Human/Mouse/Rat Neural Lineage Functional Identification Kit

Catalog Number SC028

Reagents for the identification of human/mouse/rat Neural Progenitor Cells (NPCs) by *in vitro* functional differentiation.

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.

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PRINCIPLE OF THE ASSAY

Progenitor cells can be found in a variety of tissues and are functionally defined by their capacity to self renew and their ability to differentiate into multiple specialized cells in specific lineages. Neural progenitor cells (NPCs) are capable of differentiating into multiple cell types including astrocytes, neurons, and oligodendrocytes. During the isolation and expansion of NPCs, these cells are best evaluated functionally by measuring their ability to differentiate into multiple neural lineages.

The Human/Mouse/Rat Neural Lineage Functional Identification Kit contains specially formulated media supplements, which can be used for the short-term maintenance and expansion of NPCs and the differentiation of NPCs into astrocyte, neuron, and oligodendrocyte lineages. An antibody panel consisting of anti-rat Nestin, anti-human Glial Fibrillary Acidic Protein (GFAP), anti-Neuron-specific beta-III Tubulin, and anti-Oligodendrocyte Marker O4 are also included to identify the phenotypes of neural precursors, astrocytes, neurons, and oligodendrocytes.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- The quality of the cells and any variations in the procedure can cause variation in the results.
- Do not mix or substitute reagents with those from other lots or sources.

PRECAUTION

The acute and chronic effects of over-exposure to the reagents in this kit are unknown. Safe laboratory handling procedures should be followed and protective clothing should be worn when handling kit reagents.

MATERIALS PROVIDED

Store unopened kit at ≤ -20 °C in a manual defrost freezer for up to 6 months from date of receipt.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED REAGENTS
Neural Maintenance Supplement	390520	100 µL of a 500X concentrated solution containing recombinant human FGF basic and recombinant human EGF; enough to supplement 50 mL of media.	Store at ≤ -20 °C in a manual defrost freezer for up to 3 months.* Avoid repeated freeze-thaw cycles.
Neural Differentiation Supplement	390521	1.0 mL of a 100X concentrated solution containing IGF-I and fetal bovine serum; enough to supplement 100 mL of media.	
Bovine Fibronectin 100X	390522	250 µL of a 100X (100 µg/mL) solution containing purified bovine Fibronectin.	
anti-rat Nestin	965224	25 µg of lyophilized anti-rat Nestin polyclonal antibody; enough to make 5 mL of staining solution when used at the suggested concentration of 0.5 µg/100 µL.	Stored at 2-8 °C for up to 1 month or aliquot and stored at ≤ -20 °C in a manual defrost freezer for up to 6 months.* Avoid repeated freeze-thaw cycles.
anti-human GFAP	965225	50 µg of lyophilized anti-human Glial Fibrillary Acidic Protein (GFAP) polyclonal antibody; enough to make 5 mL of staining solution when used at the suggested concentration of 1.0 µg/100 µL.	
anti-Neuron-specific β-III Tubulin	964673	25 µg of lyophilized anti-Neuron-specific β-III Tubulin monoclonal antibody; enough to make 5 mL of staining solution when used at the suggested concentration of 0.5 µg/100 µL.	
anti-Oligodendrocyte Marker O4	954674	10 µg of lyophilized anti-Oligodendrocyte Marker O4 monoclonal antibody; enough to make 5 mL of staining solution when used at the suggested concentration of 0.2 µg/100 µL.	

*Provided this is within 6 months from the date of receipt.

OTHER SUPPLIES REQUIRED

Materials

- Human, mouse, or rat neural progenitor cells
- 24-well culture plates
- 12 mm cover slips
- 15 mL and 50 mL centrifuge tubes
- Pipettes and pipette tips
- Serological pipettes
- Glass slides
- Slide box
- Fine pointed curved forceps

Reagents

- N-2 MAX Media Supplement (R&D Systems, Catalog # AR009)
- Poly-L-ornithine
- Phosphate Buffered Saline (PBS)
- Penicillin-Streptomycin 100X
- Bovine Serum Albumin (BSA)
- DMEM/F12
- Glucose
- L-Glutamine
- Sodium Bicarbonate ($NaHCO_3$)
- Trypan blue
- 95% Ethanol
- 4% Paraformaldehyde in PBS
- 1% BSA in PBS
- 1% BSA, 10% normal donkey serum in PBS
- 0.3% Triton™ X-100, 1% BSA, 10% normal donkey serum in PBS
- Mounting medium (R&D Systems, Catalog # CTS011)
- Secondary antibody (R&D Systems, Catalog # NL001, NL007, NL010, NL019)
- Deionized or distilled water

Equipment

- 37 °C and 5% CO_2 incubator
- Centrifuge
- Hemocytometer
- Inverted microscope
- 37 °C water bath
- Fluorescence microscope
- 0.2 µm filter unit, 250 mL

REAGENT AND MATERIAL PREPARATION

Completed Base Media - Mix the components listed in the table below with deionized or distilled water to make 200 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.

ITEM	AMOUNT
DMEM-F12	2.4 g
Glucose	0.31 g
L-Glutamine	0.0146 g
NaHCO3	0.338 g
N-2 MAX Media Supplement	2 mL

Note: *If desired, Penicillin-Streptomycin can be added to a final concentation of 1X.*

PREPARATION OF 24-WELL PLATE

Poly-L-ornithine (1000X) - Dissolve Poly-L-ornithine in sterile PBS to make a 15 mg/mL stock. Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

Poly-L-ornithine Solution (1X) - Dilute Poly-L-ornithine (1000X) 1000-fold in sterile PBS to make a 1X solution (15 µg/mL). Prepare fresh as needed.

Bovine Fibronectin Solution (1X) - Dilute the Bovine Fibronectin 100X 100-fold in sterile PBS to make a 1X solution (1 µg/mL). Mix by gentle swirling, without vortexing. Prepare fresh as needed.

PREPARATION OF LYOPHILIZED ANTIBODIES

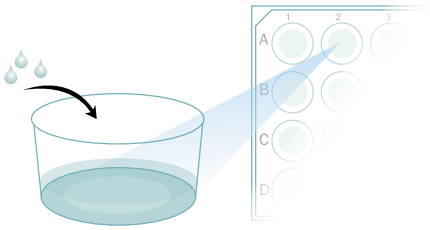
Anti-rat Nestin - Reconstitute with 500 µL of sterile PBS. Mix gently. Results in a 100 µg/mL stock solution.

Anti-human GFAP - Reconstitute with 500 µL of sterile PBS. Mix gently. Results in a 100 µg/mL stock solution.

Anti-Neruon-specific β-III Tubulin - Reconstitute with 500 µL of sterile PBS. Mix gently. Results in a 100 µg/mL stock solution.

Anti-Oligodendrocyte Marker O4 - Reconstitute with 500 µL of sterile PBS. Mix gently. Results in a 100 µg/mL stock solution.

PROTOCOL OUTLINE



Prepare Poly-L-ornithine and Fibronectin-coated coverslips.

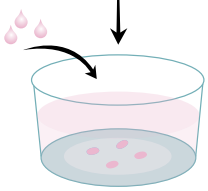
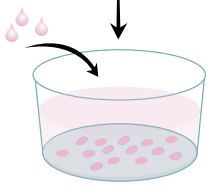


Plate 0.5–1.0 x 10^6 NPCs in media containing Neural Maintenance Supplement.

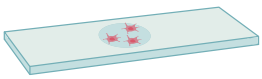


Replace with fresh media after 24 hours.
Culture cells for 48 hours after initial plating.

ICC detection of Nestin to identify NPCs.

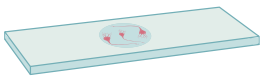
Cell Differentiation
DAY 1:
Replace media with Neural Differentiation Supplement.
DAY 4:
Repeat media change every 3 days.
DAYS 7–10:

Astrocyte Differentiation



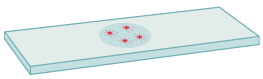
ICC detection of GFAP.

Neuron Differentiation



ICC detection of β-III Tubulin.

Oligodendrocyte Differentiation



ICC detection of Oligodendrocyte Marker O4.

PROTOCOL

PREPARATION OF POLY-L-ORNITHINE & FIBRONECTIN COATED PLATES

1. Insert a sterile cover slip (sterilized with 95% EtOH and flamed) into each well of a 24-well plate.
2. Add 0.5 mL of Poly-L-ornithine Solution (1X) to each well. Gently sink the floating cover slips with a sterile pipette tip. Incubate overnight at 37 °C.
3. Discard the Poly-L-ornithine Solution. Wash each well 3 times with 1 mL of sterile PBS each time.
4. Add 0.5 mL of sterile PBS to each well. Incubate overnight at 37 °C.
5. Discard the PBS. Wash each well once with 1 mL of sterile PBS.
6. Add 0.5 mL of 1X Bovine Fibronectin Solution to each well. Gently sink the floating cover slips with a sterile pipette tip.
7. Incubate in a 37 °C incubator for 3-24 hours.
8. Discard the 1X Bovine Fibronectin Solution and wash each well once with 1 mL of PBS before proceeding to the Cell Plating protocol.

CELL PLATING & MAINTENANCE

Fresh supplemented media should be made for each usage or media change. The recommended amount of media for a 24-well plate is 0.5 mL/well. Make 12 mL of media for 24 wells.

1. Add 24 µL of the Neural Maintenance Supplement to 12 mL of Completed Base Media. Mix gently.
2. Seed 0.5-1.0 x 10^6 NPCs in 12 mL of Completed Base Media containing the Neural Maintenance Supplement on a Poly-L-ornithine/Fibronectin Coated Plate at 0.5 mL/well.
3. Incubate the cells at 37 °C and 5% CO_2. Cells should become adherent after 24 hours.
4. Twenty-four hours after the initial plating, replace the media with fresh Completed NSC Base Media containing the Neural Maintenance Supplement.
5. Forty-eight hours after the initial plating, cells from two wells can be evaluated by immunocytochemistry for Nestin expression and the rest of the wells are ready for differentiation.

CELL DIFFERENTIATION

Differentiation of NPCs is performed 48 hours after the initial cell plating and culturing in Completed Base Media containing Neural Maintenance Supplement.

Fresh supplemented media should be made for each usage or media change (12 mL of media is required for each media change).

1. Add 120 µL of the Neural Differentiation Supplement to 12 mL of Completed Base Media. Mix gently.
2. Remove the media from the wells and wash once with sterile PBS.
3. Add 0.5 mL of Completed Base Media containing the Neural Differentiation Supplement to each well.
4. Replace the media with fresh Completed Base Media containing the Neural Differentiation Supplement every three days.
5. Cells can be fixed for characterization after seven days of differentiation in Completed Base Media containing the Neural Differentiation Supplement.

CHARACTERIZATION OF CELLS BY IMMUNOCYTOCHEMISTRY

Nestin, Neuron-specific β-III Tubulin, and GFAP are used as markers for neural progenitor cells, neurons, and astrocytes, respectively.

1. Remove the media from wells selected for immunocytochemistry characterization and wash the cells twice with 1 mL of PBS.
2. Fix the cells with 0.5 mL of 4% paraformaldehyde in PBS for 20 minutes at room temperature.
3. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
4. Permeabilize and block the cells with 0.5 mL of 0.3% Triton X-100, 1% BSA, and 10% normal donkey serum in PBS at room temperature for 45 minutes.
5. During step 4, when the cells are being blocked, dilute the reconstituted anti-rat Nestin, anti-Neuron-specific β-III Tubulin, or anti-human GFAP antibody in PBS containing 1% BSA and 10% normal donkey serum to the suggested final concentration.
6. After blocking, incubate the cells with 300 µL/well of 1X anti-rat Nestin, anti-Neuron-specific β-III Tubulin or anti-human GFAP overnight at 2-8 °C.

   Note: *A negative control should be performed using PBS containing 1% BSA and 10% normal donkey serum with no primary antibody.*
7. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
8. Dilute the appropriate secondary antibody (*e.g.,* NL557-conjugated donkey anti-mouse IgG, donkey anti-goat IgG, or donkey anti-sheep IgG secondary antibody) at 1:200 in PBS containing 1% BSA.
9. Incubate the cells with 300 µL/well of secondary antibody **in the dark** at room temperature for 60 minutes.
10. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
11. Wash the cells once with 0.5 mL of PBS for 5 minutes.
12. Aspirate the PBS from the wells and add 0.5 mL of deionized or distilled water. Carefully remove the cover slips with forceps and mount cell side down onto a drop of mounting media on a glass slide.
13. The slides are ready for microscopic observation.

IMMUNOCYTOCHEMISTRY OF OLIGODENDROCYTES

1. Remove the media from wells selected for immunocytochemistry characterization and wash the cells twice with 1 mL of PBS.
2. Fix the cells with 0.5 mL of 4% paraformaldehyde in PBS for 20 minutes at room temperature.
3. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
4. Block the cells with 0.5 mL of 1% BSA and 10% normal donkey serum in PBS at room temperature for 45 minutes.
5. During step 4 when the cells are being blocked, dilute the reconstituted anti-Oligodendrocyte Marker O4 antibody in PBS containing 1% BSA and 10% normal donkey serum to a final concentration of 0.2 µg/100 µL.
6. After blocking, incubate the cells with 300 µL/well of anti-Oligodendrocyte Marker O4 overnight at 2-8 °C.

   Note: *A negative control should be performed using PBS containing 1% BSA and 10% normal donkey serum with no primary antibody.*
7. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
8. Dilute the secondary antibody (*e.g.,* NL557-conjugated donkey anti-mouse IgM secondary antibody) at 1:200 in PBS containing 1% BSA.
9. Incubate the cells with 300 µL/well secondary antibody **in the dark** at room temperature for 60 minutes.
10. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each.
11. Wash the cells once with 0.5 mL of PBS for 5 minutes.
12. Aspirate the PBS from the wells and add 0.5 mL of deionized or distilled water. Carefully remove the cover slips with forceps and mount cell side down onto a drop of mounting media on a glass slide.
13. The slides are ready for microscopic observation.

SAMPLE DATA

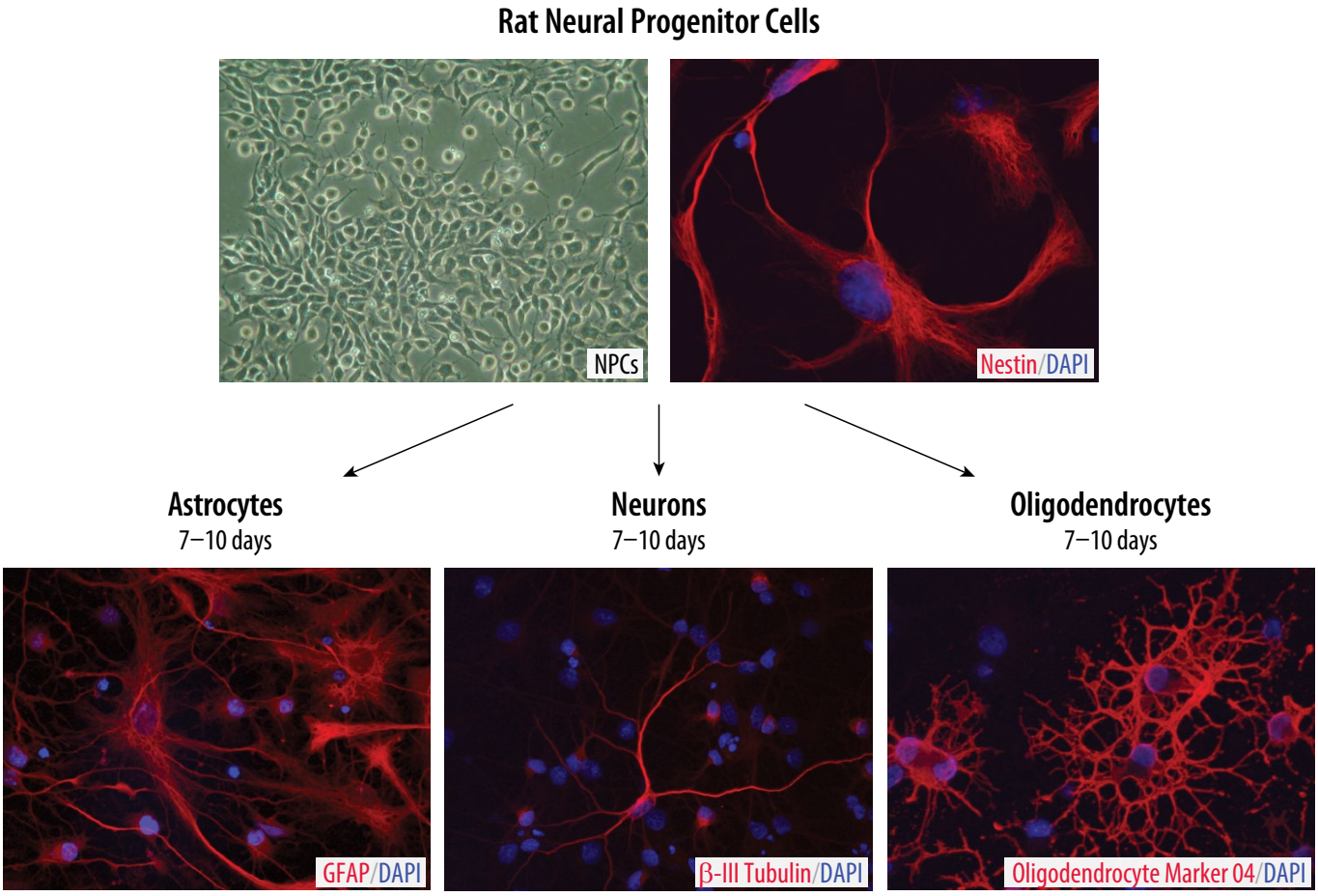


Figure 1: Verification of Neural Progenitor Cell Multipotency. Rat neural progenitor cells were maintained in culture and differentiated towards neural lineages using the specialized media supplement supplied in this kit. Neural progenitor cell multipotency was functionally verified using the antibodies supplied in the kit to detect phenotype-specific markers for undifferentiated neural precursors and the following differentiated derivatives: astrocytes, neurons, and oligodendrocytes.

NOTES