StemPro™ NSC SFM

Catalog Number A1050901

Pub. No. MAN0007329 **Rev.** 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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

Product description

Gibco™ StemPro™ NSC SFM is a serum free medium (SFM) 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.

Contents and storage

Table 1 StemPro™ NSC SFM Kit, Cat. No. A1050901

Contents	Cat. No.	Amount	Storage	Shelf Life
KnockOut™ DMEM/F-12 medium	12660012	1 × 500 mL	2°C to 8°C. Protect from light	18 months[1]
StemPro™ Neural Supplement	A10508-01	1 × 10 mL	–20°C to –5°C. Protect from light	12 months[1]
FGF-basic (AA 10–155) Recombinant Human Protein	PHG0024	1 × 10 µg	2°C to 8°C, dessicated	12 months[2]
EGF Recombinant Human Protein	PHG0314	1 × 10 µg		

[1] Shelf life duration is determined from Date of Manufacture.
[2] Shelf life duration is determined from date of receipt when stored properly.

Culture conditions

Media: StemPro™ NSC SFM complete medium

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. Ensure that proper gas exchange is achieved in culture vessels. Minimize exposure of cultures to light.

Procedural guidelines

- Thaw StemPro™ Neural Supplement at 37°C to avoid precipitate from forming. Store thawed StemPro™ Neural Supplement in the dark at 2°C to 8°C for up to 4 weeks before use or freeze for future use.
- Basic Fibroblast Growth Factor (bFGF) is unstable at 37°C. Aliquot complete medium into required working volumes. Avoid exposing the complete medium to multiple warming (37°C)/ cooling cycles.
- 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). Determine optimal growth conditions for each application.
- The procedures described in this user guide are for cultures in a T-25 culture flask (25 cm^2). Adjust volumes accordingly for other vessel sizes.

Prepare StemPro™ NSC SFM complete medium

StemPro™ NSC SFM complete medium requires supplementation of KnockOut™ DMEM/F-12 medium with StemPro™ Neural Supplement, Epidermal Growth Factor (EGF), bFGF, and GlutaMAX™ Supplement.

1. Aseptically add 10 mL of StemPro™ Neural Supplement to 484 mL of KnockOut™ DMEM/F-12 medium.
2. Aseptically reconstitute bFGF and EGF by adding 0.5 mL of KnockOut™ DMEM/F-12 medium to each tube, then aseptically transfer the lyophilized growth factors to the complete medium.

   IMPORTANT! For the cultivation of OPC and GRP, use PDGF-AA (10 ng/mL) instead of EGF.
3. Aseptically add 5 mL of 200 mM GlutaMAX™ Supplement (2 mM final concentration) to complete KnockOut™ DMEM/F-12 medium (500 mL total volume).
4. *(Optional)* 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 *StemPro™ Neural Stem Cells User Guide* (Pub. No. MAN0007861).

Coat culture vessels

For detailed coating procedures, refer to the user guides *Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix* (Pub. No. MAN0007332), *CTS™ CELLStart™* (Pub. No. MAN0007326), *Natural Mouse Laminin* (Pub. No. MAN0001423), with the following modifications.

1. Choose one of the following coating materials and dilute according to the following table.

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

2. Mix by gentle pipetting, do not vortex, then 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 for 60 minutes.
4. Immediately before use, remove all coating solution, then replace with pre-warmed complete StemPro™ NSC SFM.

Recovery of cryopreserved NSC

1. Rapidly (<2 minutes) thaw 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, then mix by gentle swirling of the tube.
4. Add additional pre-warmed complete StemPro™ NSC SFM to a final volume of 10 mL.
5. Centrifuge at 200 × *g* for 4 minutes, confirm the presence of the cell pellet, then discard the supernatant.

   Be careful not to disturb cell pellet.
6. Resuspend the cell pellet in 5 mL of pre-warmed complete StemPro™ NSC SFM, then transfer the entire contents of the conical tube into a coated tissue culture flask.
7. Incubate at 37°C in a humidified atmosphere of 5% CO_2.
8. Replace medium with fresh pre-warmed complete StemPro™ NSC SFM 24 hours 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 NSC in StemPro™ NSC SFM

Note: For suspension neurosphere cultures, refer to *StemPro™ Neural Stem Cells User Guide* (Pub. No. MAN0007861).

1. When cultures reach 90–100% confluency, aspirate then discard the medium from the flask.
2. Wash cell monolayer with 5 mL of pre-warmed DPBS without calcium and magnesium, then aspirate and discard the solution.
3. Add 1.0 mL of pre-warmed StemPro™ Accutase™ Cell Dissociation Reagent to each flask, then incubate for 2–5 minutes at room temperature.

   Ensure complete coverage of the cell monolayer before incubation.
4. 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.
6. Stop the cell dissociation reaction by adding 9 mL of pre-warmed complete StemPro™ NSC SFM, then transfer cell suspension to a sterile conical tube.
7. Centrifuge the cells at 200 × *g* for 4 minutes.
8. Discard the supernatant, then resuspend the cell pellet in a minimal volume of pre-warmed complete StemPro™ NSC SFM.

9. Determine total viable cell density with a Countess™ II Automated Cell Counter (alternative automated or manual procedures can be used).

10. Remove coating solution from each coated flask, then add 5 mL of pre-warmed complete StemPro™ NSC SFM.

11. Add 5×10^4 cells/cm^2 to each flask (for example, 1.25×10^6 cells/T-25 flask), then mix or swirl the cell suspension to ensure even distribution.

12. Incubate at 37°C in a humidified atmosphere of 5% CO_2.

    Note: For optimal performance and cell growth, replace culture medium every 2–3 days with fresh, pre-warmed complete StemPro™ NSC SFM.

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 step 7 in "Subculture NSC in StemPro™ NSC SFM" on page 2 to harvest cells for cryopreservation.

3. During centrifugation, calculate the final volume required to give a cell density of 2×10^6 viable cells/mL.

   Note: The next steps require one half-volume of room temperature StemPro™ NSC SFM and one half-volume of cold cryopreservation solution.

4. Discard the supernatant, then resuspend the pellet in one half-volume of room temperature complete StemPro™ NSC SFM.

5. Add an equal volume of cold complete StemPro™ NSC SFM + 20% DMSO, in a drop wise manner, to result in a final concentration of 10% DMSO.

6. Immediately aliquot the cell suspension into cryovials (1 mL/vial).

7. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (a decrease of 1°C per minute).

8. Transfer frozen cells to liquid nitrogen.

   We recommend vapor phase liquid nitrogen storage at –200°C to –150°C.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Cat. No.
GlutaMAX™ Supplement (200mM (100X), liquid)	35050061
Antibiotic-Antimycotic (100X), liquid	15240
PDGF-AA Recombinant Human	PHG0035
CTS™ CELLStart™ Substrate	A10142
Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A14133
Laminin Mouse Protein, Natural	23017
Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium, or phenol red (1X), liquid	14190
StemPro™ Accutase™ Cell Dissociation Reagent	A11105
StemPro™ Neural Stem Cells	A15654 A15655
Rat Fetal Neural Stem Cells	N7744100
Trypan Blue Solution, 0.4%	15250
Countess™ Automated Cell Counter	AMQAX1000 AMQAF1000

Limited product warranty

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