### STEMdiff™ SMADi Neural Induction Kit

Serum-free medium kit for highly efficient SMAD inhibitionmediated neural induction of human ES and iPS cells



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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713 INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## **Product Description**

STEMdiff™ SMADi Neural Induction Kit consists of a defined, serum-free medium and supplement for the highly efficient neural induction of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. This kit combines STEMdiff™ Neural Induction Medium (Catalog #05835) with STEMdiff™ SMADi Neural Induction Supplement, which directs differentiation by blocking TGF-β/BMP-dependent SMAD signaling, resulting in efficient neural induction of even hard-to-differentiate cell lines. Neural progenitor cells (NPCs) can be generated using STEMdiff™ SMADi Neural Induction Kit with either an embryoid body (EB) protocol or monolayer culture protocol. The resulting cultures are enriched for central nervous system (CNS)-type NPCs, which express SOX1, Nestin, and PAX6. NPCs generated using this kit can be passaged as single cells and expanded in STEMdiff™ Neural Progenitor Medium (Catalog #05833). The NPCs can also be differentiated into neurons and glia using STEMdiff™ Neuron Differentiation Kit (Catalog #08500), STEMdiff™ Dopaminergic Neuron Differentiation Kit (Catalog #08520), or STEMdiff™ Astrocyte Differentiation Kit (Catalog #08540).

# Ordering Information

PRODUCT NAME	CATALOG #	SIZE	KIT COMPONENTS
STEMdiff <sup>™</sup> Neural Induction Medium	05835	250 mL	Not applicable.
STEMdiff™ Neural Induction Medium	05839	2 x 250 mL	STEMdiff™ Neural Induction Medium, 2 x 250 mL
STEMdiff™ SMADi Neural Induction Kit	08581	1 Kit	STEMdiff™ Neural Induction Medium, 250 mL     STEMdiff™ SMADi Neural Induction Supplement, 0.5 mL
STEMdiff™ SMADi Neural Induction Kit, 2 Pack	08582	1 Kit	STEMdiff™ Neural Induction Medium, 2 x 250 mL     STEMdiff™ SMADi Neural Induction Supplement, 2 x 0.5 mL

# Component Storage and Stability

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COMPONENT NAME	COMPONENT#	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Neural Induction Medium*†	05835	250 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ SMADi Neural Induction Supplement**	08580	0.5 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

<sup>\*</sup>This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

If product is received thawed, immediately place at -20°C or aliquot and store at -20°C. Product performance will not be affected.

\*\*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product. This component is sold as part of a kit (Catalog #08581 or 08582) and is not available for individual sale.



## Preparation of Media

NOTE: If STEMdiff™ Neural Induction Medium is received thawed, immediately place at -20°C or aliquot and store at -20°C. Product performance will not be affected. For thawing instructions, refer to section A or B below.

#### A. STEMdiff™ Neural Induction Medium + SMADi

Use sterile technique when preparing STEMdiff™ Neural Induction Medium + SMADi (STEMdiff™ Neural Induction Medium + STEMdiff™ SMADi Neural Induction Supplement). The following example is for preparing approximately 250 mL of medium. If preparing other volumes, adjust accordingly.

- 1. Thaw STEMdiff™ Neural Induction Medium and STEMdiff™ SMADi Neural Induction Supplement at room temperature (15 25°C) or overnight at 2 8°C. Mix thoroughly.
  - NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the product shelf life. Alternatively, thawed Neural Induction Medium may be stored at 2 8°C for up to 2 weeks. After thawing aliquots, use immediately. Do not re-freeze.
- 2. Add 0.5 mL of STEMdiff™ SMADi Neural Induction Supplement to 250 mL of STEMdiff™ Neural Induction Medium. Mix thoroughly. Warm medium to room temperature (15 25°C) before use.

NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the individual components. After thawing aliquots, use immediately. Do not re-freeze.

### B. STEMdiff™ Neural Induction Medium (Without SMADi)

Use sterile technique when preparing STEMdiff™ Neural Induction Medium.

Thaw STEMdiff™ Neural Induction Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C.

NOTE: If not used immediately, store at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the expiry date (EXP) as indicated on the label. After thawing aliquots, use immediately. Do not re-freeze.

### Directions for Use

For complete instructions on neural differentiation using STEMdiff™ Neural Induction Medium and STEMdiff™ SMADi Neural Induction Supplement, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System, available at www.stemcell.com or contact us to request a copy.

### Assessment of Neural Induction

Antibodies for PAX6, SOX1, and Nestin (e.g. Anti-Human Nestin Antibody, Clone 10C2; Catalog #60091) can be used alone or in combination to evaluate the phenotype of neural progenitor cells during neural induction. STEMdiff™ Human Neural Progenitor Antibody Panel (Catalog #69001) provides primary antibodies that are immunoreactive toward marker proteins highly expressed either by neural progenitor cells (Nestin, PAX6, SOX1) or by undifferentiated human ES and iPS cells (OCT4/OCT3). To determine the presence of potentially contaminating neural crest cells, anti-SOX10 or anti-CD271 antibodies can be used. Additionally, Anti-Beta-Tubulin III Antibody, Clone TUJ1 (Catalog #60052) can be used to evaluate premature neuronal differentiation. For evaluating neural induction, the optimal timepoint for assessment is between days 10 - 12 for the EB protocol or days 6 - 7 for the monolayer protocol. For complete protocols, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System, available at www.stemcell.com or contact us to request a copy. Results may vary depending on cell line used.

### Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow or contact us at techsupport@stemcell.com.

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