STEMdiff™ SMADi Neural Induction Kit

Serum-free medium kit for highly efficient SMAD inhibitionmediated neural induction of human ESCs and iPSCs



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Product Description

STEMdiffTM SMADi Neural Induction Kit consists of a defined, serum-free medium and supplement for the highly efficient neural induction of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs; e.g. Healthy Control Human iPSC Line, Female, SCTi003-A, Catalog #200-0511). This kit combines STEMdiffTM Neural Induction Medium (Catalog #05835) with STEMdiffTM 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 STEMdiffTM 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 STEMdiffTM Neural Progenitor Medium (Catalog #05833). The NPCs can also be differentiated into neurons and glia using STEMdiffTM Forebrain Neuron Differentiation Kit (Catalog #08600), STEMdiffTM Midbrain Neuron Differentiation Kit (Catalog #100-0013).

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	KIT COMPONENTS	
STEMdiff™ 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	2 Kits	STEMdiff [™] Neural Induction Medium (2 x 250 mL) STEMdiff [™] SMADi Neural Induction Supplement (2 x 0.5 mL)	

Component Storage and Stability

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 4 years from date of manufacture (MFG) on label.

^{*} 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.

Preparation of Media

STEMdiffTM Neural Induction Medium may be shipped with dry ice or ice packs and may be received thawed.

STEMdiff™ SMADi Neural Induction Supplement may be shipped with dry ice, ice packs, or under ambient conditions and may be received thawed.

If these products are 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.

^{**} 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.

STEMdiff™ SMADi Neural Induction Kit



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.

- Thaw STEMdiff[™] Neural Induction Medium at room temperature (15 25°C) or overnight at 2 8°C. Thaw STEMdiff[™] SMADi Neural Induction Supplement at room temperature. Mix thoroughly.
 - NOTE: If not using immediately, aliquot and store at -20°C. Do not exceed the shelf life of the individual components. 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 before use.

NOTE: If not using 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

Neural progenitor cell phenotype may be assessed by immunocytochemistry (ICC) at the end of neural induction (i.e. days 18 - 21 for monolayer cultures, or days 17 - 19 for EB cultures) after labelling with the following antibodies:

- Anti-Human Nestin Antibody, Clone 10C2 (Catalog #60091), and/or
- Anti-Human PAX6 (AN) Antibody, Polyclonal (Catalog #100-1340), and/or
- Anti-human SOX1 antibody, polyclonal (e.g. R&D Systems #AF3369)

To confirm that the proportion of neural crest cells in the final population is minimal (< 10%), the following antibodies may be used as a negative control:

- · Anti-human SOX10 antibody, clone A-2 (e.g. Santa Cruz #sc-365692), or
- Anti-human CD271 antibody, clone ME20.4 (e.g. Biolegend #345105)

Additionally, Anti-Beta-Tubulin III Antibody, Clone TUJ1 (Catalog #60052) can be used to evaluate premature neuronal differentiation. For complete protocols, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System (Document #10000005588), available at www.stemcell.com, or contact us to request a copy. Results may vary depending on the 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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