

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-SSEA-1:

Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: MC-480

Isotype: mouse IgM

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

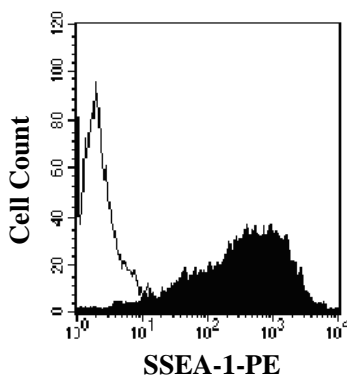
Designed to quantitatively determine the percentage of cells bearing the stage-specific embryonic antigen-1 (SSEA-1) within a population and qualitatively determine the density of SSEA-1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing SSEA-1. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing SSEA-1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of SSEA-1. Cell surface expression of SSEA-1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-SSEA-1: Use as is; no preparation necessary.



Mouse D3 embryonic stem cell line was stained with PE-conjugated anti-SSEA-1 (Catalog # FAB2155P, filled histogram) or isotype control (open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 µL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (up to 1 x 10⁶ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated anti-SSEA-1 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted anti-SSEA-1 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgM antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Background Information

Stage-specific embryonic antigen-1 (SSEA-1), an antigenic epitope defined as a Lewis X carbohydrate structure, is expressed during early mouse embryogenesis on murine embryonal carcinoma cells (EC), murine embryonic stem cells (ES) and murine and human germ cells (1 - 3). Expression of SSEA-1 is down regulated following differentiation of murine EC and ES cells (4). In contrast, the differentiation of human EC and ES cells is accompanied by an increase in SSEA-1 expression (5 - 7). SSEA-1 has also been used to identify adult mouse neural stem cells *in vivo* (8).

References

1. Solter, D. and B.B. Knowles (1978) Proc. Natl. Acad. Sci. USA **75**:5565.
2. Knowles, B.B. *et al.* (1978) Curr. Top. Microbiol. Immunol. **81**:51.
3. Ozawa, M. *et al.* (1985) Cell Differ. **16**:169.
4. Matsui, Y. *et al.* (1992) Cell **70**:841.
5. Fox, N. *et al.* (1983) Cancer Res. **43**:669.
6. Shablott, M.J. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:13726.
7. Thomson, J.A. *et al.* (1998) Science **282**:1145.
8. Capela, A. and S. Temple (2002) Neuron **35**:865.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.