

# 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 #05835).

## 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.

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

## Materials Required But Not Included

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

## Preparation of STEMdiff™ Neural Progenitor Medium

NOTE: If STEMdiff™ Neural Progenitor Basal Medium is received thawed, immediately place at -20°C or aliquot and store at -20°C. Product performance will not be affected.

Use sterile technique to prepare 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. Do not exceed the product shelf life. Alternatively, thawed Basal Medium may be stored at 2 - 8°C for up to 3 weeks. 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 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: For complete instructions on coating plates with poly-L-ornithine (PLO)/laminin or Corning® Matrigel®, refer to the Technical Manual: Generation and Culture of Neural Progenitor Cells using the STEMdiff™ Neural System available at [www.stemcell.com](http://www.stemcell.com) or contact us to request a copy.

The following are instructions for passaging NPCs from one well of a 6-well plate and plating them onto a matrix-coated well of a new 6-well plate. Indicated volumes are for a single well. If using other cultureware, adjust volumes accordingly.

1. Coat one well of a 6-well tissue culture-treated plate with either PLO/laminin or Corning® Matrigel®. Incubate at room temperature (15 - 25°C) for at least 1 hour prior to use.
2. Warm (37°C) sufficient volumes of complete STEMdiff™ Neural Progenitor Medium, DMEM/F-12 with 15 mM HEPES, and ACCUTASE™.  
OPTIONAL: Wash cells to be passaged with 1 mL of DMEM/F-12 with 15 mM HEPES.
3. Aspirate medium and add 1 mL of ACCUTASE™.
4. Incubate at 37°C for 5 - 10 minutes.
5. Using a 1 mL pipettor, dislodge remaining attached cells by pipetting up and down.
6. Add 5 mL of DMEM/F-12 with 15 mM HEPES and transfer the cell suspension to a 15 mL tube.
7. Centrifuge at 300 x g for 5 minutes.
8. Carefully aspirate the supernatant and add 1 mL of complete STEMdiff™ Neural Progenitor Medium.
9. Count viable cells using Trypan Blue and a hemocytometer.
10. Using a serological pipette or by aspiration, gently remove the matrix solution from the plate prepared in step 1. Ensure that the coated surface is not scratched.
11. Plate cells at desired density (e.g.  $1.25 \times 10^5$  cells/cm<sup>2</sup>) in complete STEMdiff™ Neural Progenitor Medium onto the new matrix-coated plate.
12. Place the plate in a 37°C incubator with 5% CO<sub>2</sub> 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.
13. Perform daily medium changes using STEMdiff™ Neural Progenitor Medium.
14. Visually assess cultures to monitor growth and to determine timing of the next passage. Cultures are ready for passaging after approximately 7 days of culture; however, depending on rate of cell growth, passaging can be performed on Day 6 - 9.

## 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, and 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](http://www.stemcell.com/hPSCNCworkflow) or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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