Human Pluripotent Stem Cell Marker Antibody Panel Plus

Catalog Number SC009

Reagents for the identification of human pluripotent 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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PRINCIPLE OF THE ASSAY

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). More recently, it has been discovered that somatic cells are able to be reprogrammed to an ES cell-like state. These induced pluripotent stem (iPS) cells are able to be cultured under similar conditions as ES cells and also have the ability to give rise to all three germ layers: ectoderm, mesoderm, and endoderm (3-5). Gene expression of undifferentiated human pluripotent stem 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 pluripotent-specific or highly expressed genes and candidates that can serve as markers for human pluripotent cells and may also contribute to the "stemness" phenotype (6-13).

The Human Pluripotent Stem Cell Marker Antibody Panel Plus is designed for users who are interested in characterizing the status of undifferentiated human pluripotent stem cells. The panel contains antibodies specific for the following human protein markers: CD9, E-Cadherin, Nanog, Oct-3/4, PODXL (GCTM antigen), SOX2, SSEA-1, and SSEA-4.

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 pluripotent stem cells and any variation in the procedure can cause variation in the efficiency of cell differentiation.

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 & STORAGE CONDITIONS

Store unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF RECONSTITUTED MATERIAL
anti-hCD9 Purified Mouse Monoclonal IgG_{2B} Clone 209306	963490	25 µg of a monoclonal antibody specific for human CD9; lyophilized.	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.
Anti-hE-Cadherin Purified Mouse Monoclonal IgG_{2B} Clone 180224	963491	25 µg of a monoclonal antibody specific for human E-Cadherin; lyophilized.	
anti-hNanog Affinity Purified Goat IgG	963488	25 µg of a polyclonal antibody specific for human Nanog; lyophilized.	
anti-hOct-3/4 Affinity Purified Goat IgG	962649	25 µg of a polyclonal antibody specific for human Oct-3/4; lyophilized.	
anti-hPodocalyxin Purified Mouse Monoclonal IgG_{2A} Clone 222328	963492	25 µg of a monoclonal antibody specific for human PODXL; lyophilized.	
anti-hSOX2 Purified Mouse Monoclonal IgG_{2A} Clone 245610	963493	25 µg of a monoclonal antibody specific for human SOX2; lyophilized.	
anti-hSSEA-1 Purified Mouse Monoclonal IgM Clone MC-480	963489	25 µg of a monoclonal antibody specific for human SSEA-1; lyophilized.	
anti-h/mSSEA-4 Purified Mouse Monoclonal IgG_3 Clone MC-813-70	962648	25 µg of a monoclonal antibody specific for human SSEA-4; lyophilized.	

*Provided this is within the expiration of the kit.

OTHER SUPPLIES REQUIRED

Materials

- 24-well culture plates
- 12 mm coverslips (Carolina Biologicals, Catalog # 633009 or equivalent)
- 15 mL centrifuge tubes
- Pipettes and pipette tips
- Serological pipettes
- Fine pointed curved forceps
- Glass slides
- Liquid barrier pen
- 5 mL FACS™ tubes

Reagents

- Flow Cytometry Staining Buffer (Catalog # FC001)
- Sterile Phosphate-Buffered Saline (PBS)
- 4% Paraformaldehyde in PBS
- 1% BSA in PBS
- 0.3% Triton® X-100, 1% BSA, 10% normal donkey serum in PBS
- Mounting medium (Catalog # CTS011)
- Flow cytometry secondary antibodies (Catalog # F0101B, F0102B, F0103B, F0114, F0116, F0117, F0118, and F0119)
- Immunocytochemistry secondary antibodies (Catalog # NL001, NL002, NL003, NL007, NL008, and NL009)
- Flow cytometry isotype controls (Catalog # MAB0041 and MAB007)
- Deionized or distilled water

Equipment

- Fluorescence microscope
- Benchtop centrifuge
- 2-8 °C refrigerator
- Hemocytometer
- Flow Cytometer

REAGENT & MATERIAL 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.*

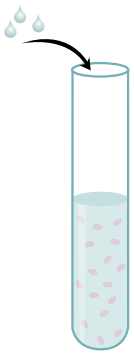
FLOW CYTOMETRY SURFACE STAINING PROTOCOL

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

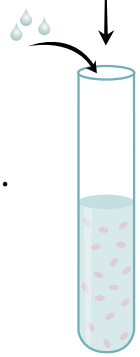
1. Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1×10^6 cells/mL.
2. For each marker, transfer 90 µL of the cell suspension into a separate 5 mL FACS tube. Add 10 µL of either anti-CD9, anti-E-Cadherin, anti-PODXL, anti-SSEA-1, or anti-SSEA-4.
   Note: *As a control for analysis, cells in a separate tube should be treated with a flow cytometry isotype control.*
3. Incubate for 30 minutes at room temperature.
4. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
5. Resuspend the cells in 100 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 room temperature **in the dark.**
7. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
8. Resuspend the cells in 200-400 µL of Flow Cytometry Staining Buffer for flow cytometric analysis.

FLOW CYTOMETRY SURFACE STAINING OUTLINE

Perform a cell count on harvested cells.
Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1 x 10^6 cells/1 mL.

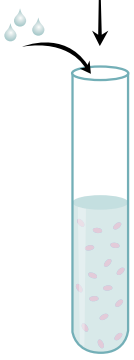


Aliquot 90 µL of cells into 5 mL flow cytometry tubes.

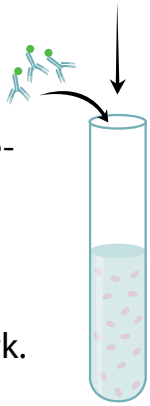


Add 10 µL of antibody or isotype control (10 µL for up to 10^6 cells or a previously titrated amount).
Vortex and incubate for 30 minutes at room temperature.

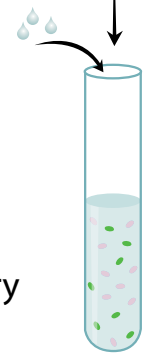
Centrifuge the samples at 300 x g for 5 minutes.
Wash the samples three times with Flow Cytometry Staining Buffer.
Resuspend each sample in 100 µL Flow Cytometry Staining Buffer.



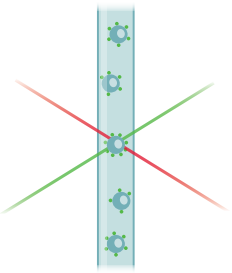
Add 10 µL of a fluorochrome-conjugated secondary developing reagent (or a previously titrated amount).
Incubate for 30 minutes at room temperature in the dark.



Centrifuge the samples at 300 x g for 5 minutes.
Wash the samples with Flow Cytometry Buffer.
Resuspend the cells in 200-400 µL of Flow Cytometry Staining Buffer.



Analyze the cells by flow cytometry.



IMMUNOCYTOCHEMISTRY FIXING & STAINING PROCEDURE

1. Wash the cells twice with PBS (1 mL/well of a 24-well plate).
2. Fix the cells with 4% paraformaldehyde in PBS for 20 minutes at room temperature.
3. Wash the cells 3 times with 1% BSA in PBS for 5 minutes (0.5 mL/well of a 24-well plate).
4. Permeabilize and block the cells with 0.3% Triton X-100, 1% BSA, and 10% normal donkey serum in PBS at room temperature for 45 minutes (0.5 mL/well of a 24-well plate).
5. During the blocking, dilute the reconstituted antibody in PBS containing 0.3% Triton X-100, 1% BSA, and 10% normal donkey serum to a final concentration of 10 µg/mL.

   Note: *A negative control should be performed using PBS containing 0.3% Triton X-100, 1% BSA, and 10% normal donkey serum in the absence of a primary antibody.*
6. After blocking, incubate the cells with diluted antibody (300 µL/well of a 24-well plate) for 3 hours at room temperature or overnight at 2-8 °C.
7. Wash the cells 3 times with 1% BSA in PBS for 5 minutes (0.5 mL/well of a 24-well plate).
8. Dilute the appropriate secondary antibody at 1:200 in PBS containing 1% BSA.
9. Incubate the cells with diluted secondary antibody in the dark for 60 minutes at room temperature (300 µL/well of a 24-well plate).
10. Wash the cells 3 times with 1% BSA in PBS for 5 minutes (0.5 mL/well of a 24-well plate).
11. Cover the cells with PBS (1 mL/well of a 24-well plate) and visualize with a fluorescence microscope. Alternatively, aspirate the PBS and add distilled or deionized water (0.5 mL/well of a 24-well plate). Carefully remove each coverslip with forceps and mount cell-side down onto a drop of mounting medium on a glass slide.
12. Slides are ready for microscopic observation.

FIGURES & IMAGES OF PLURIPOTENT 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

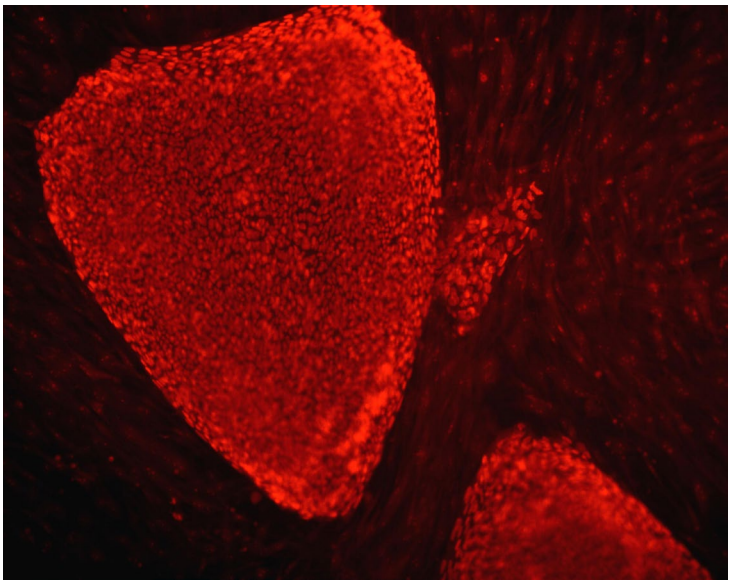


Figure 1A: Detection of Oct-3/4 in Human Embryonic Stem Cells. Human embryonic stem cells were labeled with the Anti-Human Oct-3/4 Affinity Purified Polyclonal Antibody provided in the Human Pluripotent Stem Cell Marker Antibody Panel Plus. The cells were stained using a Rhodamine Red-conjugated Donkey Anti-Goat IgG Secondary Antibody.

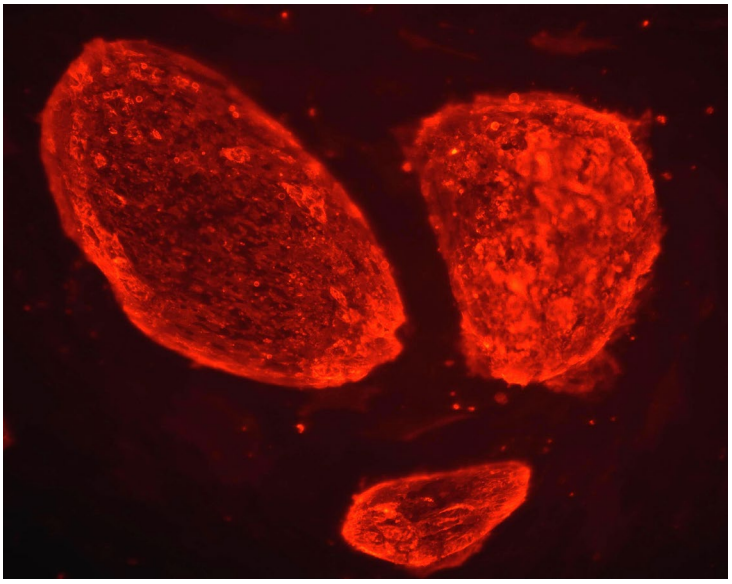


Figure 1B: Detection of SSEA-4 in Human Embryonic Stem Cells. Human embryonic stem cells were labeled with the Anti-Human/Mouse SSEA-4 Monoclonal Antibody provided in the Human Pluripotent Stem Cell Marker Antibody Panel Plus. The cells were stained using a Rhodamine Red-conjugated Donkey Anti-Mouse IgG Secondary Antibody.

DATA EXAMPLES

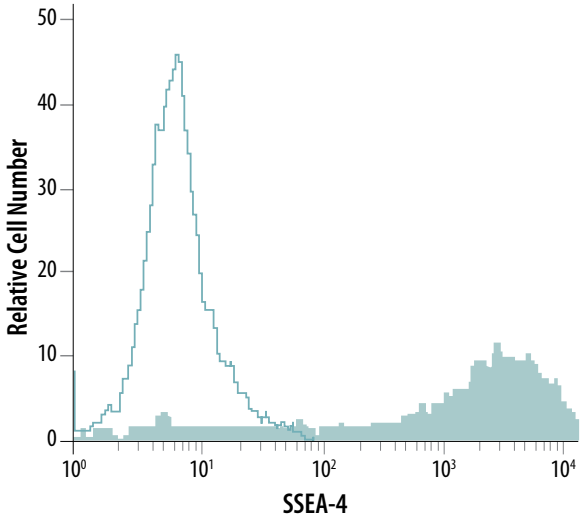


Figure 2: Detection of SSEA-4 in BG01V Human Embryonic Stem Cells. BG01V Human embryonic stem cells were stained with the Anti-Human SSEA-4 Monoclonal Antibody provided in the Human Pluripotent Stem Cell Marker Antibody Panel Plus (filled histogram) or a Mouse IgG3 Isotype Control Antibody (R&D Systems, Catalog # MAB007; open histogram). The cells were stained using a PE-conjugated Goat Anti-Mouse Secondary Antibody (R&D Systems, Catalog # F0102B).

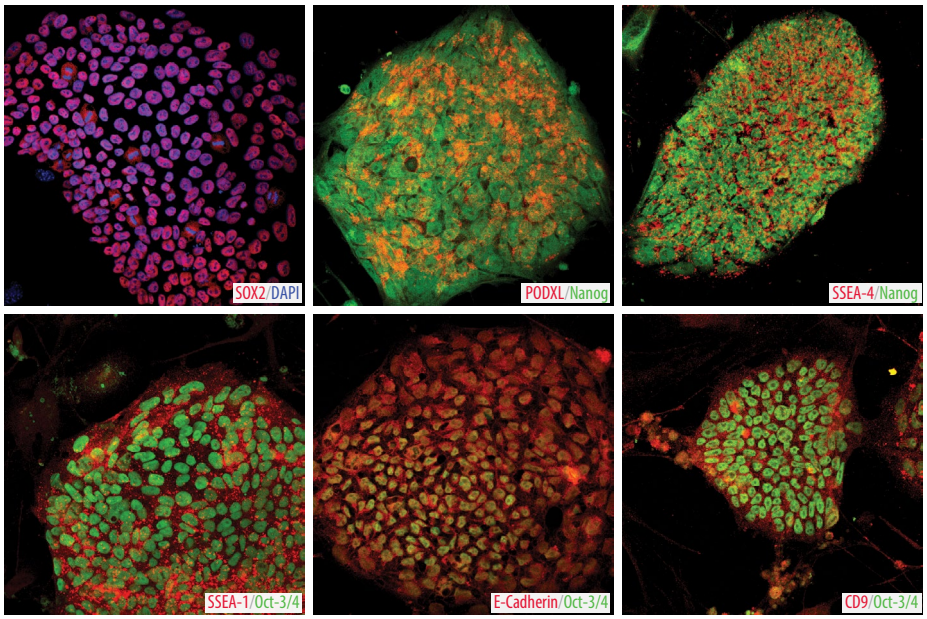


Figure 3: Expression of Pluripotency Markers in Human Induced Pluripotent Stem Cells. iPS2 human induced pluripotent stem cells were cultured on irradiated Mouse Embryonic Fibroblasts (iMEFs) (R&D Systems, Catalog # PSC001) and labeled with antibodies from the Human Pluripotent Stem Cell Marker Antibody Panel Plus. Pluripotency marker expression was analyzed by dual immunofluorescence with the indicated primary antibodies supplied in the panel. The cells were stained using NorthernLights™ (NL)493- and NL557-conjugated Secondary Antibodies (green and red, respectively). Where indicated, the nuclei were counterstained with DAPI (blue).

RELATED REAGENTS

Product Description	R&D Systems Catalog Number
Anti-CD9 Antibody	FAB1880F, FAB1880P, MAB1880
Anti-E-Cadherin Antibody	AF648, AF748, BAF648, BAF748, BAM18381, FAB18381A, FAB18381P, MAB748, MAB7481, MAB1838, MAB18381, NL648R, NL648G
Anti-Nanog Antibody	AF1997, BAF1997, NL1997G, NL1997R
Anti-Oct-3/4 Antibody	AF1759, BAF1759, MAB1759, NL1759G
Anti-PODXL Antibody	AF1556, AF1658, BAF1556, BAF1658, FAB1556A, FAB1556N, FAB1556P, FAB1658A, FAB1658C, FAB1658G, FAB1658N, FAB1658P, MAB1556, MAB1658, NL1658R
Anti-SOX2 Antibody	AF2018, BAF2018, IC2018A, IC2018C, IC2018P, MAB2018, NL20181G, NL2018R, NL20181V
Anti-SSEA-1 Antibody	FAB2155A, FAB2155C, FAB2155G, FAB2155N, FAB2155P, MAB2155, NL2155G, NL2155R, NLLC2155G, NLLC2155R
Anti-SSEA-4 Antibody	BAM1435, FAB1435A, FAB1435C, FAB1435F, FAB1435P, MAB1435, NL1435G, NL1435R, NL1435V, NLLC1435G, NLLC1435R
Mouse IgG_{2B} Flow Cytometry Isotype Control (Clone 133303)	IC0041C, IC0041G, IC0041P, MAB0041
Mouse IgG_3 Isotype Control (Clone 133316)	IC007A, 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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