

StemPro® Neural Supplement

GIBCO® StemPro Neural Supplement has been developed for the growth and expansion of neural stem and progenitor cells. Mammalian neural stem cells and Glial restricted progenitors can be expanded for multiple passages while maintaining their characteristics in the medium supplemented with StemPro Neural supplement.

Description	Cat. No.	Size
StemPro Neural Supplement	A1050801	1 x 10 mL

Intended Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Precautions

To avoid precipitate from forming it is recommended to thaw StemPro Neural Supplement at 37°C until thawed completely prior to use. Thawed material can be used for up to 4 weeks when stored in the dark at 2° to 8°C or can be refrozen for future use.

It is recommended to aliquot complete medium into required working amounts. Avoid exposing the complete medium to 37°C multiple times.

Storage

Store at -5° to -20°C, in the dark.

Shelf Life

12 months

Physical Conditions

Standard physical conditions for neural stem and progenitor cells grown in medium supplemented with StemPro Neural Supplement are 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air. Using standard aseptic conditions, cultures may be grown in medium on appropriately coated tissue culture vessels (i.e. T25 flask) Ensure that proper gas exchange is achieved in culture vessels. Avoid overexposure of cultures to light.

The following procedures are developed for proliferation of human Glial Progenitor Cells (hGPC). KnockOut DMEM/F12, bFGF, PDGF-AA, and GlutaMAX-1 are sold separately. For purchasing information, See Related Products section.

Medium Preparation

hGPC medium requires supplementation of KNOCKOUT DMEM/F12 with StemPro Neural Supplement, bFGF, PDGF-AA and GlutaMAX-I. Complete medium is stable for 2 weeks when stored in the dark at 2 to 8°C.

- For 500 mL of complete medium, aseptically add 10 mL of StemPro Neural Supplement to 485 mL of KNOCKOUT DMEM/F12.
- Aseptically add 10 µg of bFGF and 5 µg of PDGF-AA to complete medium.
- Aseptically add 5.0 mL (10mL/L) of 200 mM GlutaMAX-I to complete medium.
- If so desired, add Antibiotic-Antimycotic solution (Cat. No. 15240) at 10mL/L to the complete medium.

Recovery of Cryopreserved Human GPCs:

NOTE: hGPC can adhere to the bare plastic and glass pipettes. To maximize cell recovery and yield, it is recommended to rinse all plastic and glassware that will come in contact with the cell suspension in advance with complete medium.

1. Rapidly thaw frozen vial of cells in a 37°C water bath.
2. Pipet the entire contents of the cryovial into a 50 mL conical tube.
3. Carefully add to conical tube 4 mL of pre-warmed (37°C) medium at an approximate rate of 1 drop per second while swirling the tube. Then add 5 mL of pre-warmed medium.
4. To remove cryoprotectant, centrifuge the tube at 300 x g for 7 minutes and remove the supernatant above the cell pellet.
5. Resuspend cells gently in pre-warmed medium and transfer the entire contents of the tube into a Poly-L-Ornithine coated tissue culture flask. **See Poly-L-Ornithine Coating of Culture Flask section.**
6. Incubate at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.
7. Fluid change flasks 24 hours post-thaw. **See hGPC Passaging section.**

NOTE: For recovery of hGPC, it is recommended to seed cells at $\geq 0.8 \times 10^5$ cells/cm² for the initial recovery passage.

Subculturing hGPC

The complete medium has been developed for the multi-passage expansion of human GPCs either isolated from fetal tissue or derived from embryonic stem cells. Optimal growth conditions must be determined for each application.

NOTE: The following procedures apply to adherent cultures in a T25 culture flask (25 cm²). Volumes should be adjusted accordingly for desired vessel size.

A. Poly-L-Ornithine Coating of Culture Flask:

1. Dilute Poly-L-Ornithine (Sigma-Aldrich® Cat. No. P3655) in distilled water (Cat. No. 15230) to make 15 µg/mL solution. Coat T25 culture flasks by adding 3 mL of the Poly-L-Ornithine solution to each flask.
2. Place culture flasks with Poly-L-Ornithine solution in the incubator at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air for 60 minutes.
3. After incubation, remove flasks from the incubator and wash three times with distilled water.
4. After the third wash, allow flasks to dry before using. Coated flasks can be stored at 4°C up to 2 weeks.

B. hGPC Passaging:

1. Observe stock culture flask under the microscope and confirm that the cells are ready to be sub-passaged (~80% confluent).
2. Pre-warm cell dissociation reagent (TrypLE™ or StemPro Accutase®) and complete medium to 37°C before use.
3. Remove spent medium from the flask using a pipet and transfer to a conical tube.

NOTE: Do not discard spent medium. To be used as a washing buffer in Step 7.

4. Gently rinse flask with 2 mL of DPBS (Cat. No. 14190) and transfer to the conical tube used in Step 3.
5. Add 1.0 mL cell dissociation reagent to the T25 flask, tilt flask in all directions to evenly distribute. Incubate for 2 to 5 minutes at room temperature.
6. Once cell detachment is observed (detached cells will move with tilting of the flask) gently pipette up and down to break clumps into a single cell suspension.
7. Transfer dissociated cells to the new conical tube. If cell clumps still appear, repeat Steps 5 and 6.
8. Rinse flask with wash buffer (from Steps 3 and 4) and transfer dissociated cells to the conical tube in Step 7.
9. Centrifuge tube at 300 x g for 7 minutes.
10. Resuspend the cells in a minimal volume of warmed complete medium. Take a sample from the cell suspension for cell counting using a preferred counting method (i.e. hemocytometer).
11. Add 5 mL of complete medium to Poly-L-Ornithine coated flask and enough cell suspension to provide a final seeding density of 5×10^4 cells/cm² (i.e. 1.25×10^6 cells/T25 flask). Mix or swirl cell suspension to ensure even distribution.
12. Place culture flask in the incubator at 36 to 38°C in a humidified atmosphere of 4 to 6% CO₂ in air.
13. For optimal performance and cell growth, cultures should be fed every two to three days with fresh complete medium.

Cryopreservation of hGPC

1. Prepare GPC cryopreservation solution by supplementing complete medium with 20% DMSO and store at 4°C until use; make cryopreservation medium on day of intended use.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a desired final cell density (i.e. 1.0×10^6 cells/mL).
3. Centrifuge cells at 300 x g for 7 minutes.
4. Resuspend the pellet in half the final volume required of complete medium. Add the other half of the final volume of the 20% DMSO containing medium in a drop wise manner to result in a final concentration of 10% DMSO.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, (vapor phase); storage at -125°C to -200°C is recommended.

For human neural stem cell culture protocols using StemPro NSC SFM (A10509-01) which contains StemPro Neural Supplement, refer to Product Insert # 5020.

For rat neural stem cell culture protocol using the Rat Fetal Neural Stem Cell Kit (N7744-200) which contains StemPro Neural Supplement, refer to Product Manual # A11229.

For rat glial progenitor cell culture protocols using Rat Glial Precursor Cells (N7746-100) refer to Product Manual # A11232.

For the expansion of Neural Stem Cells (NSCs) please refer to www.invitrogen.com/stemcells for detailed protocols.

Related Products

StemPro NSC SFM kit (A10509-01)

FGF Basic REC HU (PHG0024)

PDGF-AA REC HU (PHG0035)

GlutaMAX-I, 200mM (100X), liquid (35050)

Knockout DMEM/F12 (12660)

Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium (1X), liquid (14190)

Antibiotic-Antimycotic (100X) (15240)

StemPro Accutase (A11105)

Mouse anti-A2B5 (433110)

TrypLE Express (1X), liquid without phenol red (12604)

Water, distilled, (15230)

Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at www.invitrogen.com. For further assistance, please email our Technical Support team at Techsupport@Invitrogen.com.

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