Human/Mouse Pluripotent Stem Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC001
Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains four conjugated antibodies and four corresponding isotype controls that can be used for single-step staining of human/mouse pluripotent stem cells (PSCs) (1-7).

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C in the dark. Use within 6 months of receipt.

PART	PART #	DESCRIPTION
Positive Markers	965654	250 µL of SOX2-PE Mouse IgG_{2A}; Clone 245610
	965655	250 µL of Oct-3/4-APC Rat IgG_{2B}; Clone 240408
Marker (Positive for human; Negative for mouse)	965656	250 µL of SSEA-4-CFS Mouse IgG_3; Clone MC-813-70
Marker (Negative for human; Positive for mouse)	965657	250 µL of SSEA-1-PerCP Mouse IgM; Clone MC-480
Isotype Controls	965658	250 µL of Mouse IgG_{2A}-PE Isotype Control
	965659	250 µL of Rat IgG_{2B}-APC Isotype Control
	965660	250 µL of Mouse IgG_3-CFS Isotype Control
	965661	250 µL of Mouse IgM-PerCP Isotype Control
Fixation/Permeabilization Buffer	895029	30 mL of 1X Fixation/Permeabilization Buffer
Permeabilization/Wash Buffer	895030	30 mL of 1X Permeabilization/Wash Buffer

INTENDED USE

This product is designed for the flow cytometric analysis of human/mouse PSCs using four fluorochrome-conjugated antibodies.

PRECAUTIONS

Formaldehyde is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes, and avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice.

Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

INTRACELLULAR STAINING PROTOCOL WITH SIMULTANEOUS FIXATION/PERMEABILIZATION

1. Harvest cells of interest and wash twice in PBS or Hanks' Balanced Salt Solution (HBSS).
2. Resuspend approximately 5 x 10^5 washed cells in 0.5 mL of Fixation/Permeabilization Buffer and incubate at 2-8 °C for 30 minutes. The cells should be vortexed intermittently in order to maintain a single cell suspension.
3. Centrifuge the cells, and resuspend the pellet in 100-200 µL of the Permeabilization/Wash Buffer.
4. Add 10 µL of each antibody, or add 10 µL of each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30-45 minutes at room temperature **in the dark**.
6. Following the incubation, remove any excess antibody by washing the cells in 2 mL of Permeabilization/Wash Buffer. The final cell pellet is resuspended in 200-400 µL of PBS for flow cytometric analysis.

   Notes: *Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular staining. Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (8).*

DATA EXAMPLES

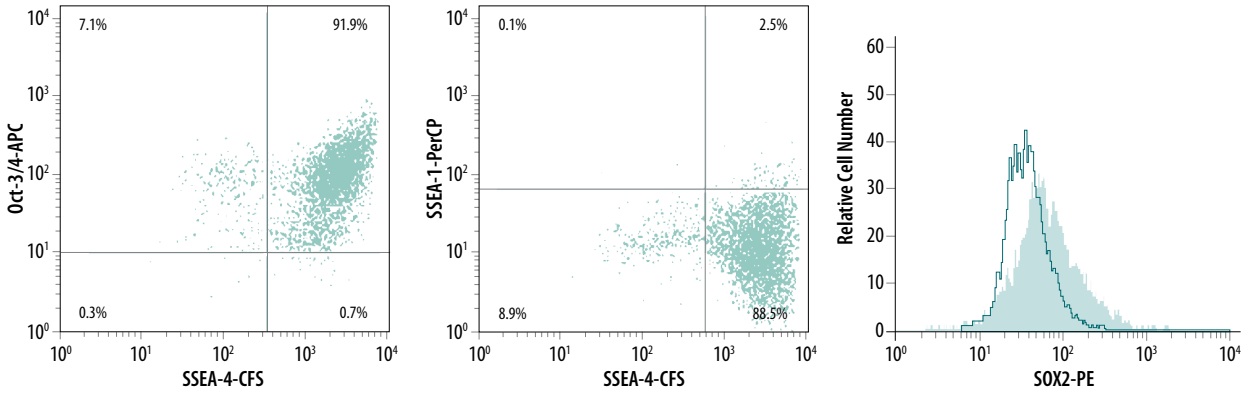

Figure 1: BG01V human embryonic stem cells were stained using the antibodies provided in the Human/Mouse Pluripotent Stem Cell Multi-Color Flow Cytometry Kit. Cells were analyzed simultaneously for their expression of SSEA-1, SSEA-4, Oct-3/4, and SOX2.

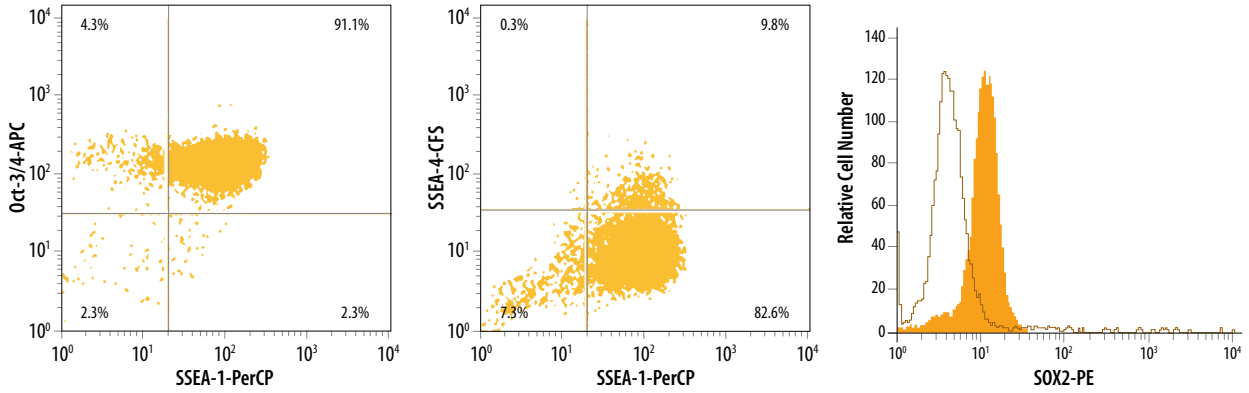

Figure 2: D3 mouse embryonic stem cells were stained using the antibodies provided in the Human/Mouse Pluripotent Stem Cell Multi-Color Flow Cytometry Kit. Cells were analyzed simultaneously for their expression of SSEA-1, SSEA 4, Oct-3/4, and SOX2.

REFERENCES

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