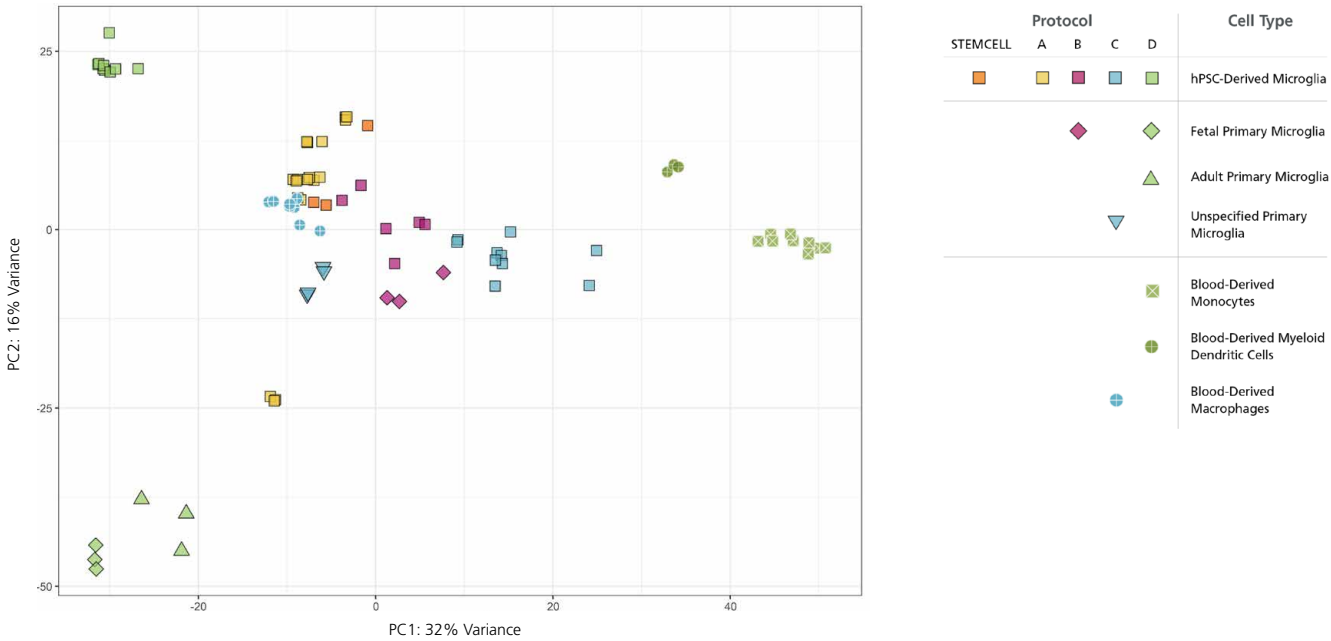


Figure 17. Microglia Generated with STEMdiff™ Microglia Culture System Are Typical PSC-Derived Microglial Cells

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

Accessory Products

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[10] result in EBs that are heterogeneous in size and shape (Figure 18A), leading to inefficient and uncontrolled differentiation.[11]

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 18B) and to ensure reproducibility of differentiation experiments.[12]

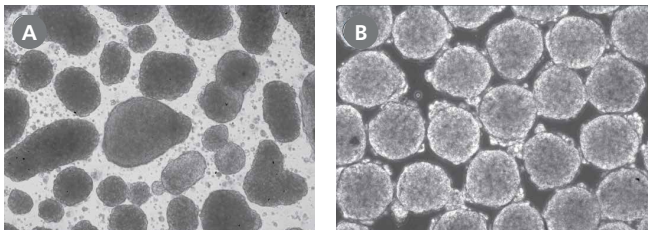


Figure 18. AggreWell™ Plates Are Used to Generate Uniform EBs

(A) Human EBs formed using conventional methods are heterogeneous in size and shape. In contrast, (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.

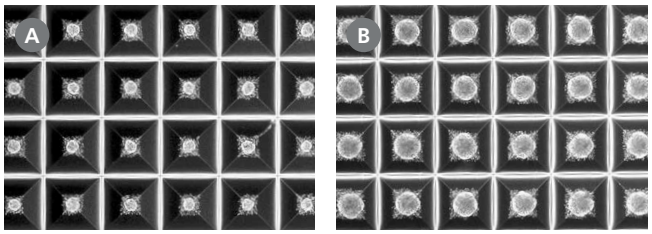


Figure 19. The Size of EBs Can Be Controlled in AggreWell™

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 in AggreWell™400 formed by seeding (A) 250 cells per microwell and (B) 1000 cells per microwell.

Why Use AggreWell™?

EASY-TO-USE. Simple EB generation.

REPRODUCIBLE. Produce large numbers of uniformly sized EBs.

CONTROLLABLE. Generate EBs consisting of 50 to 20,000 cells.

CONSISTENT. Reduce variability in differentiation protocols.

HIGH-YIELDING. Obtain up to 5900 EBs per well.

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.

Supplementary Reagents

Small Molecules

Regulate developmental signaling pathways related to neural differentiation of hPSCs with small molecules. Chemically defined with high purity and low lot-to-lot variability in activity, small molecules are increasingly being used to selectively activate or inhibit key targets for the differentiation of hPSCs into neural cell types. For a complete listing of small molecules available, visit **www.stemcell.com/smallmolecules**.

Small Molecules for Neural Differentiation

PRODUCT	FUNCTION	CATALOG #
Purmorphamine	Hedgehog pathway activator Activates smoothened	72202
All-Trans Retinoic Acid	Activates retinoic acid receptor (RAR)	72262
CHIR99021	WNT pathway activator Inhibits GSK3	72052
DAPT	Notch pathway inhibitor Inhibits γ-secretase	72082
SB431542	TGF-β pathway inhibitor Inhibits ALK5, ALK4, ALK7	72232
Dibutyryl-cAMP	Activates cAMP-dependent protein kinases	73882
Ascorbic Acid	Improves yield of neural cells during directed differentiation	72132

*selected products only, see **www.stemcell.com/smallmolecules** for more

Cytokines and Proteins for Your Neural Workflows

Induce, expand, and differentiate hPSCs with cytokines and growth factors. These high-quality reagents support NPC cultures and ensure reproducibility across a variety of assays. Choose from a large selection of cytokines and growth factors to incorporate into your research workflow. To view the full list of cytokines for hPSC-derived neural cell research, visit **www.stemcell.com/cytokines.**

Cytokines for Neural Differentiation*

PRODUCT	SIZE	CATALOG #
BDNF, Human, Recombinant[1]	10 µg	78005
GDNF, Human, Recombinant[1]	10 µg	78058
bFGF, Human, Recombinant[1]	50 µg	78003
EGF, Human, Recombinant[1]	500 µg	78006
Human Amyloid-β (1-42) Peptide (Trifluoroacetate Salt)	0.5 mg	100-0899

1. Animal Component-Free version available

*selected products only, see **www.stemcell.com/cytokines** for more

Antibodies

Analyze cells with antibodies that are verified to work with STEMCELL's cell culture reagents for select applications. These primary antibodies ensure consistent results for downstream applications, including immunofluorescence and immunocytochemistry. Choose from a wide range of antibodies selected for hPSC-derived neural cell research. For a complete listing of available antibodies, visit **www.stemcell.com/antibodies**.

Antibodies for Neural Differentiation and Characterization*

PRODUCT	HOST	CATALOG #
Neuronal Class III Beta-Tubulin Antibody, Clone TUJ1	Mouse	60052
Anti-Human Nestin Antibody, Clone 10C2	Mouse	60091
GFAP Antibody	Rabbit	60128
Anti-Rat NGF Receptor/p75NTR (CD271) Antibody, Clone 192-IgG	Mouse	60101

*selected products only, see **www.stemcell.com/antibodies** for more

For more related products, including primary cells, dissociation reagents, and cultureware, visit **www.stemcell.com/hPSCNCworkflow** or contact us at **techsupport@stemcell.com**.

Product Listing

Differentiation

Ectoderm Differentiation Products

PRODUCT	SIZE	CATALOG #
Neural Differentiation		
STEMdiff™ SMADi Neural Induction Kit	1 Kit	08581
STEMdiff™ Neural Induction Medium	250 mL	05835
STEMdiff™ Neural Rosette Selection Reagent	100 mL	05832
STEMdiff™ Neural Progenitor Medium	1 Kit	05833
STEMdiff™ Forebrain Neuron Differentiation Kit	1 Kit	08600
STEMdiff™ Forebrain Neuron Maturation Kit	1 Kit	08605
STEMdiff™ Midbrain Neuron Differentiation Kit	1 Kit	100-0038
STEMdiff™ Midbrain Neuron Maturation Kit	1 Kit	100-0041
STEMdiff™ Astrocyte Differentiation Kit	1 Kit	100-0013
STEMdiff™ Astrocyte Maturation Kit	1 Kit	100-0016
STEMdiff™ Motor Neuron Differentiation Kit	1 Kit	100-0871
STEMdiff™ Motor Neuron Maturation Kit	1 Kit	100-0872
STEMdiff™ Neural Progenitor Freezing Medium	100 mL	05838
STEMdiff™ Cerebral Organoid Kit	1 Kit	08570
STEMdiff™ Cerebral Organoid Maturation Kit	1 Kit	08571
STEMdiff™ Dorsal Forebrain Organoid Differentiation Kit	1 Kit	08620
STEMdiff™ Ventral Forebrain Organoid Differentiation Kit	1 Kit	08630
STEMdiff™ Neural Organoid Maintenance Kit	1 Kit	100-0120
STEMdiff™ Choroid Plexus Organoid Differentiation Kit	1 Kit	100-0824
STEMdiff™ Choroid Plexus Organoid Maturation Kit	1 Kit	100-0825
Customizable Neural Differentiation and Characterization		
BrainPhys™ Neuronal Medium	500 mL	05790
BrainPhys™ Without Phenol Red	500 mL	05791
BrainPhys™ Imaging Optimized	500 mL	05796
BrainPhys™ Neuronal Medium and SM1 Kit	1 Kit	05792
BrainPhys™ Neuronal Medium N2-A & SM1 Kit	1 Kit	05793
BrainPhys™ hPSC Neuron Kit	1 Kit	05795
NeuroFluor™ NeuO	0.1 mL	01801
PRODUCT	SIZE	CATALOG #
Neural Crest Differentiation		
STEMdiff™ Neural Crest Differentiation Kit	1 Kit	08610
STEMdiff™ Sensory Neuron Differentiation Kit	1 Kit	100-0341
STEMdiff™ Sensory Neuron Maturation Kit	1 Kit	100-0684
MesenCult™-ACF Plus Medium Kit	1 Kit	05445
MesenCult™-ACF Chondrogenic Differentiation Kit	1 Kit	05455
MesenCult™ Osteogenic Differentiation Kit (Human)	1 Kit	05465
MesenCult™ Adipogenic Differentiation Kit (Human)	1 Kit	05412
Neuroimmune Cell Differentiation		
STEMdiff™ Hematopoietic Kit	1 Kit	05310
STEMdiff™ Microglia Differentiation Kit	1 Kit	100-0019
STEMdiff™ Microglia Maturation Kit	1 Kit	100-0020

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