

February 2013

Cell surface staining of stem cell lines and other adherent cells for flow cytometry

Materials and Reagents

Full Name	Short Name	Catalog Number
Microwell plates (round bottom wells) or Tubes (12 x 75-mm polypropylene round-bottom test tubes)		N/A
PBS (without calcium or magnesium)	PBS	554781
Accutase™ Cell Detachment Solution	Accutase	561527
BD CytoFix™ fixation buffer (optional)	Fixation Buffer	554655
BD Pharmingen™ stain buffer, or equivalent, 1X PBS, 2%	Stain Buffer (FBS)	554656
FBS (or BSA) 0.1% NaN ₃ (pH 7.1–7.4)	Stain Buffer (BSA)	554657
70-µm cell strainer		352350
N/A, not applicable		

Procedural Notes

- The following protocol was used during antibody development. It has not been routinely used for Quality Control testing of antibodies.
- Investigators can determine if other detachment enzymes or procedures are suitable. Some antibody epitopes can be affected by enzyme treatment, such as the trypsin-sensitive epitope within human CD325 (N-cadherin) recognized by clone 8C11.
- After the detachment enzyme is added, cells can be incubated at 37°C instead of room temperature. However, enzyme activity will decrease over time.
- Gently tapping the side of the plate or flask can help dislodge a cell monolayer. Additional Accutase might be needed to dissociate three-dimensional structures.
- Determination of optimal antibody concentration may be necessary. For test size antibody products, add the recommended test size volume. Staining time may be increased (> 45 minutes) depending on the avidity of the fluorescent antibody.
- If analysis must be delayed, then the stained cells can be fixed with buffered paraformaldehyde (for example, BD Cytotfix Buffer; see the product Technical Data Sheet for detailed protocol) for 30 minutes at 4°C, washed, resuspended in Stain Buffer and then stored at 4°C (protected from light). The fixed cells should be analyzed as soon as possible. We have not tested all fluorescently conjugated antibodies for this fixation. Therefore, researchers might need to verify if this fixation will affect antibody binding and fluorescence intensity.

Procedure

1. Remove tissue culture medium from the plate or flask and wash the cells with room-temperature PBS (without Ca or Mg).
2. Add the detachment enzyme (for example, Accutase) to the cells to cover the surface of the well or flask.
3. Incubate at room temperature for 5-10 minutes, or until cells are detached.
4. Add culture medium or PBS (for example, 2 mL for a 6-well plate) to assist in washing off the cells, and pipette gently up and down to help disperse doublets.
5. Transfer the contents to a new 15-mL tube.



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- Optional: Remove a small amount of liquid and check it under a microscope to confirm the presence of single cells. If you observe clumps of cells, collect the cell suspension and pass it through a 70- μm cell strainer.
- Wash the cells in two to four volumes of PBS centrifuging at 300g for 5 minutes.
- Wash the cells in stain buffer (with BSA or FBS) and resuspend in a volume that is appropriate for cell counting. Determine the cell concentration using the standard method for a hemocytometer or other cell counter.
- Resuspend the cells to a concentration of 1×10^7 cells/mL. Add 100 μL to 12 x 75-mm tubes or to round-bottomed wells of microwell plates.
- Add fluorescently conjugated antibodies (or purified, or biotin-conjugated antibodies) and incubate for 20-45 minutes on ice or at room temperature, protected from light.
- Wash the cells twice with either 100-200 μL (for microwell plates) or 1-2 mL (for tubes) of Stain Buffer.
- For indirect immunofluorescent staining of cells, repeat steps 10 and 11 with either a labeled appropriate secondary antibody or a labeled streptavidin conjugate in 100 μL of Stain Buffer.
- Resuspend the cell pellet in either 200- μL (for microwell plates) or 0.5-mL (for tubes) volumes of Stain Buffer.
- Analyze stained cell samples by flow cytometry.

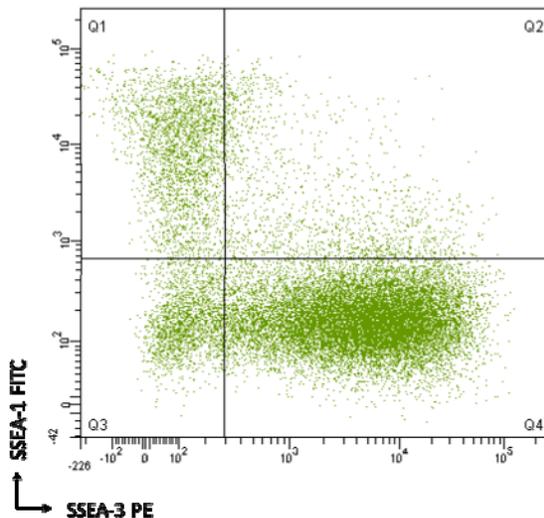


Figure 1. Differentiating H9 cells were harvested and stained with antibodies against SSEA-1 and SSEA-3 (as part of the BD Stemflow™ human pluripotent stem cell sorting and analysis kit, Cat. No. 560461).

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23-14720-02

