Rat Cortical Stem Cells

Catalog Number NSC001

For cortical stem cell expansion and differentiation into neurons, astrocytes, and oligodendrocytes.

This package insert must be read in its entirety before using this product.
For laboratory research use only. Not for diagnostic use.
The safety and efficacy of this product in diagnostic or
other clinical uses has not been established.

TABLE OF CONTENTS

SECTION **PAGE**

DESCRIPTION 1
PRECAUTION 1
STORAGE 1
QUALITY CONTROL 1
REFERENCES 1
OTHER SUPPLIES REQUIRED 2
OTHER SUPPLIES REQUIRED (MONOLAYER SYSTEM) 2
OTHER SUPPLIES REQUIRED (NEUROSPHERE SYSTEM) 2
REAGENT & MEDIA PREPARATION 3
THAWING CRYOPRESERVED RAT CORTICAL STEM CELLS 3
MONOLAYER SYSTEM PROCEDURE 4
MONOLAYER SYSTEM PROCEDURE OUTLINE 6
NEUROSPHERE SYSTEM PROCEDURE 7
NEUROSPHERE SYSTEM PROCEDURE OUTLINE 8
DATA EXAMPLES 9

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DESCRIPTION

Neural stem/progenitor cells are self renewing, multipotent cells, which are capable of differentiating into all cell types of the nervous system. These cells provide an excellent model for the study of neural development processes as well as neurological disorders. Rat primary cortical stem cells were isolated from the cortex of E14.5 Sprague-Dawley rats. Cells were cultured in a monolayer system (1, 2) in media supplemented with N-2 Plus Media Supplement (R&D Systems, Catalog # AR003) and Recombinant Human FGF basic (R&D Systems, Catalog # 233-FB or 4114-TC). Cells were then harvested and cryopreserved. These cells are designated as passage 0 (P0) cells.

Rat cortical stem cells can be reliably passaged for a limited number of times *in vitro* before their multipotency is compromised. The number of passages is dependent on whether a monolayer or neurosphere culture system is used. P0 cells can be expanded for 3 passages using the monolayer system and for 4 passages using the neurosphere system.

PRECAUTION

This product contains trace amounts of DMSO and human transferrin. The transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV 1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection.

STORAGE

Store in liquid nitrogen for up to 1 year from date of receipt.

QUALITY CONTROL

Cells from this lot have been thawed and tested for their ability to proliferate using either a monolayer system (for 3 passages) or a neurosphere system (for 4 passages). Stem and progenitor cells expanded from the end of passage 3 (monolayer system) or passage 4 (neurosphere system) have been examined for Nestin and SOX2 expression. They were also tested for their ability to differentiate into astrocytes, neurons, and oligodendrocytes.

The cells tested negative for mycoplasma using the MycoProbe™ Mycoplasma Detection Kit (R&D Systems, Catalog # CUL001B). The cells also tested negative for microbial contamination.

Note: *Testing of the cells was performed using R&D Systems Neural Stem Cell Expansion Reagents mentioned above. Performance of the cryopreserved cells cannot be guaranteed if reagents from other manufacturers are substituted.*

REFERENCES

1. Johe, K.K. *et al.* (1996) Genes & Development **10**:3129.
2. Kim, J.H. *et al.* (2003) Methods Enzymol **365**:303.

OTHER SUPPLIES REQUIRED

Reagents

- N-2 Plus Media Supplement (R&D Systems, Catalog # AR003) or N-2 MAX Media Supplement (R&D Systems, Catalog # AR009)
- Recombinant Human FGF basic (R&D Systems, Catalog #233-FB or 4114-TC)
- PBS
- DMEM/F12
- Glucose
- Glutamine
- $NaHCO_3$
- Penicillin-Streptomycin 100X
- BSA, very low endotoxin
- Trypan Blue
- Deionized (DI) water

Materials

- 15 mL centrifuge tubes
- 0.2 µm, 500 mL filter units
- Pipettes and pipette tips

Equipment

- 37 °C and 5% CO_2 incubator
- Centrifuge
- Hemocytometer
- Microscope
- Water bath

OTHER SUPPLIES REQUIRED (MONOLAYER SYSTEM)

Reagents

- Bovine Fibronectin (R&D Systems, Catalog # 1030-FN)
- Poly-L-ornithine
- Ca^{2+}/Mg^{2+}-free Hank's Balanced Salt Solution (HBSS) (10X)
- HEPES

Materials

- 10 cm tissue culture plates
- 0.2 µm, 1000 mL filter unit

OTHER SUPPLIES REQUIRED (NEUROSPHERE SYSTEM)

Materials

- 6-well plates

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REAGENT & MEDIA PREPARATION

Note: *Sterile technique is required when handling the reagents.*

Completed Base Media - Mix the components listed in the chart below with DI water to make 500 mL of Completed Base Media. Adjust the pH to 7.2 ± 0.2. Sterile filter the solution using a 0.2 μm filter unit and store in the dark at 2-8 °C for up to 2 weeks.

COMPONENT	AMOUNT
DMEM/F12	6 g
Glucose	0.775 g
Glutamine	0.0365 g
$NaHCO_3$	0.845 g
N-2 Plus Media Supplement	5 mL

Buffered HBSS (1X) - Add 100 mL of HBSS (10X) and 3.9 g of HEPES to 900 mL of deionized water to make 1000 mL of Buffered HBSS (1X). Adjust the pH to 7.2 ± 0.2. Alternately, add 100 mL of HBSS (10X) and 6.9 mL 1M HEPES solution to 831 mL deionized H_2O; no pH adjustment. Sterile filter the solution using a 0.2 μm filter unit. Store at room temperature for up to 6 months.

FGF basic Stock (1000X) - Add sterile 0.1% BSA in PBS to the human FGF basic vial to make a 20 μg/mL stock. Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

THAWING CRYOPRESERVED RAT CORTICAL STEM CELLS

Note: *Review the following protocol in detail before thawing the cells.*

1. Warm 20 mL of Completed Base Media supplemented with 1X FGF basic in a 37 °C water bath.
2. Add 10 mL of pre-warmed Completed Base Media with FGF basic to a 15 mL tube.
3. Carefully remove the cryovial containing frozen Rat Cortical Stem Cells from the liquid nitrogen. Immediately pipette 1 mL of fresh pre-warmed media to the vial by gently pipetting up and down. As cells begin to thaw, transfer the thawed portion into the pre-warmed media in the 15 mL tube. Repeat this process with the warmed media until all of the cells have thawed.

   Note: *Most of the frozen cells will be at the bottom of the cryovial. Rapid resuspension of frozen cells in warmed media during thawing is critical. Allowing cells to thaw in the DMSO will dramatically reduce viability.*
4. Mix 10 μL of the cell suspension with 10 μL of Trypan Blue and count the live cells on a hemocytometer.
5. Seed cells according to the expansion protocol.

MONOLAYER SYSTEM PROCEDURE

Note: *Use serological pipettes to transfer and remove solutions.*

I. Poly-L-ornithine and Fibronectin-coated Plates

1. Dissolve Poly-L-ornithine in sterile PBS to make a 15 mg/mL stock (1000X). Aliquot and store at ≤-20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.
2. Dilute the 1000X Poly-L-ornithine Stock 1000-fold in sterile PBS to make a 15 µg/mL (1X) solution. Prepare fresh as needed.
3. Add 10 mL of the (1X) Poly-L-ornithine solution to each 10 cm tissue culture dish. Incubate 3 hours to overnight at 37 °C and 5% CO_2.
4. Discard the Poly-L-ornithine solution. Wash each dish 3 times with 10 mL of PBS.
5. Add 10 mL of PBS to each dish. Incubate overnight at 37 °C and 5% CO_2.
6. Allow the vial of Bovine Fibronectin to warm to room temperature **without agitation.** Make a 1 µg/mL solution by pipetting the Bovine Fibronectin into sterile PBS and gently inverting the tubes. Prepare fresh as needed.
7. Discard the PBS from each Poly-L-ornithine-coated dish. Wash each dish once with 10 mL of PBS.
8. Add 10 mL of 1 µg/mL Bovine Fibronectin solution to each dish. Incubate at 37 °C and 5% CO_2 for 3 hours to overnight.
9. Discard the Bovine Fibronectin solution. Wash each dish once with 10 mL of PBS before use.

II. Cell Expansion

1. Seed 1.0-1.5 x 10^6 Rat Cortical Stem Cells in 10 mL of Completed Base Media supplemented with 1X of FGF basic on a Poly-L-ornithine/Fibronectin-coated 10 cm plate.
2. Incubate the cells at 37 °C and 5% CO_2. After cells become adherent (3 hours to overnight), replace the media with fresh Completed Base Media supplemented with 1X FGF basic.
3. After 24 hours, add 10 µL of 1000X FGF basic stock to the culture.
4. Every second day, replace the media with fresh Completed Base Media.
5. Supplement the media with 10 µL of 1000X stock FGF basic each day.
6. Passage the cells when they reach 70-80% confluency according to the procedure described below.

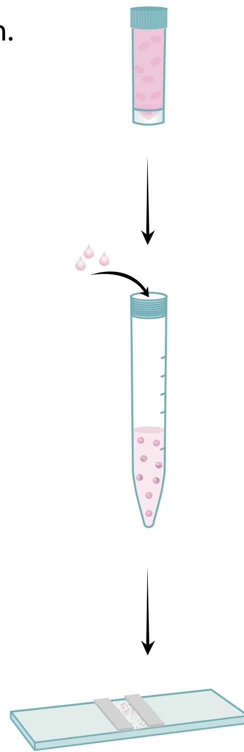
III. Passaging Cells

1. Warm the Buffered HBSS (1X) and Completed Base Media supplemented with 1X FGF basic to 37 °C.
2. Remove the media from the cells and wash once in 10 mL of Buffered HBSS (1X).
3. Add 5 mL of Buffered HBSS (1X). Incubate at room temperature for 15-45 minutes until the cells round up (check frequently).
4. Dislodge as many of the cells as possible from the plate by pipetting. Transfer the cells to a 15 mL centrifuge tube.
5. Centrifuge for 5 minutes at 200 x g. Remove the supernatant.
6. Resuspend the cells with 5 mL of Completed Base Media containing 1X FGF basic by slowly pipetting up and down approximately 5 times with a 5 mL pipette.
7. Mix 10 µL of the cell suspension with 10 µL Trypan Blue and count the live cells on a hemocytometer.
8. Seed 0.8-1.0 x 10^6 viable cells in 10 mL of Completed Base Media containing FGF basic on a Poly-L-ornithine/Fibronectin-coated plate.
9. Incubate the cells at 37 °C and 5% CO_2. Repeat steps 4 and 5 in the Expansion section (see above). Passage the cells after 3 days or when cells reach 70-80% confluency.

MONOLAYER SYSTEM PROCEDURE OUTLINE

Thawing Cryopreserved Rat Cortical Stem Cells

Coat cell culture plates with Poly-L-ornithine and Fibronectin.
Add 1 mL of fresh pre-warmed media to the vial of frozen rat cortical stem cells.

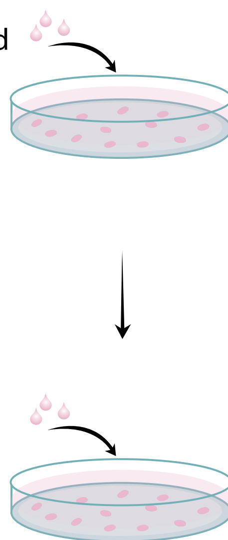


Pipette up and down as cells thaw.
Transfer the thawed portion into a 15 mL tube containing pre-warmed Completed Base Media supplemented with FGF basic.

Perform a cell count.

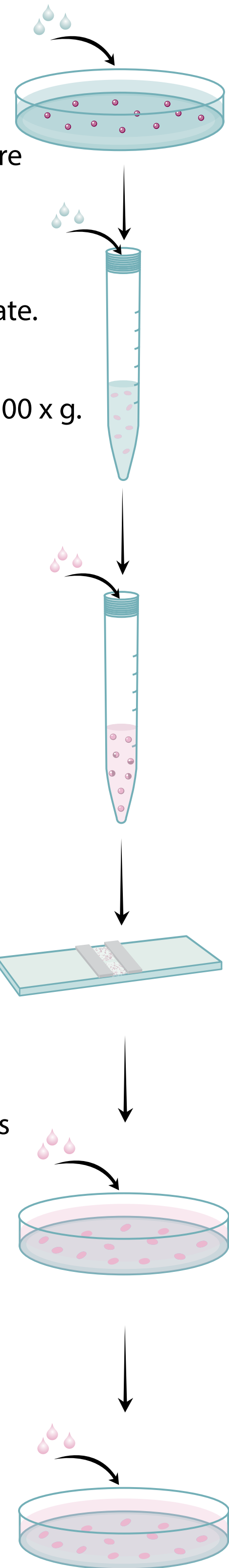
Cell Expansion

Plate 1.0-1.5 x 10^6 Rat Cortical Stem Cells in 10 mL of Completed Base Media supplemented with FGF basic onto a Poly-L-ornithine/ Fibronectin-coated 10 cm plate.
Incubate the cells at 37 °C and 5% CO_2.



Replace the media once cells become adherent.
After 24 hours, **supplement** the media with FGF basic.
Replace the media with fresh Completed Base Media every second day.
Supplement the media daily with FGF basic.
Passage the cells when they reach 70–80% confluency.

Passaging Cells



Wash the cells once with pre-warmed Buffered HBSS.
Add 5 mL of Buffered HBSS.
Incubate at room temperature until the cells round up.

Pipette the cells off of the plate.
Transfer the cells to a 15 mL centrifuge tube.
Centrifuge for 5 minutes at 200 x g.
Remove the supernatant.

Resuspend the cells in 5 mL of Completed Base Media containing FGF basic.

Perform a cell count.

Plate 0.8-1.0 x 10^6 viable cells in 10 mL of Completed Base Media containing FGF basic onto a Poly-L-ornithine/ Fibronectin-coated plate.
Incubate the cells at 37 °C and 5% CO_2.

Replace the media with fresh Completed Base Media every second day.
Supplement the media with FGF basic daily.
Passage the cells after 3 days or when the cells reach 70–80% confluency.

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NEUROSPHERE SYSTEM PROCEDURE

I. Neurosphere Expansion

1. Seed approximately 1 x 10^5 Rat Cortical Stem Cells in 5 mL of Completed Base Media supplemented with 1X FGF basic per well in a 6-well plate.

2. Incubate the cells at 37 °C and 5% CO_2.

3. Add fresh FGF basic to the media each day. Every fourth day, based on the number of neurospheres, replace the media according to the steps described below.

    a. Less than 50 neurospheres - Transfer the neurospheres directly into 2.5 mL of Completed Base Media containing and FGF basic in one well of a 6-well plate. **DO NOT DISCARD THE CONDITIONED MEDIA.** Add 2.5 mL of this conditioned media to the well. When there are fewer neurospheres, conditioned media is required. Only half of the media is replaced with fresh Completed Base Media containing FGF basic.

    b. 50 neurospheres or more - Transfer the media containing the neurospheres to a 15 mL tube. Allow the neurospheres to settle for 10 minutes. Remove the media. Gently resuspend the neurospheres using a small quantity of fresh Completed Base Media containing FGF basic. Add the neurosphere suspension to 5 mL of fresh Completed Base Media containing and FGF basic in one well of a 6-well plate.

4. Passage the cells at 5-7 days, or when the neurospheres have a dark clump inside or ruffling on the outside of the neurosphere.

II. Passaging Neurospheres

1. Transfer the media containing the floating neurospheres to a 15 mL tube.
   DO NOT DISLODGE ATTACHED NEUROSPHERES FOR PASSAGE.

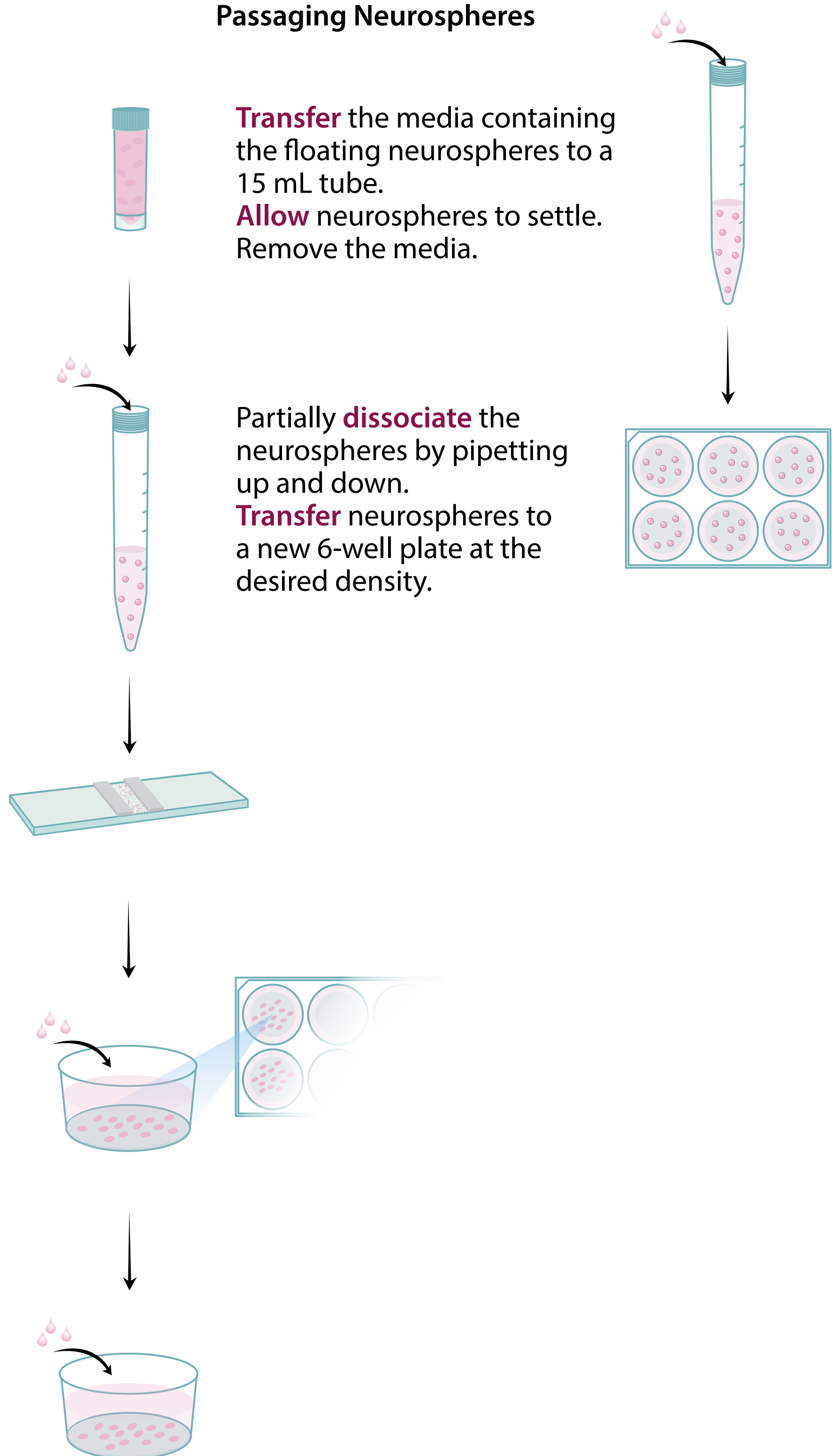
2. Allow neurospheres to settle for 10 minutes. Remove the media.

3. Partially dissociate the neurospheres by pipetting up and down 20 times, being careful not to create bubbles in the suspension.

   Note: *For optimal dissociation of the neurospheres, it is recommended that a P200 pipette be used.*

4. At passages 1 and 2 the cells should be split 1:1. After passage 2 the cells can be split 1:2.

NEUROSPHERE SYSTEM PROCEDURE OUTLINE



Thawing Cryopreserved Rat Cortical Stem Cells

Add 1 mL of fresh pre-warmed media to the vial of frozen rat cortical stem cells.

Pipette up and down as cells thaw.
Transfer the thawed portion into a 15 mL tube containing pre-warmed Completed Base Media supplemented with FGF basic.

Neurosphere Expansion

Perform a cell count.

Plate cells at approximately 1.0×10^5 cells in 5 mL of Completed Base Media supplemented with FGF basic per well in a 6-well plate.
Incubate the cells at 37 °C and 5% CO_2.
Add fresh FGF basic to the media each day.

Replace the media every 4 days according to the number of neurospheres present.
Passage the cells at 5-7 days or when the neurospheres have a dark clump inside or ruffling on the outside.

Passaging Neurospheres

Transfer the media containing the floating neurospheres to a 15 mL tube.
Allow neurospheres to settle.
Remove the media.

Partially **dissociate** the neurospheres by pipetting up and down.
Transfer neurospheres to a new 6-well plate at the desired density.

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DATA EXAMPLES

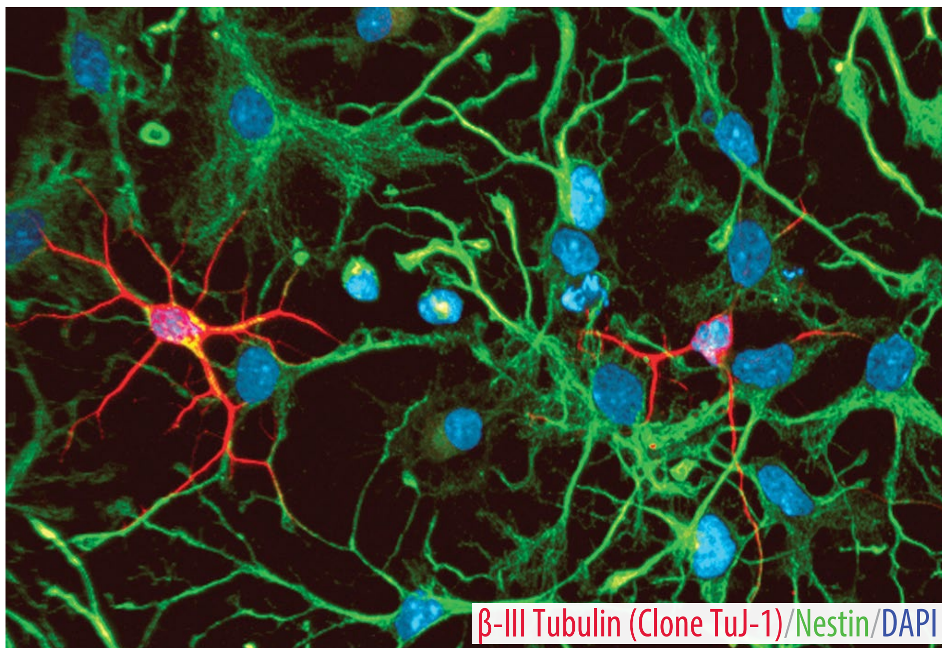

Figure 1: Differentiation of Rat Cortical Stem Cells. Rat Cortical Stem Cells were cultured for seven days in DMEM/F12 containing N-2 Plus Media Supplement (R&D Systems, Catalog # AR003) without FGF basic to induce differentiation. The differentiated cells were labeled with Goat Anti-Rat Nestin Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF2736) followed by NorthernLights™ (NL)493-conjugated Donkey Anti-Goat IgG Secondary Antibody (R&D Systems, Catalog # NL003; green) and Mouse Neuron-specific Anti-beta-III Tubulin Monoclonal Antibody (R&D Systems, Catalog # MAB1195) followed by NL-557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (R&D Systems, Catalog # NL007; red). Nuclei were counterstained with DAPI (blue).

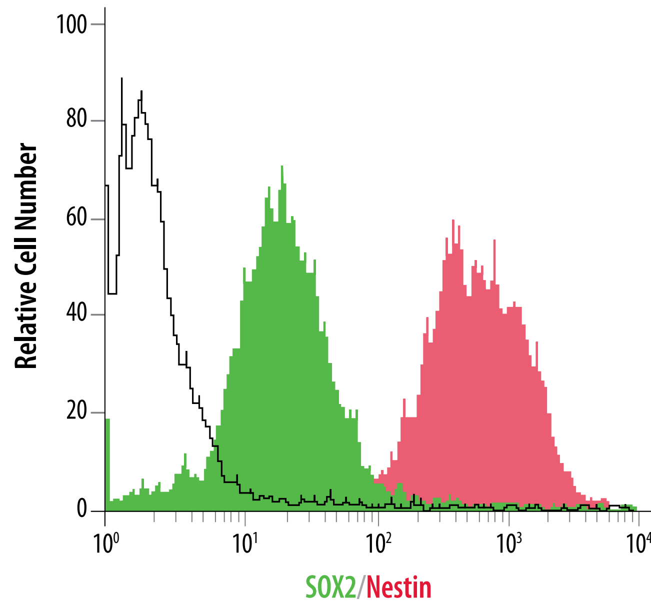

Figure 2: Rat Cortical Stem Cells Expanded with N-2 Plus Media Supplement Express Nestin and SOX2. Rat Cortical Stem Cells (R&D Systems, Catalog # NSC001) were cultured for 7 days in media supplemented with 1X N-2 Plus Media Supplement and 20 ng/mL of Recombinant Human FGF basic (R&D Systems, Catalog # 233-FB). The cells were stained with PE-conjugated Mouse Anti-Human Nestin Monoclonal Antibody (R&D Systems, Catalog # IC1259P; red histogram), PE-conjugated Mouse Anti-Human/Mouse SOX2 Monoclonal Antibody (R&D Systems, Catalog # IC2018P, green histogram), or PE-conjugated Mouse IgG_{2A} Isotype Control (R&D Systems, Catalog # IC003P; open histogram). Under these conditions, cells were shown to express high levels of the neural multipotency markers Nestin and SOX2.

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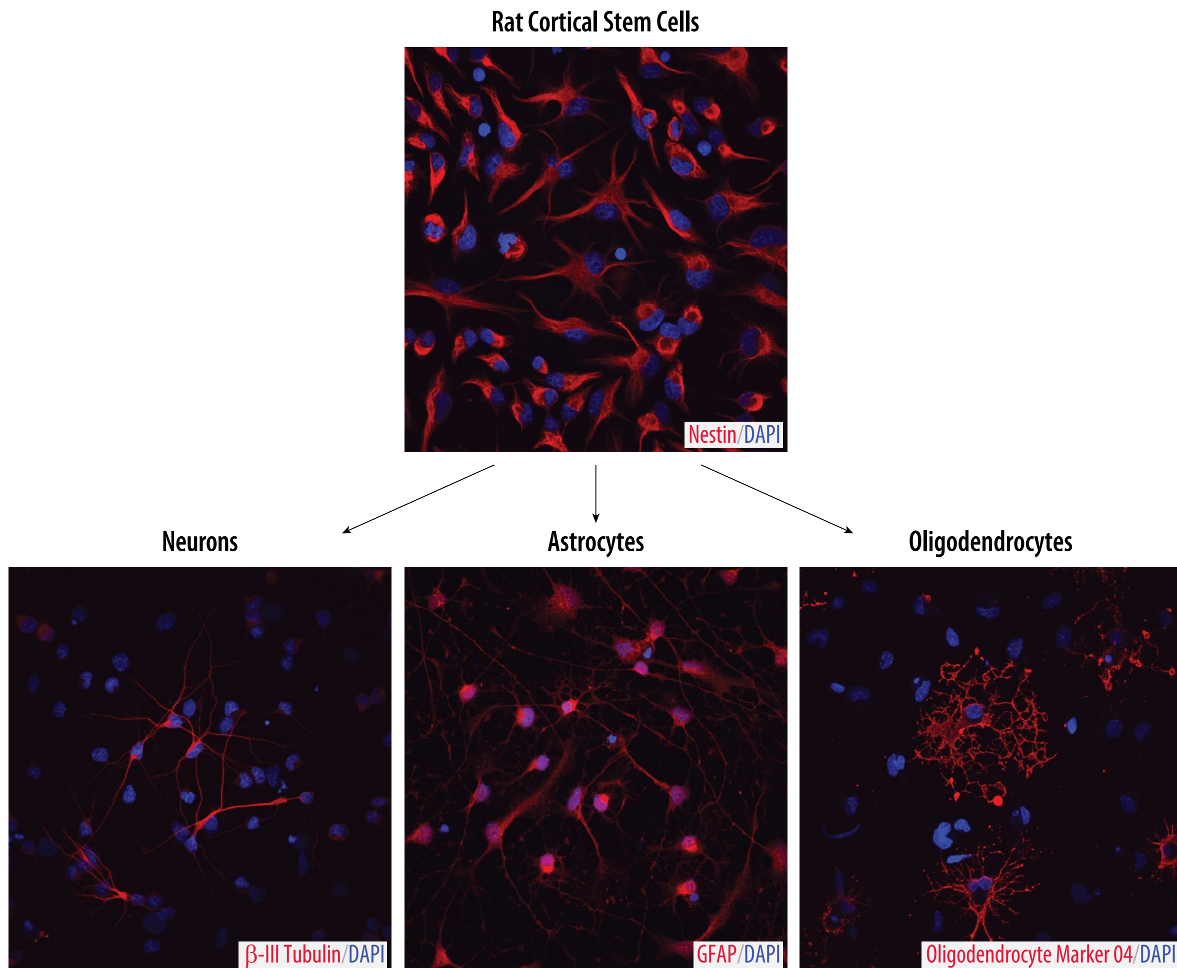

Figure 3: Verification of Neural Progenitor Cell Multipotency Following Expansion with N-2 MAX Media Supplement. Rat Cortical Stem Cells were differentiated for 7 days in media supplemented with N-2 MAX Media Supplement (Catalog # AR009). Differentiated cells were stained with Mouse Neuron-specific Anti-beta-III Tubulin Monoclonal Antibody (R&D Systems, Catalog # MAB1195) followed by NL557-conjugated Donkey Anti-Mouse IgG Secondary Antibody (R&D Systems, Catalog # NL007; red) to detect neurons. The cells were stained with Sheep Anti-Human/Rat GFAP Antigen Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF2594) followed by NL557-conjugated Donkey Anti-Sheep IgG Secondary Antibody (R&D Systems, Catalog # NL010; red) to detect astrocytes; and the cells were stained with Mouse Anti-Oligodendrocyte Marker O4 Monoclonal Antibody (R&D Systems, Catalog # MAB1326), followed by NL557-conjugated Goat Anti-Mouse IgM Secondary Antibody (R&D Systems, Catalog # NL019) to detect oligodendrocytes. The nuclei were counterstained with DAPI (blue).

NOTES

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