

Human Embryonic Stem Cell Marker Antibody Panel

Catalog Number SC008

Reagents for the expansion 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). While the undifferentiated/pluripotent state of ES cells can be best defined functionally, a good number of molecular markers have been used to characterize it.

Pluripotent ES cells can be characterized by high level expression of Oct-3/4, (also termed Oct-3 or Oct-4) a member of the POU transcription factors, and Nanog. A critical amount of Oct-3/4 and Nanog expression is required to sustain stem-cell pluripotency. When ES cells are induced to differentiate, Oct-3/4 and Nanog are downregulated, which has proven to be essential for a proper and divergent developmental program (4 - 9).

The undifferentiated state of ES cells is often characterized by the expression of the cell surface antigens, SSEA-1, SSEA-3 and SSEA-4. SSEA-1 is expressed on the surface of preimplantation-stage murine embryos at the eight-cell stage and has been found on the surface of teratocarcinoma stem cells but not on their differentiated derivatives (10,11). SSEA-3 and -4 are synthesized during oogenesis and are present in the membranes of oocytes, zygotes and early cleavage-stage embryos (12, 13). Biological roles of these carbohydrate-associated molecules have been suggested in controlling cell surface interactions during development (14). Undifferentiated primate ES cells, human ES cells and human Embryonic Carcinoma (EC) cells express SSEA-3 and SSEA-4, but not SSEA-1. Undifferentiated mouse ES cells, however, do express SSEA-1 but do not express SSEA-3 or SSEA-4 (3, 15).

Alkaline Phosphatase is an enzyme in the blood, intestines, liver, and bone cells and exists as membrane-bound isoforms of glycoproteins sharing a common protein structure but differing in carbohydrate content. These enzymes are most active at alkaline pH - hence the name (15). Undifferentiated human EC, ES and embryonic germ (EG) cells have been shown to express a very high level of the liver/bone/kidney isozyme of alkaline phosphatase (16 - 18). Expression levels of alkaline phosphatase decrease following stem cell differentiation.

DESIGN OF THE PANEL

The Human Embryonic Stem Cell Marker Antibody Panel is designed for users who are interested in analyzing the undifferentiation/differentiation status of human ES cells by analyzing ES cell marker expressions. The panel contains the following antibodies; anti-alkaline phosphatase, anti-Nanog, anti-Oct-3/4, anti-SSEA-1, and anti-SSEA-4.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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-alkaline phosphatase (clone B4-78, isotype mouse IgG₁, Part # 962647) - 25 µg of lyophilized mouse anti-alkaline phosphatase monoclonal antibody, enough to make 2.5 mL of staining solution when used at the suggested concentration of 1 µg/100 µL.

Goat anti-Nanog (Part # 963488) - 25 µg of lyophilized goat anti-Nanog polyclonal antibody, enough to make 2.5 mL of staining solution when used at the suggested concentration of 1 µg/100 µL.

Goat anti-Oct-3/4 (Part # 962649) - 25 µg of lyophilized goat anti-Oct-3/4 polyclonal antibody, enough to make 2.5 mL of staining solution when used at the suggested concentration of 1 µg/100 µL.

Mouse anti-SSEA-1 (clone MC-480, isotype mouse IgM, Part # 963489) - 25 µg of lyophilized mouse anti-SSEA-1 monoclonal antibody, enough to make 2.5 mL of staining solution when used at the suggested concentration of 1 µg/100 µL.

Mouse anti-SSEA-4 (clone MC-813-70, Isotype mouse IgG₃, Part # 962648) - 25 µg of lyophilized mouse anti-SSEA-4 monoclonal antibody, enough to make 2.5 mL of staining solution when used at the suggested concentration of 1 µg/100 µL.

STORAGE AND REAGENT PREPARATION

Unopened Kit	Store at 2 - 8° C. Use within 1 year of receipt.	
Opened Reagents	Mouse anti-alkaline phosphatase	Reconstitute each vial with 250 µL of sterile PBS. Mix gently. Aliquot and store the 10X stocks at ≤ -20° C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.
	Goat anti-Nanog	
	Goat anti-Oct-3/4	
	Mouse anti-SSEA-1	
	Mouse anti-SSEA-4	

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

OTHER SUPPLIES REQUIRED

- FACS Buffer - 2% fetal bovine serum, 0.1% sodium azide in Hank's buffer
- 4% paraformaldehyde in PBS
- 1% BSA in PBS
- 0.1% Triton X-100, 1% BSA, 10% normal donkey serum in PBS
- 1% BSA, 10% normal donkey serum in PBS
- Secondary developing reagents
- Fluorescence microscope
- Sterile PBS

PROCEDURE

Use serological pipettes to transfer and remove solutions.

Surface Marker Analysis of Alkaline Phosphatase, SSEA-1, and SSEA-4 by Flow Cytometry

1. Resuspend the cells in FACS buffer at the concentration of 1×10^6 cells/mL.
2. Transfer 90 μ L of cell suspension into a 5 mL tube. Add 10 μ L of either anti-SSEA-1, anti-SSEA-4, or anti-alkaline phosphatase.
3. Incubate for 30 minutes at 2 - 8° C.
4. Following incubation, wash the sample twice in 3 mL of FACS buffer.
5. Resuspend the cells in 200 μ L of FACS buffer and add a secondary developing reagent [e.g. PE-conjugated goat anti-mouse IgG secondary antibody (Caltag, Catalog # M35004-3) for alkaline phosphatase and SSEA-4 or PE-conjugated goat anti-mouse IgM secondary antibody (Caltag, Catalog # M31504) for SSEA-1] according to the manufacturer's instructions.
6. Incubate for 30 minutes at 2 - 8° C **in the dark**.
7. Following the incubation, wash the sample twice in 3 mL of FACS buffer.
8. Resuspend the cells in 400 μ L of FACS buffer for flow cytometric analysis.

Note: *As a control for analysis, cells in a separate tube should be treated with fluorochrome-labeled mouse IgG₁ isotype control for anti-alkaline phosphatase, mouse IgM isotype control for SSEA-1 antibody, and mouse IgG₃ isotype control for SSEA-4 antibody.*

Immunocytochemistry of Nanog and Oct-3/4

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

1. Wash the cells twice with 1 mL of PBS.
2. Fix the cells with 0.5 mL of 4% paraformaldehyde (prepared in PBS) for 20 minutes at room temperature.
3. Wash the cells twice with 1 mL of PBS for 5 minutes.
4. Permeabilize and block the cells with 0.5 mL of 0.1% Triton X-100, 1% BSA, 10% normal donkey serum in PBS at room temperature for 45 minutes.
5. During the blocking, prepare the goat anti-Nanog antibody or goat anti-Oct-3/4 antibody working solution by diluting the 10X stock with PBS containing 1% BSA, 10% normal donkey serum to a final concentration of 10 $\mu\text{g/mL}$.

Note: *For staining 5 sample wells at 300 μL per well, add 150 μL of goat anti-Nanog antibody (10X) or goat anti-Oct-3/4 antibody (10X) into 1.35 mL of PBS containing 1% BSA, 10% normal donkey serum.*

6. After blocking, incubate the cells with 300 μL /well of diluted goat anti-Nanog antibody or goat anti-Oct-3/4 antibody working solution overnight at 2 - 8° C.
7. Wash the cells three times with 1 mL of PBS containing 1% BSA for 5 minutes.
8. Dilute the secondary antibody [e.g. Rhodamine Red-conjugated donkey anti-goat secondary antibody (Jackson ImmunoResearch, Catalog # 705-026-147) or equivalent] according to the manufacturer's instructions in PBS containing 1% BSA. Incubate the cells with diluted secondary antibody at 300 μL per well for 60 minutes at room temperature **in the dark**.
9. Wash the cells three times with 1 mL of PBS containing 1% BSA for 5 minutes.
10. Cover the cells with 1 mL of PBS and visualize with a fluorescence microscope.

Immunocytochemistry of Alkaline Phosphatase, SSEA-1, and SSEA-4

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

1. Wash the cells twice with 1 mL of PBS.
2. Fix the cells with 0.5 mL of 4% paraformaldehyde (prepared in PBS) for 20 minutes at room temperature.
3. Wash the cells twice with 1 mL of PBS for 5 minutes.
4. Block the cells with 0.5 mL of 0.1% BSA, 10% normal donkey serum in PBS at room temperature for 45 minutes.
5. During the blocking, prepare the anti-alkaline phosphatase, anti-SSEA-1, or anti-SSEA-4 primary antibody working solution by diluting the 10X stock with PBS containing 1% BSA and 10% normal donkey serum to a final concentration of 10 $\mu\text{g}/\text{mL}$.

Note: *For staining 5 sample wells at 300 μL per well, add 150 μL of anti-SSEA-1 (10X), anti-SSEA-4 (10X), or anti-alkaline phosphatase antibody (10X) into 1.35 mL of PBS containing 1% BSA and 10% normal donkey serum.*

6. After blocking, incubate the cells with 300 μL /well of diluted primary antibody working solution overnight at 2 - 8° C.
7. Wash the cells three times with 1 mL of PBS containing 1% BSA for 5 minutes.
8. Dilute the secondary antibody [e.g. Rhodamine Red-conjugated donkey anti-mouse IgG secondary antibody (Jackson Immunoresearch, Catalog # 715-295-150 or equivalent) for alkaline phosphatase and SSEA-4 or Rhodamine Red-conjugated donkey anti-mouse IgM secondary antibody (Jackson Immunoresearch, Catalog # 715-295-020 or equivalent) for SSEA-1] according to the manufacturer's instructions in PBS containing 1% BSA. Incubate the cells with secondary antibody at 300 μL per well for 60 minutes at room temperature **in the dark**.
9. Wash the cells three times with 1 mL of PBS containing 1% BSA for 5 minutes.
10. Cover the cells with 1 mL of PBS and visualize with a fluorescence microscope.

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

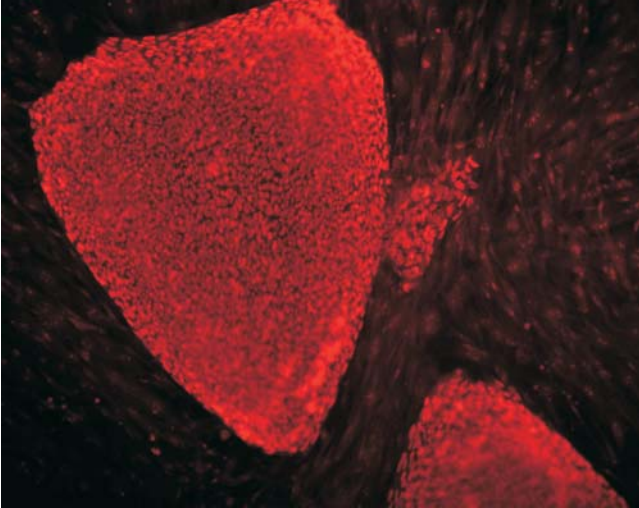


Figure 1A: Detection of Oct-3/4 in human embryonic stem cells using R&D Systems' goat anti-Oct-3/4 affinity-purified polyclonal antibody (Catalog # AF1759). Cells were stained using a Rhodamine Red-conjugated donkey anti-goat IgG secondary antibody.

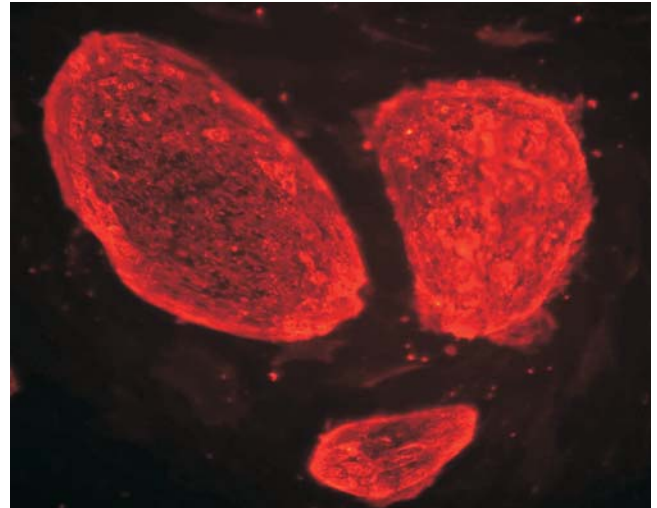


Figure 1B: Detection of SSEA-4 in human embryonic stem cells using R&D Systems' mouse anti-SSEA-4 monoclonal antibody (Catalog # MAB1435). Cells were stained using a Rhodamine Red-conjugated donkey anti-mouse IgG secondary antibody.

Flow Cytometry

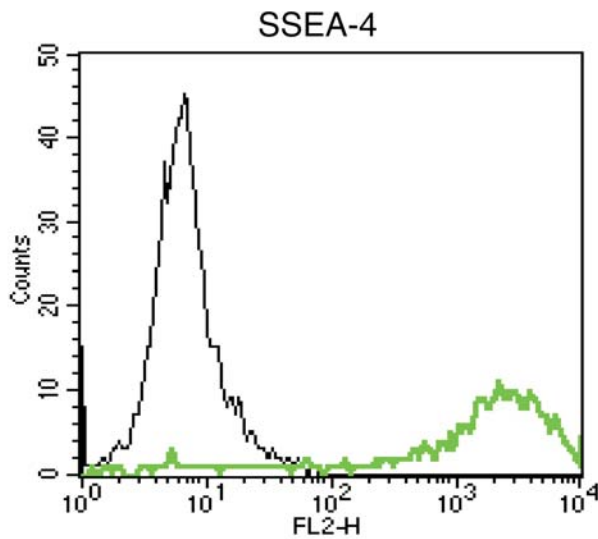


Figure 2A: Reactivity of human embryonic stem cells stained with mouse anti-SSEA-4 monoclonal antibody (Catalog # MAB1435) (right) or isotype control (left). Cells were stained using a PE-conjugated goat anti-mouse IgG secondary antibody.

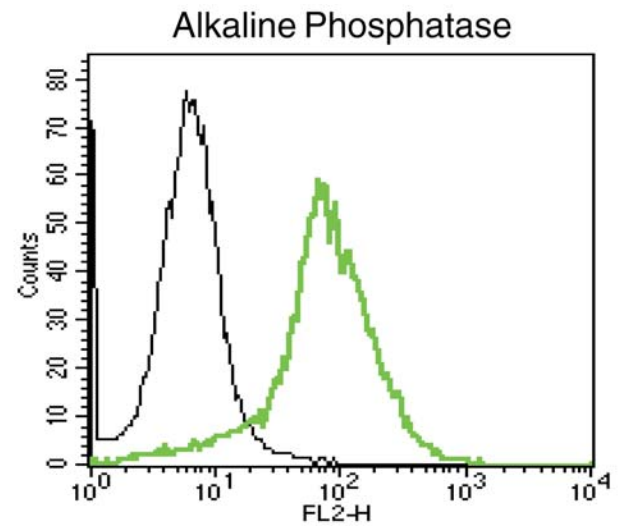


Figure 2B: Reactivity of human embryonic stem cells stained with mouse anti-Alkaline Phosphatase monoclonal antibody (Catalog # MAB1448) (right) or isotype control (left). Cells were stained using a PE-conjugated goat anti-mouse IgG secondary antibody.

Please refer to the website (www.RnDSystems.com) for additional data regarding alkaline phosphatase, Nanog, Oct-3/4, SSEA-1, and SSEA-4.

RELATED REAGENTS

Reagent	R&D System's Catalog Number
Anti-Alkaline Phosphatase Antibody	BAM1448, FAB1448A, MAB1448
Anti-Nanog Antibody	AF1997, BAF1997
Anti-Oct-3/4 Antibody	AF1759, BAF1759, MAB1759
Anti-SSEA-1 Antibody	MAB2155
Anti-SSEA-4 Antibody	BAM1435, FAB1435P, MAB1435

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NOTES