PRODUCT DESCRIPTION

This kit contains three conjugated antibodies and three corresponding conjugated isotype controls for the positive identification of human mesenchymal stem cells (MSCs). A Negative Marker Cocktail containing five conjugated antibodies and a Negative Isotype Cocktail containing the corresponding isotypes are included for exclusion of non-MSCs. This combination of eight markers is included in the minimal criteria set by the International Society for Cellular Therapy to define an MSC (1). These reagents provide single-step staining for the verification of human MSCs.

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C. Refer to the kit label for date of expiration.

<table>
<thead>
<tr>
<th>PART</th>
<th>PART #</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Markers</td>
<td>967542</td>
<td>500 μL of CD90-APC Mouse IgG2; Clone Thy-1A1</td>
</tr>
<tr>
<td></td>
<td>967544</td>
<td>500 μL of CD73-CFS Mouse IgG2; Clone 606112</td>
</tr>
<tr>
<td></td>
<td>967546</td>
<td>500 μL of CD105-PerCP Mouse IgG1; Clone 166707</td>
</tr>
</tbody>
</table>
| Negative Marker Cocktail | 967548 | 500 μL of Negative Marker Cocktail:  
• CD45-PE Mouse IgG1; Clone 2D1  
• CD34-PE Mouse IgG1; Clone QBEnd10  
• CD11b-PE Mouse IgG1; Clone 238446  
• CD79A-PE Mouse IgG1; Clone 706931  
• HLA-DR-PE Mouse IgG1; Clone L203 |
| Positive Isotype Controls | 967543 | 500 μL of Mouse IgG2A-APC Isotype Control; Clone 20102 |
| | 967545 | 500 μL of Mouse IgG2B-CFS Isotype Control; Clone 133303 |
| | 967547 | 500 μL of Mouse IgG1-PerCP Isotype Control; Clone 11711 |
| Negative Isotype Control Cocktail | 967549 | 500 μL of Negative Isotype Cocktail:  
• Mouse IgG2a-PE Isotype Control; Clone 11711  
• Mouse IgG2b-PE Isotype Control; Clone 133303 |
| Staining Buffer | 895091 | 125 mL of 1X Staining Buffer |

INTENDED USE

This product is designed for the flow cytometric analysis of human MSCs using three fluorochrome-conjugated antibodies and one fluorochrome-conjugated antibody cocktail.

PRECAUTIONS

The Staining Buffer contains 0.09% sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

REFERENCES

SURFACE STAINING PROTOCOL

1. Cell samples should be washed with 2 mL of Staining Buffer, spinning the tube at 300 x g for 5 minutes.
2. Washed cells should be counted and then Fc receptor blocking reagents may be added if desired. If using excess pre-immune IgG to block Fc receptor, use 1 μg of IgG per 1 x 10^5 cells to be stained. The excess IgG does not need to be washed from the cells following the incubation period and can be carried into the staining reaction.
3. Transfer a small volume of cells (1 x 10^5 cells in 100 μL is recommended) into a 5 mL Flow Cytometry tube.
4. Add 10 μL of each positive antibody and 10 μL of the Negative Marker Cocktail to a tube of cells, or add 10 μL of each corresponding isotype control antibody and 10 μL of the Negative Isotype Cocktail to a tube of 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 with 2 mL of Staining Buffer. The final cell pellet is resuspended in 200-400 μL of Staining Buffer for flow cytometric analysis.

**Note:** Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (2).

DATA EXAMPLES

Figure 1: Human bone marrow-derived mesenchymal stem cells were stained using this kit. Cells demonstrate positive expression of CD73, CD105, and CD90 as well as negative expression of all markers included in the Negative Marker Cocktail. Quadrants have been set based on isotype controls.

Figure 2: KG-1 human acute myelogenous leukemia cells and peripheral blood mononuclear cells (PBMCs) were stained using this kit. Cells demonstrate positive expression for the antibodies included in the Negative Marker Cocktail (filled histogram) over the Negative Isotype Cocktail (open histogram).