hPSC-DERIVED NEURAL CELLS
Products for Your Research
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.

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.

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

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-positive neuronal precursors, yielding neurons that can be maintained long-term in culture (Figure 3).

**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.

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**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 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).

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

(A) NPCs generated from hPSCs in TeSR™-EB™ 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 and 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-Dopa](http://www.stemcell.com/STEMdiff-Dopa)

Learn more at [www.stemcell.com/STEMdiff-Astro](http://www.stemcell.com/STEMdiff-Astro)
hPSC-DERIVED NEURAL CELLS

**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.

**Figure 6. 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.

**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 efficiency of organoid formation with a formulation based on a popular published protocol.

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

Learn more at [www.stemcell.com/COKit](http://www.stemcell.com/COKit)
**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.

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**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.

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**Figure 2. STEMdiff™ Dorsal Forebrain Organoid Kit and STEMdiff™ Ventral Forebrain Organoid Kit Support the Generation of Organoids**

Both dorsal and ventral forebrain organoids form on a multielectrode array (MEA) and further used in co-culture to form assemblies or "assembloids" with other 2D or 3D systems.

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**Figure 7. 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 are seen by day 75.

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**Figure 8. 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 Paçca².

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Learn more at [www.stemcell.com/dorsal-forebrain-organoid](http://www.stemcell.com/dorsal-forebrain-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.¹

Published protocols using a basal medium together with neural supplements, such as NeuroCult™ SM1 Neuronal Supplement (based on the published B27 formulation⁴) and N2 supplement,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

NeuroFluor™ NeuO 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.

Why Use BrainPhys™ Neuronal Medium?

- PHYSIOLOGICAL. More representative of the brain’s extracellular environment.
- ACTIVE. Improved neuronal function and a higher proportion of synaptically active neurons.
- STREAMLINED. Perform functional assays without replacing media.
- VERSATILE. Supports 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.⁶ Labeling with this probe is non-permanent; it can be washed off, providing unlabeled, viable cells for downstream applications.

Figure 9. 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 mM 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

Figure 10. 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). Due to their migratory capabilities and transience during development, NCCs remain difficult to isolate from tissues.

With the STEMdiff™ Neural Crest Differentiation Kit, multipotent NCCs can be generated from hPSC monolayers in 6 days. Further expansion of this 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. Peripheral neurons expressing peripherin and BRN3A may be generated using established protocols (Figure 12B), with potential applications for pain and sensory neuron research.

Passaging NCCs into MesenCult™-ACF Plus Medium allows for differentiation to the chondrogenic lineage after using the MesenCult™-ACF Chondrogenic Differentiation Kit (Figure 12C), to the osteogenic lineage after using MesenCult™ Osteogenic Differentiation Kit (Figure 12D), and to the adipogenic lineage using MesenCult™ Adipogenic Differentiation Kit.

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**Why Use the STEMdiff™ Neural Crest Differentiation Kit?**

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

**EFFICIENT.** Obtain greater than 70% purity of multipotent SOX10+CD271+ neural crest cells.

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

**VERSATILE.** Compatible with human ES and iPS cells maintained in mTeSR™ Plus, mTeSR™1, or TeSR™-E8™.

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**Figure 11.** STEMdiff™ Neural Crest Differentiation Kit Generates a Highly Pure Population of NCCs with Minimal CNS-type Progenitors

After 6 days in culture, neural crest cells (SOX10+, red; CD271+, light blue) outnumber CNS-type progenitors (PAX6+, green). (A) Channel merge of cells fixed 2 days after being passaged on day 6. Individual immunofluorescence channels show (B) DAPI, (C) PAX6, (D) SOX10, and (E) CD271. The NCCs also express expected neural crest markers, such as FOXD3 and HNK1 (CD57) (data not shown). Scale bar = 100 μm.

**Figure 12.** NCCs Generated with the STEMdiff™ Neural Crest Differentiation Kit Are Multipotent

NCCs (A) were cultured for 6 days and display typical morphology. (B) Culturing NCCs using established protocols generates peripheral neurons (Peripherin, green; BRN3a, red; DAPI, blue). (C) 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. (D) 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) 500 μm, (D) 1 mm.

Learn more at [www.stemcell.com/NCKit](http://www.stemcell.com/NCKit)
**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.** Upstream-compatible with HPCs generated using STEMdiff™ Hematopoietic Kit.

**FUNCTIONAL.** Produce microglia capable of phagocytosis and activation.

**CONVENIENT.** Easy-to-use culture system can replace workflows based on various published differentiation protocols.

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**Figure 13. 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, 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 14. 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](http://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 result in EBs that are heterogeneous in size and shape (Figure 15A), leading to inefficient and uncontrolled differentiation.

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 15B) and to ensure reproducibility of differentiation experiments.

Why Use AggreWell™?

EASY-TO-USE. Simple EB generation.

REPRODUCIBLE. Produce large numbers of uniformly sized EBs.

CONTROL OF EB SIZE. 50 to 20,000 cells per EB.

CONSISTENCY. Reduces variability in differentiation protocols.

HIGH YIELD. 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).

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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

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Cytokines
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*

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*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*

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*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.
**References**


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