

Reagent Information

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human/mouse SSEA-1: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: MC-480

Ig Class: mouse IgM

Additional Reagents Required

- 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

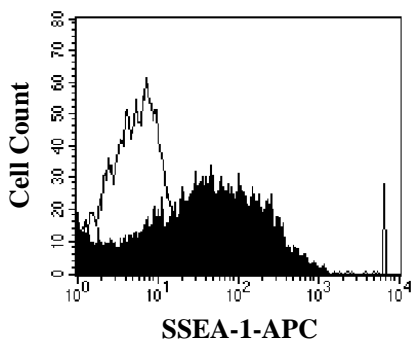
Designed to quantitatively determine the percentage of cells bearing SSEA-1 within a population and qualitatively determine the density of SSEA-1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the APC-labeled monoclonal antibody, which binds to the cells expressing SSEA-1. Unbound APC-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 SSEA-1. Cell surface expression of SSEA-1 is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

APC-conjugated mouse anti-human/mouse SSEA-1: Use as is; no preparation is necessary.



Mouse D3 embryonic stem cell line stained with APC-conjugated anti-SSEA-1 (Catalog # FAB2155A, 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. 50 µL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells 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) are 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) Transfer cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 2) Add 10 µL of APC-conjugated anti-SSEA-1 reagent.
- 3) Incubate for 30 - 45 minutes at 2° - 8° C.
- 4) Following this incubation, remove unreacted anti-SSEA-1 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Human Erythrocyte Lysing Kit, Catalog # WL1000*).
- 5) Resuspend the cells in 200 - 400 µL of PBS buffer for final flow cytometric analysis.
- 6) As a control for analysis, cells in a separate tube should be treated with APC-labeled mouse IgM antibody.

This procedure may need to be modified, depending upon final utilization.

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. Shambloott, 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.