



Immunostaining Protocols for Flow Cytometric Analysis of Adherent Cells

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ABSTRACT

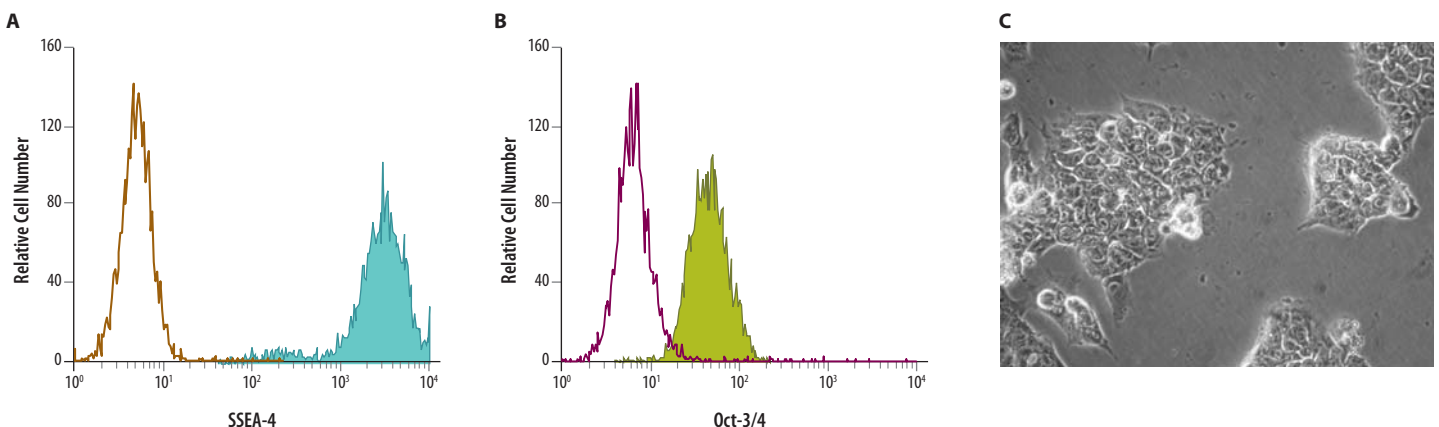
Flow cytometry is a powerful technique for assessing the expression of multiple proteins simultaneously in individual cells within a heterogeneous population. This technique is most commonly used with non-adherent cells, but it can also be an effective tool for analyzing protein expression in adherent cells. The following immunostaining

protocols outline the steps required for detecting surface or intracellular antigens in adherent cells by flow cytometry. Data is presented to show the staining of SSEA-4 (surface) and Oct-3/4 (intracellular) in BG01V embryonic stem cells using this protocol.

MATERIALS

- ✓ PBS or Hanks' Balanced Salt Solution (HBSS)
- ✓ PBS/EDTA (PBS, 2 g/L EDTA, Sigma Catalog # E9884)
- ✓ Trypsin solution [cell media with 5 g/L Trypsin (Sigma, Catalog # T1426) and 2 g/L EDTA]; or Gibco 0.05% Trypsin-EDTA 1X (Invitrogen Catalog # 25200-056), or Trypsin EDTA 1X (Irvine Scientific Catalog # 9341), or other cell detachment solutions [Accutase™ (Millipore Catalog # SCR005), Detachin™ (Genlantis Catalog # T100100) or Cellstripper™ (MediaTech Catalog # 25-056-Cl)]
- ✓ Flow Cytometry Staining Buffer (R&D Systems Catalog # FC001)
- ✓ Flow Cytometry Fixation Buffer (R&D Systems Catalog # FC004)
- ✓ Flow Cytometry Permeabilization/Wash Buffer I (R&D Systems Catalog # FC005)
- ✓ Flow Cytometry Fixation/Permeabilization Buffer I (R&D Systems Catalog # FC007)
- ✓ Scraper
- ✓ Pipettes
- ✓ Cell media
- ✓ Adherent cell lines: HeLa, MCF-7, HEK293, HUVEC, RAW264, BG01V (licensed from BresaGen, Inc.), etc.
- ✓ Antibodies: APC-conjugated anti-human/mouse SSEA-4 (R&D Systems Catalog # FAB1435A) and PE-conjugated anti-human/mouse Oct-3/4 (R&D Systems Catalog # IC1759P)

RESULTS: Detection of SSEA-4 and Oct 3/4 in BG01V cells by Flow Cytometry



The human embryonic stem cell line BG01V was stained with (A) APC-conjugated anti-human/mouse SSEA-4 (Catalog # FAB1435A; surface antigen) and (B) PE-conjugated anti-human/mouse Oct-3/4 (Catalog # IC1759P; intracellular antigen) monoclonal antibodies (filled histograms), or respective isotype control antibodies (open histograms), using the simultaneous fixation/permeabilization procedure described above. (C) shows a bright-field micrograph of BG01V cells at 20x magnification.

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METHODS

A. FOR SURFACE ANTIGENS:

1. Remove media from culture flask or plate and rinse cells with PBS or HBSS.
2. To remove adherent cells:
 - a. Add Trypsin solution (a volume large enough to cover the bottom of the flask), keep at room temperature for 10 min. or less (incubation time should be optimized by the investigator). Pipette up and down to gently remove the adherent cells. (Note: Trypsinization may significantly remove or decrease some surface antigens. This should be determined by the investigator prior to the experiment.)
 - b. Alternatively, use a scraper or pipette the cells up and down gently with PBS/EDTA solution.
 - c. Some cell lines may work better with a gentler cell detachment solution, (several are listed above). When using these commercial solutions, follow the manufacturer's instructions.
3. Following cell detachment, check under a light microscope to make sure the cells are not in clumps. Pipette up and down 3x to break up any clumps.
4. Centrifuge and resuspend the cells at $\sim 1 \times 10^6$ cells/100 μ L Flow Cytometry Staining Buffer in a 5 mL flow cytometry tube.
5. Add antibody to the cells (at optimal concentration) and incubate for 30 min ($2 - 8^\circ$ C).
6. Remove excess antibody by washing the cells with 2 mL of Flow Cytometry Staining Buffer and resuspend the final pellet in 200 - 400 μ L of the same buffer for flow cytometric analysis.

B. FOR INTRACELLULAR ANTIGENS (WITH FIXATION FOLLOWED BY PERMEABILIZATION):

1. Repeat steps 1 - 3 from (A).
2. Wash cells 2x with PBS or HBSS.
3. Resuspend the cells in 500 μ L of Flow Cytometry Fixation Buffer and incubate at room temperature for 10 min. Vortex cells intermittently to maintain a single-cell suspension.
4. Wash cells twice with PBS or HBSS and resuspend in 100 - 200 μ L of Flow Cytometry Permeabilization/Wash Buffer.
5. Add antibody to the cells (at optimal concentration) and incubate for 30 min ($2 - 8^\circ$ C).
6. Remove excess antibody by washing the cells with 2 mL of Flow Cytometry Permeabilization/Wash Buffer. Resuspend the final pellet in 200 - 400 μ L of Flow Cytometry Staining Buffer for flow cytometric analysis.

C. FOR SIMULTANEOUS FIXATION/PERMEABILIZATION STAINING:

1. Repeat steps 1 - 3 from (A).
2. Wash cells 2x with PBS or HBSS.
3. Resuspend the cells in 500 μ L of Flow Cytometry Fixation/Permeabilization Buffer and incubate at $2 - 8^\circ$ C for 30 min. Vortex cells intermittently to maintain a single-cell suspension.
4. Centrifuge the cells and resuspend in 100 - 200 μ L of Flow Cytometry Permeabilization/Wash Buffer.
5. Add antibody to the cells (at optimal concentration) and incubate for 30 min ($2 - 8^\circ$ C).
6. Remove excess antibody by washing the cells with 2 mL of Flow Cytometry Permeabilization/Wash Buffer. Resuspend the final pellet in 200 - 400 μ L of Flow Cytometry Staining Buffer for flow cytometric analysis.

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