Mouse/Rat Neural Stem Cell Functional Identification Kit

Catalog Number SC013

Reagents for the identification of mouse/rat Neural Stem Cells (NSCs) 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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MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems GmbH
Borsigstrasse 7
65205 Wiesbaden-Nordenstadt
Germany

TELEPHONE: +49 (0)6122 90980
FAX: +49 (0)6122 909819
E-MAIL: infogmbh@RnDSystems.co.uk

R&D Systems Europe
77 boulevard Vauban
59041 LILLE CEDEX
France

FREEPHONE: +0800 90 72 49
FAX: +0800 77 16 68
E-MAIL: info@RnDSystems.co.uk

PRINCIPLE OF THE ASSAY

Stem cells are functionally defined by their capacity to self renew and their ability to differentiate into multiple specialized progenitor cells, which can commit to further maturation along specific lineages. Neural stem cells (NSCs) are capable of differentiating into multiple cell types including astrocytes, neurons and oligodendrocytes (1 - 6). During the isolation and expansion of NSCs, the status of stem cells is best evaluated functionally by measuring their ability to differentiate into multiple neural lineages.

The Mouse/Rat Neural Stem Cell Functional Identification Kit contains specially formulated media supplements, which can be used for the short-term maintenance and expansion of NSCs and the differentiation of NSCs into astrocyte, neuron and oligodendrocyte lineages. An antibody panel consisting of goat anti-rat Nestin, sheep anti-human Glial Fibrillary Acidic Protein (GFAP), mouse anti-Neuron-specific beta-III Tubulin, and mouse 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 DIAGNOSTIC USE.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- The kit should not be used beyond the expiration date on the kit label.
- The quality of the neural stem cells and any variation in the procedure can cause variation in the efficiency of cell differentiation.

PRECAUTIONS

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.

REAGENTS

Neural Stem Cell Maintenance Supplement (Part # 390436) - 100 µL of a 500X concentrated solution containing recombinant human Fibroblast Growth Factor (FGF) basic and recombinant human Epidermal Growth Factor (EGF), enough for 50 mL of media.

Neural Differentiation Supplement (Part # 390437) - 1.0 mL of a 100X concentrated solution containing Insulin-like Growth Factor I (IGF-I) and fetal bovine serum, enough for 100 mL of media.

Bovine Fibronectin 100X Stock (Part # 390438) - 250 µL of a 100X (100 µg/mL) solution containing purified bovine fibronectin.

Goat anti-rat Nestin (Part # 965224) - 25 µg of lyophilized goat anti-rat Nestin polyclonal antibody, enough to make 5 mL of 0.5 µg/100 µL staining solution.

Sheep anti-human GFAP (Part # 965225) - 50 µg of lyophilized sheep anti-human Glial Fibrillary Acidic Protein (GFAP) polyclonal antibody, enough to make 5 mL of 1.0 µg/100 µL staining solution.

Mouse anti-Neuron-specific β-III Tubulin (Part # 964673) - 25 µg of lyophilized mouse anti-Neuron-specific β-III Tubulin monoclonal antibody, enough to make 5 mL of 0.5 µg/100 µL staining solution.

Mouse anti-Oligodendrocyte Marker O4 (Part # 964674) - 10 µg of lyophilized mouse anti-Oligodendrocyte Marker O4 monoclonal antibody, enough to make 5 mL of 0.2 µg/100 µL staining solution.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at ≤ -20° C in a manual defrost freezer. Do not use past the expiration date.</td></tr>
<tr><td rowspan="3">Opened Reagents</td><td>Media Supplements</td><td rowspan="2">Aliquot and store at ≤ -20° C in a manual defrost freezer for up to 3 months.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Bovine Fibronectin</td></tr>
<tr><td>Reconstituted Antibodies</td><td>Store at 2 - 8° C for up to 1 month or aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months.* Avoid repeated freeze-thaw cycles.</td></tr>
</table>

*Provided this is within the expiration date.

OTHER SUPPLIES REQUIRED

Materials

- Mouse or rat neural stem cells
- 24-well culture plates (Corning Costar, Catalog # 3526 or equivalent)
- 12 mm cover slips (Assistant 1001/0012 Carolina Biologicals, Catalog # BA-63-3009 or equivalent)
- 15 mL centrifuge tubes (Corning Costar, Catalog # 430052 or equivalent)
- Pipettes and pipette tips
- Serological pipettes
- Fine pointed curved forceps (Fisher, Catalog # 08-875 or equivalent)
- Glass slides
- Slide box

Reagents

- StemXVivo™ Serum-Free NSC Base Media for neural stem cell expansion and differentiation (R&D Systems, Catalog # CCM002 or equivalent)
- Poly-L-ornithine (Sigma, Catalog # P3655 or equivalent)
- Phosphate Buffered Saline (PBS) (Invitrogen, Catalog # 20012-027 or equivalent)
- Penicillin-Streptomycin 100X (Invitrogen, Catalog # 15140-122 or equivalent)
- Bovine Serum Albumin (BSA) (Serologicals Proteins, Inc., Catalog # 81-068-3 or equivalent)
- Trypan blue (Invitrogen, Catalog # 15250-061)
- 4% Paraformaldehyde in PBS
- 1% BSA in PBS
- 0.3% Triton X-100, 1% BSA, 10% normal donkey serum in PBS
- 1% BSA, 10% normal donkey serum in PBS
- Mounting medium (R&D Systems, Catalog # CTS011)
- Secondary developing reagents (e.g. anti-sheep IgG, anti-mouse IgG or IgM, anti-goat IgG)
- Deionized or distilled water

Equipment

- 37° C and 5% CO_2 incubator
- Centrifuge
- Hemocytometer
- Inverted microscope
- 37° C water bath
- Fluorescence microscope

REAGENT AND MATERIAL PREPARATION

Use serological pipettes to transfer solutions.

Base Media - Thaw the StemXVivo Serum-Free NSC Base Media at 2 - 8° C or room temperature. Aliquot any remaining thawed media and store at ≤ -20° C or use within 10 days when stored **in the dark** at 2 - 8° C.

Completed NSC Base Media - Add Penicillin-Streptomycin (100X) to Base Media at a 1:100 dilution.

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

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

Goat anti-rat Nestin (10X) - Reconstitute the goat anti-rat Nestin antibody with 500 μL of sterile PBS. Mix gently. Aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated-freeze-thaw cycles.

Sheep anti-human GFAP (10X) - Reconstitute the sheep anti-human GFAP antibody with 500 μL of sterile PBS. Mix gently. Aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated-freeze-thaw cycles.

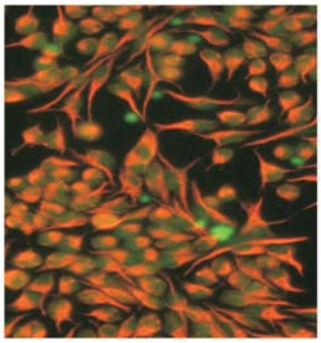
Mouse anti-Neuron-specific β-III Tubulin (10X) - Reconstitute the mouse anti-Neuron-specific β-III Tubulin antibody with 500 μL of sterile PBS. Mix gently. Aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated-freeze-thaw cycles.

Mouse anti-Oligodendrocyte Marker O4 (10X) - Reconstitute the mouse anti-Oligodendrocyte Marker O4 antibody with 500 μL of sterile PBS. Mix gently. Aliquot and store at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated-freeze-thaw cycles.

PROCEDURE OUTLINE

Neural Stem Cells

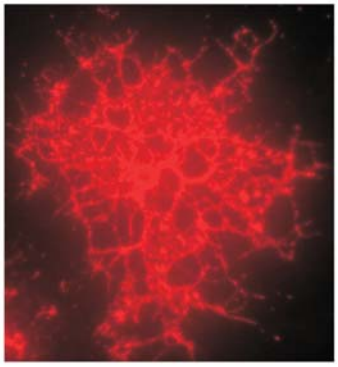
Plated in Poly-L-ornithine/fibronectin-coated plate with media containing maintenance supplement for 48 hours



Nestin

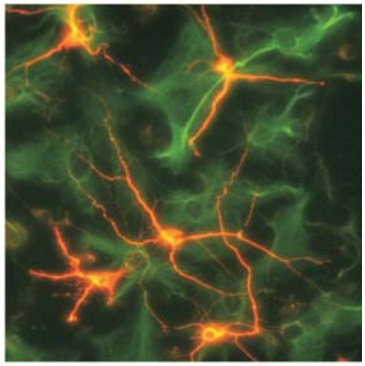
Cultured with media containing differentiation supplement for 7 -10 days

Oligodendrocytes



Oligodendrocyte Marker O4

Neurons /Astrocytes



Neuron-specific β-III Tubulin/GFAP

Please refer to the website to see full color images

(www.RnDSystems.com/pdf/SC013.pdf).

PROCEDURES

I. Preparation of Poly-L-ornithine and 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 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 Fibronectin Solution and wash each well once with 1 mL of PBS before proceeding to the Cell Plating protocol.

II. Cell Plating and 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 Stem Cell Maintenance Supplement to 12 mL of Completed NSC Base Media. Mix gently.
2. Seed 0.5 - 1.0 x 10^6 NSCs in 12 mL of Completed Base Media containing the Neural Stem Cell 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 Stem Cell Maintenance Supplement.
5. Forty-eight hours after the initial plating, cells from two wells can be evaluated by immunohistochemistry for nestin expression (step IV on page 8) and the rest of the wells are ready for differentiation (step III on page 8).

III. Cell Differentiation

Differentiation of NSCs is performed 48 hours after the initial cell plating and culturing in Completed Base Media containing Neural Stem Cell 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 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.

IV. Characterization of Cells by Immunocytochemistry

Nestin, Neuron-specific β-III Tubulin and GFAP are used as markers for neural stem 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 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 cells are being blocked, dilute the reconstituted 10X goat anti-rat Nestin, anti-Neuron-specific β-III Tubulin, or sheep anti-human GFAP antibody in PBS containing 1% BSA and 10% normal donkey serum to a final 1X concentration.
6. After blocking, incubate the cells with 300 µL/well of 1X goat anti-rat Nestin, mouse anti-Neuron-specific β-III Tubulin or sheep 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 according to the manufacturer's suggestion 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 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 (see the images in the Procedure Outline on page 6).

Immunocytochemistry of Oligodendrocytes with Mouse anti-Oligodendrocyte Marker O4

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 10X mouse 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 (1X).
6. After blocking, incubate the cells with 300 μL/well of 1X mouse 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. Rhodamine Red-conjugated donkey anti-mouse IgM secondary antibody) according to the manufacturer's suggestion 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 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 (see the images in the Procedure Outline on page 6).

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NOTES