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StemPro® NSC SFM

Description

StemPro® NSC SFM has been developed for the growth and expansion of neural stem cells (NSC) in adherent or suspension culture. Using StemPro® NSC SFM, NSC can be expanded for multiple passages while maintaining their potential to differentiate into neurons and glial cells. StemPro® Neural Supplement is based on N-2 and B-27® supplements and has been shown to support the cultivation of Oligodendrocyte Progenitor Cells (OPC) and Glial Restricted Progenitors (GRP) when used with required growth factors.

Product	Catalog no.	Amount	Storage	Shelf life
StemPro® NSC SFM Kit	A10509-01	1 kit		
Contains:				
KnockOut™ DMEM/F12	12660-012	1 × 500 mL	2°C to 8°C; Protect from light	18 months*
StemPro® Neural Supplement	A10508-01	1 × 10 mL	–20°C to –5°C; Protect from light	12 months*
FGF-basic (AA 10–105) Recombinant Human	PHG0024	1 × 10 µg	2°C to 8°C; desiccated	12 months**
EGF Recombinant Human	PHG0314	1 × 10 µg	2°C to 8°C; desiccated	12 months**

* Shelf life duration is determined from Date of Manufacture.

** Shelf life duration is determined from date of receipt when stored properly.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Important information

- Thaw StemPro® Neural Supplement at 37°C to avoid precipitate from forming. Sore thawed StemPro® Neural Supplement in the dark at 2°C to 8°C for up to 4 weeks prior to use or refreeze for future use.
- Basic Fibroblast Growth Factor (bFGF) is unstable at 37°C. We recommend aliquoting complete medium into required working volumes. Avoid exposing the complete medium to multiple warming (37°C)/ cooling cycles.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

StemPro® Neural Supplement only

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Prepare media

StemPro® NSC SFM complete medium requires supplementation of KnockOut™ DMEM/F12 with StemPro® Neural Supplement, Epidermal Growth Factor (EGF), bFGF, and GlutaMAX™-I CTS™.

1. Aseptically add 10 mL of StemPro® Neural Supplement to 484 mL of KnockOut™ DMEM/F12.
2. Aseptically add both bFGF and EGF by rinsing tubes with 0.5 mL KnockOut™ DMEM/F12 medium (20 ng/mL bFGF and EGF) and transfer to complete medium.
3. Aseptically add 5 mL 200 mM GlutaMAX™-I CTS™ (2 mM final concentration) to complete KnockOut™ DMEM/F12 medium (500 mL total volume).
4. If desired, add Antibiotic-Antimycotic solution at 10 mL/L to the complete medium.

Note: Complete StemPro® NSC SFM is stable for up to 4 weeks when stored in the dark at 2°C to 8°C within the expiration date of all components.

Note: Adding 200 µM ascorbic acid is optional especially for suspension culture. For more information, refer to the StemPro® Neural Stem Cells product insert.

Culture Conditions

Media: StemPro® NSC SFM complete medium

Cells: Human neural stem cells

Culture type: Adherent or suspension (neurosphere)

Culture vessels: Geltrex®-, CellStart™- or Laminin-coated tissue culture dish or flask (adherent cultures), or untreated culture vessels (suspension neurosphere cultures); can be used for cultivation of neural stem cells.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Note: The following procedures are for cultures in a T-25 culture flask (25 cm^2). Adjust volumes accordingly for other vessel sizes.

Coat culture vessels

Note: For detailed coating procedures, refer to the product insert with the following modifications.

1. Dilute the coating material to:

Coating material	Dilution
Geltrex® Basement Membrane Matrix	1:100
CELLstart™ CTS™ attachment substrate	1:50
Laminin	10 µg/mL

2. Mix by gentle pipetting, **do not vortex**. Add 5 mL of the coating material solution to each flask, ensure complete surface coverage.
3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 60 minutes.
4. Immediately before use, remove all coating solution and replace with pre-warmed complete StemPro® NSC SFM.

Recovery of cryopreserved NSC

1. Rapidly thaw (<2 minute) frozen cells in a 37°C water bath.
2. Pipet the entire contents of the cryovial into a sterile 15-mL conical tube.

3. Carefully, by dropwise addition (≈1 drop per second), add 4 mL of pre-warmed complete StemPro® NSC SFM. Mix by gentle swirling of the tube. Add additional pre-warmed complete StemPro® NSC SFM to a final volume of 10 mL.
4. Centrifuge at 200 × *g* for 4 minutes, confirm presence of cell pellet, aspirate and discard supernatant being careful not to disturb cell pellet.
5. Resuspend cell pellet in 5 mL pre-warmed complete StemPro® NSC SFM and transfer entire contents of the conical tube into a coated tissue culture flask (see **Coat culture vessels).**
6. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
7. Exchange spent medium with fresh pre-warmed complete StemPro® NSC SFM 24 hrs post-thaw.

Note: For recovery of cells grown in StemPro® NSC SFM, we recommend seeding cells at ≥1 × 10^5 cells/cm^2 for the initial passage.

Subculture cells

StemPro® NSC SFM has been developed for the multi-passage expansion of NSC isolated from fetal tissue or derived from pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells). Optimal growth conditions must be determined for each application.

For the cultivation of OPC and GRP, substitute PDGF-AA (10 ng/mL) for EGF in preparing complete StemPro® NSC SFM as outlined in **Prepare media**.

Note: For suspension neurosphere cultures, refer to the StemPro® Neural Stem Cells product insert.

Subculture NSC in StemPro® NSC SFM

1. When cultures reach 90–100% confluency, aspirate spent medium from the flask and discard.
2. Wash cell monolayer with 5 mL pre-warmed DPBS without calcium and magnesium, aspirate and discard.
3. Add 1.0 mL pre-warmed StemPro® Accutase® to each flask; ensure complete coverage of cell monolayer. Incubate for 2–5 minutes at room temperature.
4. After incubation, check with an inverted microscope to see if the cells have detached. Firmly tap the flask as necessary to facilitate cell detachment.
5. Gently pipet up and down to disperse clumps into a single cell suspension. Stop cell dissociation reaction by adding 9 mL pre-warmed complete StemPro® NSC SFM. Transfer cell suspension to a sterile conical tube.
6. Centrifuge tube at 200 × *g* for 4 minutes. Aspirate supernatant and discard. Resuspend cell pellet in a minimal volume of pre-warmed complete StemPro® NSC SFM. Determine total viable cell density with a Countess® Automated Cell Counter (alternative automated or manual procedures may be used).
7. Remove coating solution from each coated flask (see **Coat culture vessels**), then add 5 mL pre-warmed complete StemPro® NSC SFM.
8. Add 5 × 10^4 cells/cm^2 to each flask (for example, 1.25 × 10^6 cells/T-25 flask). Mix or swirl cell suspension to ensure even distribution.
9. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
10. Exchange spent culture medium every 2–3 days with fresh, pre-warmed complete StemPro® NSC SFM for optimal performance and cell growth.

Cryopreserve NSC in StemPro® NSC SFM

1. Prepare cryopreservation solution on day of use by supplementing complete StemPro® NSC SFM with 20% Dimethyl Sulfoxide (DMSO). Keep on ice until use.
2. Follow step 1 through 7 in **Subculture NSC in StemPro® NSC SFM** to harvest cells for cryopreservation.
3. Calculate the volume of cryopreservation solution required to give cell density of 2 × 10^6 viable cells/mL. Resuspend the pellet in half the final volume required of pre-warmed complete StemPro® NSC SFM. Add an equal volume of cold complete StemPro® NSC SFM + 20% DMSO in drop wise manner to result in a final concentration of 10% DMSO. Immediately dispense suspension into cryovials (1 mL/vial).
4. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
5. Transfer frozen cells to liquid nitrogen; we recommend vapor phase storage at –200°C to –150°C.

Related products

Product	Catalog no.
GlutaMAX™-I CTS™, 200mM (100X), liquid	A12860
Antibiotic-Antimycotic (100X), liquid	15240
PDGF-AA Recombinant Human	PHG0035
CELLstart™ CTS™	A10142
Geltrex® LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A14133
Laminin	23017
Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium, or phenol red (1X), liquid	14190
StemPro® Accutase®	A11105
StemPro® Neural Stem Cells, 1 × 10^6 cells	A15654
StemPro® Neural Stem Cells, 5 × 10^6 cells	A15655
Trypan Blue Solution, 0.4%	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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