Neural Progenitor Medium 2

Medium for the maintenance and expansion of cryopreserved human PSC-derived neural progenitor cells

Catalog #08560 1 Kit

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Product Description

Neural Progenitor Medium 2 is a serum-free medium for the expansion of STEMCELL's cryopreserved Human PSC-Derived Neural Progenitor Cells (Catalog #70901 and 70902). Neural progenitor cells (NPCs) expanded in this medium remain highly pure (≥ 90% SOX1+/Nestin+) over more than 10 passages, while retaining high neuronal and glial differentiation capacity.

NOTE: Neural Progenitor Medium 2 is recommended for use only with STEMCELL NPCs (Catalog #70901 and 70902). STEMdiff™ Neural Progenitor Medium (Catalog #05833) is recommended for the expansion of NPCs generated using STEMdiff™ Neural Induction Medium (Catalog #05831) and/or cryopreserved using STEMdiff™ Neural Progenitor Freezing Medium (Catalog #05838).

Product Information

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Neural Progenitor Medium 2, Basal Medium	08561	250 mL	Store at 2 - 8°C.	Stable for 18 months from date of manufacture (MFG) on label.
Neural Progenitor Medium 2, Supplement A	08562	5 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.
Neural Progenitor Medium 2, Supplement B	08563	50 µL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
50 mL conical tubes	38010
DMEM/F-12 with 15 mM HEPES	36254
ACCUTASE™	07920
15 mL conical tubes	38009
Trypan Blue	07050

Preparation of Reagents and Materials

A. COATING CELL CULTURE VESSELS WITH MATRIGEL®

Corning® Matrigel® hESC-Qualified Matrix should be aliquoted and frozen. Consult the Certificate of Analysis supplied with the Matrigel® for the recommended aliquot size ("Dilution Factor") to prepare 24 mL of diluted matrix. Ensure to always keep Matrigel® on ice when thawing and handling to prevent it from gelling.

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Matrigel® on ice.
2. Dispense 24 mL of cold DMEM/F-12 into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.

4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. See Table 1 for recommended coating volumes.

Table 1: Recommended Volumes for Coating Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF MATRIGEL®
96-well plate	0.33 cm^2/well	50 µL/well
4- or 24-well plate	2 cm^2/well	250 µL/well
6-well plate	10 cm^2/well	1.5 mL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 cm^2	2.5 mL

5. Swirl the cultureware to spread the Matrigel® solution evenly across the surface.

   NOTE: If the surface of the cultureware is not fully coated by the Matrigel® solution, it should not be used.

6. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature (15 - 25°C) for 30 minutes before moving onto the next step.

7. Immediately prior to plating cells, gently tilt the cultureware onto one side and allow the excess Matrigel® solution to collect at the edge. Remove the excess Matrigel® solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched and do not let it dry out.

B. PREPARATION OF COMPLETE NEURAL PROGENITOR MEDIUM 2

Use sterile techniques to prepare complete Neural Progenitor Medium 2 (Basal Medium + Supplement A + Supplement B). The following example is for preparing 50 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw 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 shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.

2. Add 1 mL of Supplement A and 10 µL of Supplement B to 50 mL of Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete Neural Progenitor Medium 2 at 2 - 8°C for up to 1 week. Warm complete medium to 37°C before use.

Directions for Use

Please read the entire protocol before proceeding.

A. PLATING AND EXPANDING NPCs

1. Thaw Human PSC-Derived Neural Progenitor Cells as described in the Product Information Sheet (PIS) for the cells (Document #DX21378), available at www.stemcell.com or contact us to request a copy.

   NOTE: Neural Progenitor Medium 2 is recommended for use only with STEMCELL NPCs (Catalog #70901 and 70902).

2. Calculate the volume of cell suspension required to seed 80,000 - 100,000 cells/cm^2 in the chosen Corning® Matrigel®-coated cultureware.
3. Aspirate Matrigel® solution from wells and add complete Neural Progenitor Medium 2.
4. Seed 80,000 - 100,000 cells/cm^2.
5. Distribute cells evenly. Incubate at 37°C and 5% CO_2 for 24 hours.
6. Change medium on Day 2 post-seeding and then every other day until NPCs reach 95 - 100% confluence (typically Day 6 - 7).

B. PASSAGING NPCs

NPCs are ready for passaging when they reach 95 - 100% confluence.

NOTE: This protocol is for a single well of a 6-well plate. Pre-warm cultureware, medium, and reagents to 37°C.

1. Remove medium and wash cells with sterile phosphate-buffered saline (PBS).
2. Remove PBS and add 1 mL of ACCUTASE™.
3. Incubate cells at 37°C for 5 minutes.
4. Using a 1 mL pipettor, gently resuspend the cells by pipetting up and down slowly 3 - 4 times. Transfer cell suspension to a sterile 15 mL conical tube.
5. Add 4 mL of DMEM/F-12 to the cell suspension.
6. Centrifuge at 300 x *g* for 5 minutes.
7. Remove supernatant and resuspend pellet in 1 - 2 mL of complete Neural Progenitor Medium 2.
8. Count cells using Trypan Blue and a hemocytometer.
9. Calculate the volume of cell suspension required to seed 80,000 - 100,000 cells/cm^2 in the chosen culture vessel.
10. Aspirate Matrigel® solution from well and add complete Neural Progenitor Medium 2.
11. Seed 80,000 - 100,000 cells/cm^2.
12. Distribute cells evenly and incubate at 37°C and 5% CO_2 for 24 hours.
13. Evaluate cell survival. Incubate at 37°C and 5% CO_2.
14. Perform a full medium change every other day until NPCs reach 95 - 100% confluence (typically 6 - 7 days after seeding).

NOTE: Perform SOX1 and Nestin staining every 5 passages to ensure neural pluripotency is retained. NPCs can be passaged more than 10 times without loss of differentiation capacity.

C. DIFFERENTIATING NPCs

For differentiation to mixed neurons, dopaminergic neurons, or astrocytes, refer to the PIS for STEMdiff™ Neuron Differentiation Kit (Document #DX20341), STEMdiff™ Dopaminergic Neuron Differentiation Kit (Document #DX20343), or STEMdiff™ Astrocyte Differentiation Kit (Document #DX20345), respectively, available at www.stemcell.com or contact us to request a copy.