STEMdiff™ Neural Progenitor Freezing Medium

For cryopreservation of neural progenitor cells generated using STEMdiff™ Neural Induction Medium

Catalog # 05838 100 mL

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Product Description

STEMdiff™ Neural Progenitor Freezing Medium is a serum-free medium for cryopreservation of neural progenitor cells (NPCs) derived from human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. This freezing medium is optimized for the cryopreservation of NPCs generated using STEMdiff™ Neural Induction Medium (Catalog #05835) and cultured in STEMdiff™ Neural Progenitor Medium (Catalog #05833). NPCs can be frozen at any point post neural induction, with reproducibly high recovery rates. Post thawing, NPCs display healthy morphology, express NPC markers, and retain the potential to expand and differentiate into neurons.

- Serum-free
- Optimized for cryopreservation of NPCs, with reproducibly high recovery rates
- Supports cryopreservation of NPCs generated using STEMdiff™ Neural Induction Medium and cultured in STEMdiff™ Neural Progenitor Medium
- Preserves NPC multipotency and expansion capacities
- Convenient, user-friendly format and protocol

Properties

Storage:	Store at 2 - 8°C.
Shelf Life:	Stable for 9 months from date of manufacture (MFG) on label.
Contains:	• Dimethyl sulfoxide (DMSO) • Other ingredients

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 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.

Directions For Use

For complete instructions on generating and culturing NPCs, 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.

FREEZING NEURAL PROGENITOR CELLS

1. Prepare a single-cell suspension of NPCs using a dissociation protocol of your choice and centrifuge cells appropriately to obtain a cell pellet.
   For example, use ACCUTASE™ (Catalog #07920) to dissociate cells (incubate at 37°C for 5 - 10 minutes), then centrifuge cells at 300 x *g* for 5 minutes.
2. Add cold (2 - 8°C) STEMdiff™ Neural Progenitor Freezing Medium to obtain a cell suspension of 2 - 4 x 10^6 cells/mL. Resuspend cells.
3. Transfer 1 mL of the cell suspension into each cryovial.
4. Freeze cells using a standard slow rate-controlled cooling protocol that reduces temperatures at approximately -1°C/minute, followed by long-term storage at -135°C (liquid nitrogen) or colder. Long-term storage at -80°C is not recommended.

THAWING NEURAL PROGENITOR CELLS

NOTE: Coat plates with poly-L-ornithine/laminin or Corning® Matrigel® and bring to room temperature (15 - 25°C) for at least 30 minutes prior to use. For complete instructions on coating plates, 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.

In general, one frozen vial containing 2 - 4 x 10^6 NPCs can be successfully thawed into one well of a 6-well plate.

1. Warm DMEM/F-12 with 15 mM HEPES (Catalog #36254) and STEMdiff™ Neural Progenitor Medium (Catalog #05833) to 37°C before starting the protocol to ensure that the thawing procedure is performed as quickly as possible.
2. Add 10 mL of warm DMEM/F-12 to a 15 mL conical tube (e.g. Catalog #38009).
3. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains.
4. Remove the cryovial from the water bath and wipe it with 70% ethanol or isopropanol.
5. Transfer cells from the cryovial to the 15 mL tube containing DMEM/F-12. Mix gently.
6. Centrifuge cells at 300 x *g* for 5 minutes.
7. Aspirate medium, leaving the cell pellet intact. Gently resuspend the cell pellet in 2 mL of STEMdiff™ Neural Progenitor Medium.
8. Add cells to one well of a coated 6-well plate.
9. 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.