DATA EXAMPLES

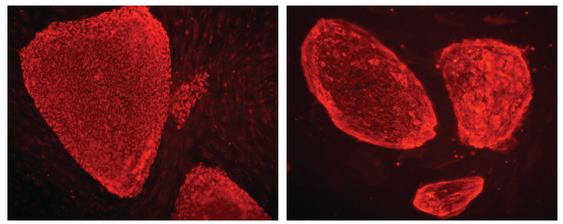

Figure 1: Detection of Oct-3/4 and SSEA-4 in Human Embryonic Stem Cells. Human embryonic stem cells were labeled with Anti-Human Oct-3/4 Affinity Purified Polyclonal Antibody **(A)** or Anti-Human/Mouse SSEA-4 Monoclonal Antibody **(B)** provided in this kit. The cells were stained using Rhodamine Red-conjugated secondary antibodies (red). *Courtesy of Dr. Jong-Hood Kim and Dr. Ron McKay from the National Institute of Neurological Disorders and Stroke & Stem Cell Unit at NIH.*

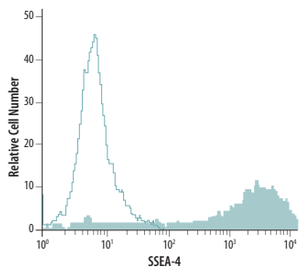

Figure 2: Detection of SSEA-4 in BG01V Human Embryonic Stem Cells. BG01V human embryonic stem cells were stained with the Anti-Human SSEA-4 Monoclonal Antibody provided in this kit (filled histogram) or a Mouse IgG_3 Isotype Control Antibody (R&D Systems®, Catalog # MAB007; open histogram). The cells were stained using a PE-conjugated Goat Anti-Mouse Secondary Antibody (R&D Systems®, Catalog # F0102B).

DATA EXAMPLES *CONTINUED*

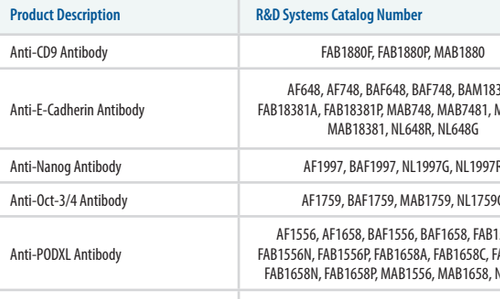

Figure 3: Expression of Pluripotency Markers in Human Induced Pluripotent Stem Cells. iPS2 human induced pluripotent stem cells were cultured on irradiated Mouse Embryonic Fibroblasts (iMEFs) (R&D Systems®, Catalog # PSC001) and labeled with antibodies from this kit. Pluripotency marker expression was analyzed by single or 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).

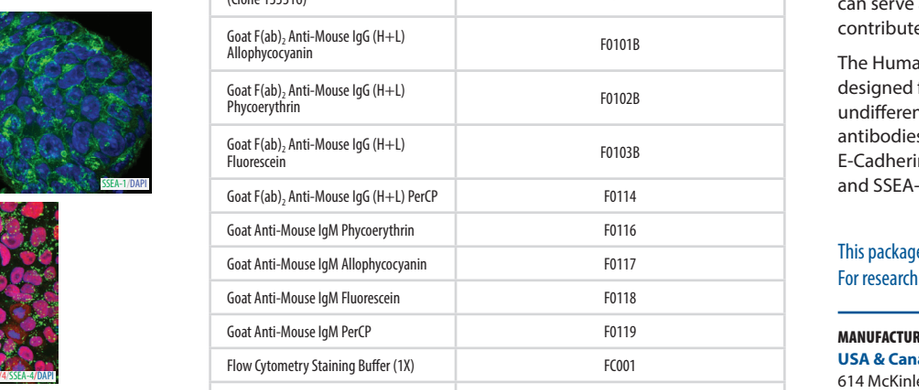

Figure 4: Expression of Pluripotency Markers in Embryonic and Induced Pluripotent Stem Cells. A,B. iBJ6 human induced pluripotent stem cells were cultured in mTeSR™ **C.** D3 mouse embryonic stem cells were cultured in StemXVivo® Mouse Pluripotent Stem Cell Media (R&D Systems®, Catalog # CCM025). **D,E.** BG01V human embryonic stem cells were cultured in MEF Conditioned Media (R&D Systems®, Catalog # AR005). Cultured cells were then labeled with antibodies provided in this kit. The cells were visualized using NorthernLights™ (NL)493- and NL557-conjugated Secondary Antibodies (green and red, respectively) and counterstained with DAPI (blue). Pluripotency marker expression was analyzed by dual (**A,B,D,E**) or single (**C**) immunofluorescence with the indicated primary antibodies supplied in the panel.

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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Human Pluripotent Stem Cell Marker Antibody Panel Plus

Catalog Number: SC009

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.

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:
USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:
UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at 2-8 °C for up to 6 months from date of receipt.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ 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	963942	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.	

OTHER MATERIALS & SUPPLIES REQUIRED

Materials

- 24-well culture plates
- 12 mm coverslips
- 15 mL centrifuge tubes
- Pipettes and pipette tips
- Serological pipettes
- Fine pointed curved forceps
- Glass slides
- 5 mL FACS™ tubes

Equipment

- Fluorescence microscope
- Benchtop centrifuge
- Hemocytometer
- Flow Cytometer

Reagents

- Flow Cytometry Staining Buffer (R&D Systems®, 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 (R&D Systems®, Catalog # CTS011)
- Flow cytometry secondary antibodies (R&D Systems®, Catalog # F0101B, F0102B, F0103B, F0114, F0116, F0117, F0118, and F0119)
- Immunocytochemistry secondary antibodies (R&D Systems®, Catalog # NL001, NL002, NL003, NL007, NL008, and NL009)
- Flow cytometry isotype controls (R&D Systems®, Catalog # MAB0041 and MAB007)
- Deionized or distilled water

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.

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 reagents sufficient for processing 25 flow cytometry samples or 8 immunocytochemistry samples.

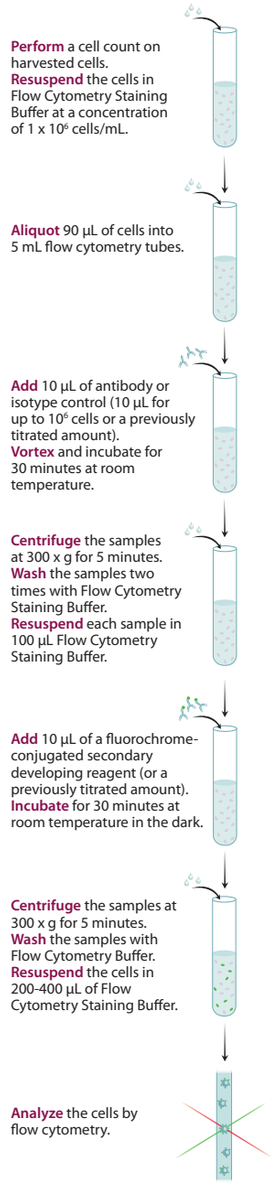
Note: *Optimal dilutions should be determined by each laboratory for each application.*

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 x 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. Vortex and centrifuge the samples at 300 x g for 5 minutes.
5. Wash the samples twice in 2 mL of Flow Cytometry Staining Buffer.
6. Resuspend the cells in 100 µL of Flow Cytometry Staining Buffer, and add 10 µL of a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome according to the manufacturer's instructions.
7. Incubate for 30 minutes at room temperature **in the dark.**
8. Vortex and centrifuge the samples at 300 x g for 5 minutes.
9. Wash the samples twice in 2 mL of Flow Cytometry Staining Buffer.
10. Resuspend the cells in 200-400 µL of Flow Cytometry Staining Buffer for flow cytometric analysis.

FLOW CYTOMETRY SURFACE STAINING OUTLINE



IMMUNOCYTOCHEMISTRY FIXING & STAINING PROTOCOL

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.

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