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. Refer to the kit label for date of expiration.

<table>
<thead>
<tr>
<th>PART</th>
<th>PART #</th>
<th>DESCRIPTION</th>
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</thead>
<tbody>
<tr>
<td>Positive Markers</td>
<td>965654</td>
<td>250 μL of SOX2-PE Mouse IgG2a; Clone 245610</td>
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<td></td>
<td>965655</td>
<td>250 μL of Oct-3/4-APC Rat IgG2b; Clone 240408</td>
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<tr>
<td>Marker (Positive for human; Negative for mouse)</td>
<td>965656</td>
<td>250 μL of SSEA-4-CFS Mouse IgG3; Clone MC-813-70</td>
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<td></td>
<td>965657</td>
<td>250 μL of SSEA-1-PerCP Mouse IgM; Clone MC-480</td>
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<tr>
<td>Isotype Controls</td>
<td>965658</td>
<td>250 μL of Mouse IgG2a-PE Isotype Control</td>
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<tr>
<td></td>
<td>965659</td>
<td>250 μL of Rat IgG1-APC Isotype Control</td>
</tr>
<tr>
<td></td>
<td>965660</td>
<td>250 μL of Mouse IgG1-CFS Isotype Control</td>
</tr>
<tr>
<td></td>
<td>965661</td>
<td>250 μL of Mouse IgM-PerCP Isotype Control</td>
</tr>
<tr>
<td>Fixation/Permeabilization Buffer</td>
<td>895029</td>
<td>30 mL of 1X Fixation/Permeabilization Buffer</td>
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<tr>
<td>Permeabilization/Wash Buffer</td>
<td>895030</td>
<td>2 bottles (30 mL/bottle) of 1X Permeabilization/Wash Buffer</td>
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</tbody>
</table>

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**