

Human Embryonic Stem Cell Marker Antibody Panel Plus

Catalog Number SC009

Reagents for the identification of human embryonic stem cells.

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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INTRODUCTION

Embryonic stem (ES) cells, derived from the inner cell mass of pre-implantation embryos, have been recognized as the most pluripotent stem cell population (1, 2). Human ES cells can be maintained and propagated on mouse fibroblast feeders for extended periods in media containing fibroblast growth factor basic (FGF basic) (3). Gene expression of undifferentiated human ES cells has been investigated in several cell lines through a variety of techniques including comparison with databases, reverse transcriptase-polymerase chain reaction, focused cDNA microarrays, and immunocytochemistry. A list of molecules has been established which is comprised of known ES-specific or highly expressed genes and candidates that can serve as markers for human ES cells and may also contribute to the “stemness” phenotype (3 - 11).

DESIGN OF THE PANEL

The Human Embryonic Stem Cell Marker Antibody Panel Plus is designed for users who are interested in characterizing the status of undifferentiated human ES cells. The panel contains a group of antibodies; anti-CD9, anti-E-Cadherin, anti-Nanog, anti-Oct-3/4, anti-PODXL (GCTM antigen), anti-SOX2, anti-SSEA-1, and anti-SSEA-4.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses have not been established.

PRECAUTION

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

MATERIALS PROVIDED

Mouse anti-CD9 (clone 209306, isotype mouse IgG_{2B}, Part # 963490) - 25 µg of lyophilized mouse anti-CD9 monoclonal antibody.

Mouse anti-E-Cadherin (clone 180224, isotype mouse IgG_{2B}, Part # 963491) - 25 µg of lyophilized mouse anti-E-Cadherin monoclonal antibody.

Goat anti-Nanog (Part # 963488) - 25 µg of lyophilized goat anti-Nanog polyclonal antibody.

Goat anti-Oct-3/4 (Part # 962649) - 25 µg of lyophilized goat anti-Oct-3/4 polyclonal antibody.

Mouse anti-PODXL (GCTM antigen) (clone 222328, isotype mouse IgG_{2B}, Part # 963492) - 25 µg of lyophilized mouse anti-PODXL monoclonal antibody.

Mouse anti-SOX2 (clone 245610, isotype mouse IgG_{2A}, Part # 963493) - 25 µg of lyophilized mouse anti-SOX2 monoclonal antibody.

Mouse anti-SSEA-1 (clone MC-480, isotype mouse IgM, Part # 963489) - 25 µg of lyophilized mouse anti-SSEA-1 monoclonal antibody.

Mouse anti-SSEA-4 (clone MC-813-70, isotype mouse IgG₃, Part # 962648) - 25 µg of lyophilized mouse anti-SSEA-4 monoclonal antibody.

STORAGE

Unopened Kit	Store at 2 - 8° C. Use within 1 year of receipt.
Opened/Reconstituted Reagents	May be stored for up to 1 month at 2 - 8° C or aliquoted and stored at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

OTHER SUPPLIES REQUIRED

- Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001)
- Sterile PBS
- 4% paraformaldehyde in PBS
- 1% BSA in PBS
- Triton[®] X-100
- 10% Normal donkey serum
- Flow cytometry secondary developing reagents (R&D Systems, Catalog # F0101B, F0102B, F0103B, F0114, F0116, F0117, F0118, and F0119)
- Immunocytochemistry secondary developing reagents (R&D Systems, Catalog # NL001, NL002, NL003, NL007, NL008, and NL009 or Jackson ImmunoResearch, Catalog # 715-506-020, 715-486-020, and 715-496-020)
- Flow cytometry isotype controls (R&D Systems, Catalog # MAB0041 and MAB007 or Caltag[®], Catalog # MGM00)
- Fluorescence microscope
- Benchtop centrifuge
- 2 - 8° C refrigerator

Triton is a registered trademark of Union Carbide, Inc.

Caltag is a registered trademark of Invitrogen, Inc.

REAGENT PREPARATION

Reconstitute each vial with 250 μL of sterile PBS. This provides reagents sufficient for processing 25 flow cytometry samples or 8 immunocytochemistry samples.

Note: *Optimal dilutions should be determined by each laboratory for each application.*

PROCEDURE

Surface Marker Analysis of CD9, E-Cadherin, PODXL, SSEA-1, and SSEA-4 by Flow Cytometry

1. Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1×10^6 cells/mL.
2. For each marker, transfer 90 μL of the cell suspension into a separate 5 mL tube. Add 10 μL of either anti-CD9, anti-E-Cadherin, anti-PODXL, anti-SSEA-1, or anti-SSEA-4.
3. Incubate for 30 minutes at 2 - 8° C.
4. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
5. Resuspend the cells in 200 μL of Flow Cytometry Staining Buffer and add a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome according to the manufacturer's instructions.
6. Incubate for 30 minutes at 2 - 8° C **in the dark**.
7. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
8. Resuspend the cells in 400 μL of Flow Cytometry Staining Buffer for flow cytometric analysis.

Note: *As a control for analysis, cells in a separate tube should be treated with a flow cytometry isotype control.*

Immunocytochemistry

Note: *This protocol is for cells grown in a 24-well tissue culture plate.*

1. Wash the cells twice with 1 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.
4. 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.

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.*

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.
7. Wash the cells three times with 0.5 mL of 1% BSA in PBS for 5 minutes.
8. Dilute the secondary antibody (R&D Systems, Catalog # NL001, NL002, NL003, NL007, NL008, NL009 or Jackson Immunoresearch, Catalog # 715-506-020, 715-486-020, 715-496-020) 1:200 in 1% BSA in PBS.
9. Incubate the cells with secondary antibody at 300 µL per well 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.
11. Aspirate the PBS from the wells and add 0.5 mL of 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.

FIGURES AND IMAGES OF EMBRYONIC STEM CELL STAINING

Courtesy of Dr. Jong-Hoon Kim and Dr. Ron McKay from the National Institute of Neurological Disorders and Stroke & Stem Cell Unit at NIH.

Immunocytochemistry

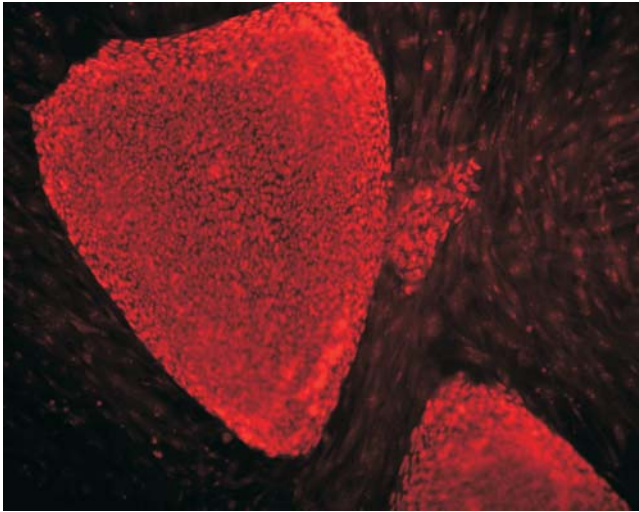


Figure 1A: Detection of Oct-3/4 in human embryonic stem cells using R&D Systems' goat anti-Oct-3/4 affinity-purified polyclonal antibody (Catalog # AF1759). Cells were stained using a Rhodamine Red-conjugated donkey anti-goat IgG secondary antibody.

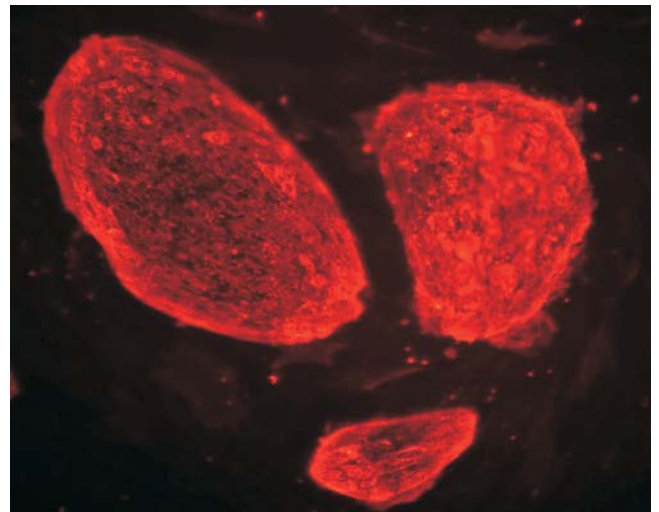


Figure 1B: Detection of SSEA-4 in human embryonic stem cells using R&D Systems' mouse anti-SSEA-4 monoclonal antibody (Catalog # MAB1435). Cells were stained using a Rhodamine Red-conjugated donkey anti-mouse IgG secondary antibody.

Please refer to the website to see full color images

(www.RnDSystems.com/pdf/SC009.pdf).

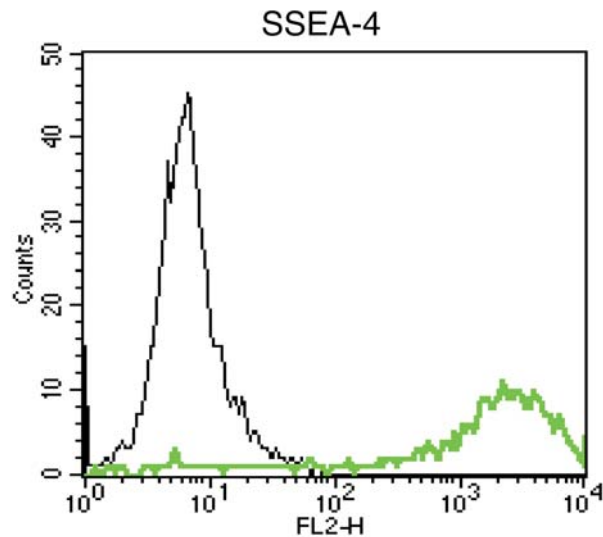


Figure 2: Reactivity of human embryonic stem cells stained with mouse anti-SSEA-4 monoclonal antibody (Catalog # MAB1435) (right) or isotype control (left). Cells were stained using a PE-conjugated goat anti-mouse IgG secondary antibody.

Please refer to www.RnDSystems.com for additional data regarding CD9, E-Cadherin, Nanog, Oct-3/4, PODXL, SOX2, SSEA-1, and SSEA-4.

RELATED REAGENTS

Reagent	Catalog Number
Anti-CD9 Antibody	FAB1880F, FAB1880P, MAB1880
Anti-E-Cadherin Antibody	AF648, AF748, BAF648, BAF748, BAM18381, FAB18381A, FAB18381P, MAB748, MAB7481, MAB1838, MAB18381
Anti-Nanog Antibody	AF1997, BAF1997
Anti-Oct-3/4 Antibody	AF1759, BAF1759, MAB1759
Anti-PODXL Antibody	AF1556, AF1658, BAF1556, BAF1658, FAB1658P, MAB1556, MAB1658
Anti-SOX2 Antibody	AF2018, BAF2018, IC2018P, MAB2018
Anti-SSEA-1 Antibody	MAB2155
Anti-SSEA-4 Antibody	BAM1435, FAB1435P, MAB1435
Mouse IgG _{2B} Flow Cytometry Isotype Control (Clone 133303)	MAB0041
Mouse IgG ₃ Isotype Control (Clone 133316)	MAB007
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Allophycocyanin	F0101B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Phycoerythrin	F0102B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Fluorescein	F0103B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) PerCP	F0114
Goat Anti-Mouse IgM Phycoerythrin	F0116
Goat Anti-Mouse IgM Allophycocyanin	F0117
Goat Anti-Mouse IgM Fluorescein	F0118
Goat Anti-Mouse IgM PerCP	F0119
Flow Cytometry Staining Buffer (1X)	FC001
Donkey Anti-Goat IgG NL557 Affinity Purified Polyclonal Antibody	NL001
Donkey Anti-Goat IgG NL637 Affinity Purified Polyclonal Antibody	NL002
Donkey Anti-Goat IgG NL493 Affinity Purified Polyclonal Antibody	NL003
Donkey Anti-Mouse IgG NL557 Affinity Purified Polyclonal Antibody	NL007
Donkey Anti-Mouse IgG NL637 Affinity Purified Polyclonal Antibody	NL008
Donkey Anti-Mouse IgG NL493 Affinity Purified Polyclonal Antibody	NL009

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