STEMdiff™ Neural Progenitor Medium

For maintenance and expansion of neural progenitor cells derived from human ES and iPS cells

Catalog #05833 1 Kit

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Product Description

STEMdiff™ Neural Progenitor Medium is optimized to support the growth of neural progenitor cells (NPCs). This medium was developed for NPCs derived from human embryonic stem (ES) and induced pluripotent stem (iPS) cells using STEMdiff™ Neural Induction Medium (Catalog #05831).

Product Information

The following components are sold as a complete kit (Catalog #05833) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Neural Progenitor Basal Medium*	05834	500 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neural Progenitor Supplement A (50X)*	05836	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Neural Progenitor Supplement B (1000X)	05837	500 µL	Store at -20°C.	Stable until expiry date (EXP) 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.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
DMEM/F-12 with 15 mM HEPES	36254
ACCUTASE™	07920
Trypan Blue	07050

Preparation of Complete STEMdiff™ Neural Progenitor Medium

Use sterile techniques to prepare complete STEMdiff™ Neural Progenitor Medium (Basal Medium + Supplement A + Supplement B). The following example is for preparing approximately 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Basal Medium, Supplement A, and Supplement B at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Thawed Basal Medium may be stored at 2 - 8°C for up to 3 weeks. Do not exceed the shelf life of the components. After thawing aliquots, use immediately. Do not re-freeze.

2. Add 2 mL of Supplement A and 100 µL of Supplement B to 98 mL of Basal Medium. Mix thoroughly.

NOTE: If not used immediately, store complete STEMdiff™ Neural Progenitor Medium at 2 - 8°C for up to 2 weeks. Do not freeze complete medium.

Directions for Use

Please read the entire protocol before proceeding.

NOTE: Poly-L-ornithine/laminin or Corning® Matrigel®-coated plates should be prepared in advance and brought to room temperature (15 - 25°C) for at least 30 minutes prior to use. For complete instructions on coating plates with these matrices, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System (Document #28782) available on our website at www.stemcell.com or contact us to request a copy.

NOTE: NPCs are ready for passage when cultures are approximately 80 - 90% confluent.

The following are instructions for passaging cells from one well of a 6-well plate. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

1. Warm (37°C) sufficient volumes of complete STEMdiff™ Neural Progenitor Medium, DMEM/F-12, and ACCUTASE™.
   OPTIONAL: Wash cells to be passaged with 1 mL of DMEM/F-12.
2. Aspirate medium and add 1 mL of ACCUTASE™.
3. Incubate at 37°C for 5 - 10 minutes.
4. Dislodge remaining attached cells by pipetting up and down using a 1 mL micropipette.
5. Add 5 mL of DMEM/F-12 and transfer the NPC-suspension to a 15 mL tube.
6. Centrifuge at 300 x *g* for 5 minutes.
7. Carefully aspirate the supernatant and add 1 mL of complete STEMdiff™ Neural Progenitor Medium.
8. Count viable cells using Trypan Blue and a hemocytometer.
9. Plate cells at desired density (e.g. 1.25 x 10^5 cells/cm^2) in complete STEMdiff™ Neural Progenitor Medium onto poly-L-ornithine/laminin or Corning® Matrigel®-coated plates.
10. Place the 6-well plate in a 37°C incubator with 5% CO_2 and 95% humidity. Move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the NPCs across the surface of the wells.
11. Perform daily medium changes using complete STEMdiff™ Neural Progenitor Medium.
12. Visually assess cultures to monitor growth and to determine timing of the next passage (i.e. when cells are approximately 80 - 90% confluent, typically after approximately 7 days of culture).

Assessment of Neural Progenitor Cells

Antibodies for PAX6, SOX1, and Nestin (Anti-Human Nestin Antibody, Clone 10C2; Catalog #60091) can be used alone or in combination to evaluate the phenotype of neural progenitor cells after transition to STEMdiff™ Neural Progenitor Medium. 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). Additionally, Anti-Beta-Tubulin III Antibody, Clone TUJ1 (Catalog #60052) can be used to assess premature neuronal differentiation. 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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