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StemPro® Neural Supplement

Description

StemPro® Neural Supplement has been developed as a convenient, and cost-effective serum-free medium supplement optimized for the proliferation of mammalian neural stem and progenitor cells, such as human and rat neural stem cells (NSC) and glial progenitor cells (GPC). StemPro® Neural Supplement can also be used, in combination with appropriate basal medium and growth factors, for the expansion of some neuronal progenitor cells. Mammalian neural stem cells and Glial restricted progenitors can be expanded for multiple passages while maintaining their progenitor status in medium supplemented with StemPro Neural supplement.

Product	Catalog no.	Amount	Storage	Shelf life*
StemPro® Neural Supplement	A10508-01	10 mL	-20°C to -5°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

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

Important information

- Thaw StemPro® Neural Supplement at 37°C, to avoid precipitate formation, until completed thawed before use.
- Thawed StemPro® Neural Supplement can be used for up to four weeks when stored at 2°C to 8°C protected from light.
- Thawed StemPro® Neural Supplement can be refrozen one time for future use.

Safety information

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

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.

Use

StemPro® Neural Supplement is designed, based on B-27® supplement and N-2 supplement, to support the multi-passage expansion of NSC and GPC either isolated from fetal tissue or derived from pluripotent stem cells. Optimal growth conditions must be determined for each application. The following procedures were developed for proliferation of human Glial Progenitor Cells (hGPC) in adherent T-25 flask (25 cm^2) cultures. Adjust volumes according to vessel size. For additional information and protocols relating to neural stem and progenitor cell culture visit **www.lifetechnologies.com/stemcells**.

Prepare medium

hGPC medium requires supplementation of KnockOut™ DMEM/F-12 Medium with StemPro® Neural Supplement, basic Fibroblast Growth Factor (bFGF), Platelet Derived Growth Factor (PDGF-AA) and GlutaMAX™-I prior to use for growth and maintenance of hGPCs. See the following table for the recipe for preparing 500 mL of media.

Component	Stock Concentration	Final Concentration	Volume
KnockOut™ DMEM/F12	—	1X	483.5 mL
StemPro® Neural Supplement	50 X	1 X	10 mL
bFGF*	10 µg/mL	10 ng/mL	0.5 mL
PDGF-AA*	10 µg/mL	10 ng/mL	1 mL
GlutaMAX™-I	200 mM	2 mM	5 mL
Antibiotic-Antimycotic**	100 X	1 X	5 mL

* Reconstitute both bFGF and PDGF-AA to a stock concentration of 10 µg/mL in KnockOut™ DMEM/F-12 Medium.

** If necessary.

Once supplemented, aliquot the complete medium into working volumes. The complete medium is stable up to 2 weeks when stored at 2°C to 8°C, protected from light. Avoid exposing the complete medium to 37°C multiple times.

Culture conditions

Media: Medium supplemented with StemPro® Neural Supplement

Cell line(s): mammalian NSC and GPC

Culture type: Adherent

Culture vessels: Poly-L-Ornithine coated T-25 flask.

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.

Poly-L-Ornithine coating of culture flask

1. Prepare 15 µg/mL solution Poly-L-Ornithine in distilled water.
2. Add 3 mL of the Poly-L-Ornithine solution to each T-25 culture flask. Tilt flask in all directions to ensure complete surface coverage.
3. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air for 60 minutes.
4. Remove flasks from the incubator and wash three times with sterile distilled water.
5. Air dry flasks before using. Coated dry flasks can be stored at 4°C for up to 2 weeks.

Recovery of cryopreserved hGPCs

Note: hGPCs readily adhere to bare plastic and glassware. To maximize cell recovery and yield, we recommend rinsing all plastic and glassware with complete medium before use.

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath removing when the last trace of ice has melted.
2. Pipet the entire contents of the cryovial into a sterile pre-rinsed 50-mL conical tube.
3. Carefully, by dropwise addition (one drop per second), add 4 mL of pre-warmed hGPC Medium while gently swirling the tube. Then add 5 mL pre-warmed hGPC Medium.
4. Centrifuge at 300 × *g* for 7 minutes; ascertain presence of cell pellet. Aspirate supernatant without disturbing cell pellet.
5. Resuspend cells in 5 mL pre-warmed complete hGPC Medium and transfer entire contents into a Poly-L-Ornithine coated tissue culture flask.

   Note: For recovery of hGPC, we recommend seeding cells at $\geq 0.8 \times 10^5$ cells/cm^2 for the initial recovery passage.
6. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.
7. Exchange spent medium with fresh pre-warmed complete hGPC Medium 24 hours post-thaw.

Subculture-Passage hGPCs

1. Observe culture flask on inverted microscope and confirm that the cells are ready to be subcultured (~80% confluent).
2. Pre-warm cell dissociation reagent (TrypLE™ or StemPro® Accutase®) and complete hGPC Medium to 37°C before use.
3. Pipet spent medium from the flask to a conical tube.

   Note: Reserve spent medium to be used as a washing buffer in step 8. Do not discard.
4. Rinse flask with 2 mL of DPBS without calcium and magnesium and transfer to the conical tube used in step 3.
5. Add 1.0 mL cell dissociation reagent to the T-25 flask, tilting flask in all directions to evenly distribute. Incubate for 2–5 minutes at room temperature.
6. Observe cells for detachment (detached cells will move with tilting of the flask); gently pipet up and down to disperse clumped cells into a single cell suspension.
7. Transfer cell suspension to a sterile 15-mL conical tube.
8. Rinse flask with wash buffer (from steps 3 and 4). Collect and transfer to the conical tube in step 7.
9. Centrifuge the cell suspension at 300 × *g* for 7 minutes.
10. Aspirate supernatant and resuspend the cell pellet in a minimal volume of pre-warmed complete hGPC Medium. Determine viable cell density using a Countess® Automated Cell Counter.
11. Seed cells at 5×10^4 cells/cm^2 (i.e., 1.25×10^6 cells/T-25 flask) into Poly-L-Ornithine coated flask containing 5 mL of pre-warmed complete hGPC Medium. Gently swirl cell suspension to ensure even distribution.
12. Incubate at 37°C in a humidified atmosphere of 5% CO_2 in air.

Note: For optimal performance and cell growth, re-feed cultures every two to three days with fresh complete medium.

Cryopreserve hGPCs

1. Prepare hGPC cryopreservation medium by supplementing complete medium with 20% DMSO and store at 4°C until use.

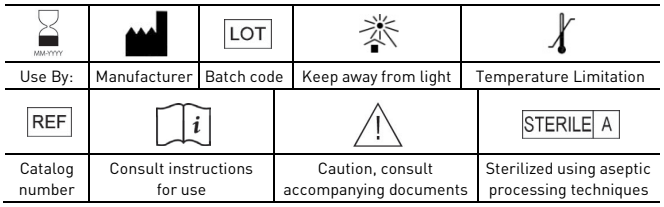
   Important: Prepare cryopreservation medium on the day of use.
2. Prepare the desired quantity of cells, harvesting when cell monolayer reaches ~80% confluency, following steps 1–9 in hGPC Passaging.
3. Resuspend the cell pellet in half the final volume required of pre-warmed complete hGPC Medium. Add an equal volume of cryopreservation solution in a drop wise manner, with constant gentle swirling to mix, to result in a final concentration of 10% DMSO.
4. Immediately dispense aliquots of cell suspension into cryovials (1 mL/vial).
5. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
6. Transfer frozen cells to liquid nitrogen; we recommend (vapor phase) storage at –200°C to –150°C.

Related products

Product	Cat. no.
GlutaMAX™-I, 200mM (100X), liquid	35050
L–Glutamine, 200mM (100X), liquid	25030
PDGF-AA Recombinant Human	PHG0035
FGF-basic (AA 10-155) Recombinant Human	PHG0024
Antibiotic-Antimycotic (100X), liquid	15240
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
KnockOut™ DMEM/F-12 (1X), liquid	12660
StemPro® NSC SFM	A10509-01
TrypLE™ Express (1X), liquid, without Phenol Red	12604
StemPro® Accutase®	A11105
A2B5 (105), Mouse Monoclonal Antibody -Unconjugated	433110
Gibco® Rat Glial Precursor Cells	N7746-100
StemPro® Neural Stem Cells, 1×10^6 cells	A15654
StemPro® Neural Stem Cells, 5×10^6 cells	A15655

Explanation of symbols and warnings

The symbols present on the product label are explained below:



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For further assistance, email **techsupport@lifetech.com**