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 defined and 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-thaw, NPCs display healthy morphology, express NPC markers, and retain the potential to expand and differentiate into neurons.

- Defined and 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.

Materials Required But Not Included

- STEMdiff™ Neural Progenitor Medium (Catalog #05833)

Handling / Directions For Use

For complete instructions on how to generate and culture NPCs, 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.

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) and 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 x10^6 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: Prepare plates coated with poly-L-ornithine/laminin or Corning® Matrigel® in advance and bring 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 of Neural Progenitor Cells from hPSCs using STEMdiff™ Neural Induction Medium (Document #28782), available on our website 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 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 tube.
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 medium (e.g. STEMdiff™ Neural Progenitor Medium).
8. Plate cells onto one well of a pre-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.