StemPro™ neural stem cells

Catalog Numbers A15654 and A15655

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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**.

Description

StemPro™ Neural Stem Cells are cryopreserved human fetal brain-derived neural stem cells (NSCs). These off-the-shelf cells are manufactured to meet Good Manufacturing Practice (GMP) manufacturing standards and are available for Research Use Only.

Product	Catalog No.	Amount	Storage
StemPro™ Neural Stem Cells	A15654	1 mL (1 × 10^6 cells/mL)	–196°C to –150°C
	A15655	1 mL (5 × 10^6 cells/mL)	

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.

Important information

Special handling is required for this product. Exposure of vial to dry ice or room temperature for more than a minute will dramatically reduce viability and cell recovery. Use forceps to transfer the vial and minimize the exposure to non-liquid nitrogen temperature especially room temperature during transfer (from dry shipper to liquid nitrogen storage upon receiving, and from liquid nitrogen storage to water bath for the thawing process).

Culture conditions

Media: StemPro™ NSC SFM™ (Cat. no. A10509-01) supplemented with 2 mM GlutaMAX™-I Supplement (Cat. no. 35050), 6 U/mL heparin (Sigma, Cat. no. H3149), and 200 µM ascorbic acid (Sigma, Cat. no. A8960)

Culture Type: Suspension culture for proliferation, adherent culture for differentiation

Recommended Substrate (for Differentiation): Geltrex™ hESC-qualified Reduced Growth Factor Basement Membrane Matrix™ (Cat. no. A1413301)

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2 in air

Prepare complete NSC culture medium

To prepare 100 mL of complete NSC culture medium, aseptically mix the components listed below. Complete NSC culture medium is stable for up to 2 weeks when stored in the dark at 2–8°C.

Component	Final concentration	Amount
KnockOut™ D-MEM™/F-12[1]	1X	97 mL
StemPro™ Neural Supplement[1]	2%	2 mL
bFGF (20 µg/mL stock)[1]	20 ng/mL	100 µL
EGF (20 µg/mL stock)[1]	20 ng/mL	100 µL
GlutaMAX™-I Supplement	2 mM	1 mL
Heparin (6000 units/mL stock)	6 units/mL	100 µL
Ascorbic acid (200 mM stock)	200 µM	100 µL

[1] Components of StemPro™ NSC SFM™ kit (Cat. no. A10509-01). bFGF and EGF stock can be prepared in 0.1% albumin PBS solution. Heparin and Ascorbic acid stock can be prepared in distilled water.

Thaw StemPro™ NSCs

The following procedure provides instructions for thawing one vial of StemPro™ NSCs containing 1 × 10^6 cells in two T25 flasks (for a total culture area of 50 cm^2).

Note: Suspension culture is recommended as plating NSCs on matrix as adherent culture would trigger differentiation.

1. Prepare 30 mL of complete NSC culture medium and warm to 37℃.
2. Transfer one vial of frozen StemPro™ NSCs from liquid nitrogen storage to 37℃ water bath, minimizing handling time.
3. Rapidly thaw (<2 minutes) the frozen vial by gently swirling it in a 37℃ water bath. Remove the vial from the water bath when only a tiny ice crystal is left. (Vial should be still cold to touch).
4. Transfer the vial to the cell culture hood and disinfect it with 70% ethanol. Allow the ethanol to evaporate before opening the vial.
5. Transfer the thawed cells into a 15-mL tube and add complete culture medium, pre-warmed to 37℃, in drop-wise manner to a total volume of 5 mL.

   Note: Do not add the entire amount of medium to the tube at once. This may lead to decreased cell viability due to osmotic shock.
6. Remove 10 µL of cell suspension and determine the viable cell density, if desired.
7. Centrifuge the thawed cells at 300 RCF for 4 minutes. Aspirate and discard the supernatant.

   Note: RCF (relative centrifugal force) = 1.118 ×10^{-5} × R × RPM^2, where R is the rotational radius measured in centimeters (cm) and RPM™ is rotating speed measured in revolutions per minute.
8. Resuspend the cells in complete NSC culture medium to a final concentration of 0.5 × 10^6 cells/mL.
9. Transfer 1 mL each of the cell suspension to two uncoated T25 flasks containing 6 mL of complete culture medium. Total volume will be 7 mL for each T25 flask (~2 × 10^4 cells/cm_2).
10. Place the culture flask in a controlled 37℃ incubator with a humidified atmosphere of 5% CO_2 in air (5% CO_2 and 5% O_2 incubator is optional).
11. Change the spent culture medium after two days and every 2–4 days thereafter.
12. You may culture the cells in suspension for up to 21 days, passaging them every 7 days.

Change medium

1. Prepare 10 mL of complete NSC culture medium per T25 flask and pre-warm to 37℃.
2. From each culture flask, tilt and pipet the medium containing the suspension cells into a corresponding pre-labeled 15-mL centrifuge tube.
3. Add 2 mL of complete NSC culture medium (pre-warmed to 37℃) to each flask and place the flasks back into the incubator.
4. Centrifuge the 15-mL tubes with the suspension cells at 300 RCF for 4 minutes and aspirate the supernatant to ~0.5 cm above pellet surface without disturbing the pellet. Discard the supernatant appropriately.
5. Retrieve flasks from incubator (from Step 3 on page 2) and transfer into the cell culture hood.
6. Add 1 mL of warm medium into each tube (from Step 4 on page 2) and dissociate the cells by gently pipetting up and down. Transfer the cell suspension back to the appropriate flask.
7. Pipet another 4 mL of pre-warmed medium into each tube in a manner that washes the sides of the tube. Pipet the medium up and down the sides several times. Transfer the cell suspension back to the appropriate flask.
8. Mix the cell suspension evenly by gently moving flasks in a left to right and then forward and backward motion several times.
9. Return flasks to the 37℃ incubator with a humidified atmosphere of 5% CO_2 in air.

Harvest cells

1. Prepare 50 mL of complete NSC culture medium and pre-warm to 37℃.
2. Remove the cells from the culture flask by pipetting the culture medium along the surface of the flask and transfer the medium containing the cells into a 15-mL conical tube. Add 3 mL of fresh complete NSC culture medium into the flask for a second wash and pipet the medium into the 15-mL tube.
3. Observe the flask to ensure that the majority of cells have been washed off of the surface of the flask. If needed, wash flask with an additional 2 mL of medium and transfer to the 15-mL tube.
4. Centrifuge the cells at 300 RCF for 4 minutes and aspirate the supernatant without disturbing cell pellet.
5. Add 1 mL of pre-warmed StemPro™ Accutase™ Cell Dissociation Reagent into each tube and dissociate the neurospheres by gently pipetting them up and down.
6. Incubate the tube at 37℃ for 10 minutes. Swirl the tube at 5 and 8 minutes to ensure that the cells do not aggregate or settle at the bottom of the tube.

7. Using a P1000 pipettor, break up the neurospheres by pipetting up and down 5 times. Place the tube back in the cell culture hood for another 5 minutes.

8. Repeat pipetting to get a single cell suspension and neutralize the dissociation reaction by adding 2 mL of warm NSC culture medium.

9. Remove a small aliquot from the cell suspension and perform a cell count.

10. Centrifuge the cells at 300 RCF for 4 minutes and aspirate the supernatant without disturbing cell pellet.

11. Resuspend the cells in complete NSC culture medium to a final concentration of 1×10^4 cells/µL.

12. You may use the cells for differentiation or other experiments, or expand further for up to an additional 14 days.

    Note: If the cells were recovered and expanded under the recommended conditions, a 2.5-fold increase in the number of total live cells is expected from a 7-day culture.

Spontaneous differentiation

1. Prepare differentiation medium and pre-warm to 37°C. Differentiation medium consists of complete NSC culture medium without bFGF and EGF.

2. Dilute Geltrex™ matrix 1:100 in KnockOut™ DMEM/F-12 (i.e., 50 µL of substrate into 10 mL of KnockOut™ DMEM/F-12).

3. Coat the surface of the culture vessel with the diluted Geltrex™ matrix solution (10 mL for T75, 5 mL for T25, 3 mL for 60-mm dish, 2 mL for 35-mm dish).

4. Incubate the culture vessel at 37°C in a humidified atmosphere of 5% CO_2 for 1 hour.

5. Remove the coated vessel from the incubator and store it until use. Remove all Geltrex™ matrix solution immediately before use, and fill the vessel with differentiation medium.

   Note: You may coat the plates in advance and store them at 2–8°C, wrapped tightly with Parafilm™ laboratory film, for up to 2 weeks. Do not remove Geltrex™ matrix solution until just prior to use. Make sure the plates do not dry out.

6. Harvest the cells as described above and resuspend them in differentiation medium to a concentration of 1×10^4 cells/µL.

7. Plate the cells on the Geltrex™ matrix-coated culture vessel to final seeding density of 5×10^4 cells/cm^2.

8. Make complete medium change after 2 days, and change half volume of medium every 2–3 days thereafter.

9. After 7 days of differentiation, all three neural lineages (astrocytes, neurons, and oligodendrocytes) can be obtained.

10. For directed differentiation to specific lineages (i.e., neural cells, astrocytes, and oligodendrocytes), refer to the Gibco™ Neurobiology Protocols handbook available at **www.lifetechnologies.com**.

Related products

Product	Cat. No.
StemPro™ NSC SFM™	A10509-01
GlutaMAX™-I CTS™, (100X), liquid	A12860
StemPro™ Accutase™ Cell Dissociation Reagent	A11105
Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix™	A14133
Countess™ Automated Cell Counter	C10227

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