

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human/mouse SSEA-4: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: MC813-70

Isotype: mouse IgG₃

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

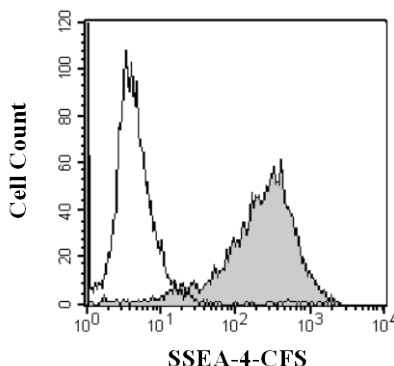
Designed to quantitatively determine the percentage of cells bearing SSEA-4 within a population and qualitatively determine the density of SSEA-4 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing SSEA-4. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing SSEA-4 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of SSEA-4. Cell surface expression of SSEA-4 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.

Reagent Preparation

Fluorescein-conjugated mouse anti-human/mouse SSEA-4: Use as is; no preparation necessary.



NTERA-2 cells were stained with CFS-conjugated anti-human/mouse SSEA-4 (Catalog # FAB1435F, filled histogram) or CFS-conjugated 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 (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of CFS-conjugated SSEA-4 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted SSEA-4 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 CFS-labeled mouse IgG₃ antibody.

This procedure may need modification, depending upon final utilization.

Background Information

SSEA-4 is expressed on the surface of human embryonal carcinoma (EC) cells (the pluripotent stem cells of teratocarcinomas), human embryonic germ cells (EG), and human embryonic stem cells (ES). Expression of SSEA-4 is down-regulated following differentiation of human EC cells. In contrast, the differentiation of murine EC and ES cells may be accompanied by an increase in SSEA-4 expression.¹⁻⁴

References

1. Shevinsky, L.H. *et al.*, 1982, *Cell* **30**:697 - 705.
2. Kannagi, R. *et al.*, 1983, *EMBO J.* **2**:2355 - 2361.
3. Thomson, J.A. and J.S. Odorico, 2000, *Trends Biotechnol.* **18**:53 - 57.
4. Draper, J.S. *et al.*, 2002, *J. Anat.* **200**:249 - 258.

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.