Figure 1: Verification of Neural Progenitor Marker Expression in Rat Neural Stem Cells. Rat cortical stem cells (R&D Systems®, Catalog # NSC001), were assessed for expression of neural progenitor markers using this kit. Rat cortical stem cells were stained with A. Mouse Anti-CXCR4 Monoclonal Antibody followed by the NorthernLights™ (NL) 557-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems®, Catalog # NL001; red) and B. Goat Anti-Musashi-1 Antibody (R&D Systems®, Catalog # NL002; green). The nuclei were counterstained with DAPI (blue) in each panel.

Figure 2: Verification of Neural Progenitor Marker Expression in Mouse Neural Stem Cells. Mouse cortical stem cells (R&D Systems®, Catalog # NSC002) were assessed for expression of neural progenitor markers using this kit. Mouse cortical stem cells were stained with A. Mouse Anti-SSEA-1 Monoclonal Antibody followed by the NorthernLights™ (NL) 557-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems®, Catalog # NL013; red) and B. Goat Anti-CXCR4 Antibody (R&D Systems®, Catalog # NSC001) using Mouse Anti-SSEA-1 Monoclonal Antibody (filled histogram) or Mouse IgG Isotype Control (empty histogram). Cells were stained using PE-conjugated secondary developing reagents.

Figure 3: Verification of Neural Progenitor Marker Expression in Human Neural Stem Cells. Human neural stem cells were assessed for expression of neural progenitor markers using this kit. Human neural stem cells were stained with A. Rat Anti-Vimentin Monoclonal Antibody followed by the NL557-conjugated Goat Anti-Rat Secondary Antibody (R&D Systems®, Catalog # NL2018; red) and B. Goat Anti-Notch-1 Antibody (R&D Systems®, Catalog # NL2018; green). The nuclei were counterstained with DAPI (blue) in each panel.

Figure 4: Detection of Neural Progenitor Markers CXCR4 and SSEA-1 by Flow Cytometry. Cells were stained with antibodies included in this kit. A. CXCR4 was detected in undifferentiated Mouse Cortical Stem Cells (R&D Systems®, Catalog # NSC002) using Mouse Anti-CXCR4 Monoclonal Antibody (filled histogram) or Mouse IgG Isotype Control (open histogram). B. SSEA-1 was detected in undifferentiated Rat Cortical Stem Cells (R&D Systems®, Catalog # NSC001) using Mouse Anti-SSEA-1 Monoclonal Antibody (filled histogram) or Mouse IgM Isotype Control (empty histogram). Cells were stained using PE-conjugated secondary developing reagents.

Data Examples

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Data Examples Continued

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Related Reagents

Human/Mouse/Rat Neural Progenitor Cell Marker Antibody Panel

Catalog Number: SC025

Introduction

Neural progenitor cells (NPCs) reside in several areas of the embryonic and adult central nervous system and can be harvested and propagated in vitro. NPCs are defined by their ability to self renew and differentiate into neurons, astrocytes, and oligodendrocytes (1, 2). Various molecular markers can facilitate identification of NPC populations in vivo and in vitro. Due to the wide expression of most markers in a variety of cell types, it is important to look at several markers to ensure you have the population of interest.

The intermediate filament protein, Nestin, has been shown to identify stem/progenitor cells in the developing nervous system (3). Nestin is now used frequently as a marker for NPCs both in vivo and in vitro. Another intermediate filament protein, Vimentin, is expressed in the developing neural tube, marking progenitor cells (4). Musashi-1 is an RNA binding protein essential for NPC self renewal by translational inhibition of its target genes, one of which is Numb. Numb is an inhibitor of Notch signaling, which is important for NPC proliferation (5).

The SRY-box transcription factors SOX1 and SOX2 are expressed in NPCs of the embryo and adult. Both of these proteins have been used to specifically sort NPCs from surrounding tissue, and are thus considered important markers for this cell population (6, 7).

Cell surface markers for stem cell populations are valuable in cell sorting progenitor cells from more differentiated phenotypes. CXCR4 and SSEA-1 are expressed on NPCs (8-10). CXCR4 plays a role in NPC migration during injury (7, 8). SSEA-1 is a useful marker for identification and sorting of NPCs from the adult brain (10).

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

Manufactured and Distributed by:

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DESIGN OF THE PANEL

The Human/Mouse/Rat Neural Progenitor Cell Marker Antibody Panel (Catalog # 967244) is designed for the identification and characterization of human/mouse/rat neural progenitor cells by marker expression. The panel contains the following antibodies: Anti-Nestin, Anti-SOX1, Anti-SOX2, Anti-Vimentin, Anti-Notch-1, Anti-Musashi-1, Anti-CXCR4, and Anti-SSEA-1.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at 2-8 °C. This kit is stable for up to one year from the date of receipt.

OTHER MATERIALS & SUPPLIES REQUIRED

- Flow Cytometry Staining Buffer (R&D Systems®, Catalog # F0117, F0118, or F0119)
- 1% BSA in PBS
- 4% paraformaldehyde in PBS
- 10% normal donkey serum
- Deionized or distilled water
- Triton X-100
- 24-well tissue culture plate

Additional supplies required are as follows:

<table>
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<tr>
<th>PRIMARY ANTIBODY</th>
<th>ISOTYPE CONTROL</th>
<th>SECONDARY DEVELOPING REAGENTS FOR IMMUNOFIJOLOGY</th>
<th>SECONDARY DEVELOPING REAGENTS FOR FLOW CYTOMETRY</th>
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*Provided this is within one year from the date of receipt.

PROCEDURES

Flow Cytometry

1. Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1 x 10^6 cells/mL.
2. For each marker, transfer 90 µL of the cell suspension into a separate 5.0 mL tube. Add 10 µL of either Anti-CXCR4 or Anti-SSEA-1.
3. Incubate for 30 minutes at room temperature.
4. Following incubation, wash the sample twice in 2.0 mL of Flow Cytometry Staining Buffer.

Surface Marker Analysis of CXCR4 and SSEA-1 by Flow Cytometry

1. Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1 x 10^6 cells/mL.
2. For each marker, transfer 90 µL of the cell suspension into a separate 5.0 mL tube. Add 10 µL of either Anti-CXCR4 or Anti-SSEA-1.
3. Incubate for 30 minutes at room temperature.
4. Following incubation, wash the sample twice in 2.0 mL of Flow Cytometry Staining Buffer.

Fluorescence microscopy

*Note: The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

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.

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.

REAGENT & MATERIAL PREPARATION

Reconstitute each vial with 250 µL of sterile PBS. This provides a 10X stock solution. Note: Optimal dilutions should be determined by each laboratory for each application.

PROCEDURE CONTINUED

Immunocytochemistry

1. Wash the cells twice with 1.0 mL of sterile 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.

PERMEABILIZE and block the cells with 0.5 mL of 1% BSA in PBS containing 10% normal donkey serum and 0.3% Triton X-100 at room temperature for 45 minutes.

5. While the cells are being blocked, dilute the reconstituted antibody in 1% BSA in PBS containing 10% normal donkey serum and 0.3% Triton X-100 to a final concentration of 10 µg/mL.

6. After blocking, incubate the cells with 300 µL/well of antibody working solution for 3 hours at room temperature or overnight at 2-8 °C.

Note: A negative control should be run using 1% BSA in PBS containing 10% normal donkey serum and 0.3% Triton X-100 with no primary antibody.

7. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each wash.
8. Dilute the secondary developing reagent 1:200 in 1% BSA in PBS.
9. Incubate the cells with 300 µL/well of secondary developing reagent for 60 minutes at room temperature in the dark.
10. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes each wash.

11. Aspirate the PBS from the wells and add 0.5 mL of destained or distilled water. Carefully remove the coverslips with forceps and mount cell side down onto a drop of mounting medium on a large slide.

12. Slides are ready for microscopic observation.

REFERENCES