Title:	**TruCytes™ Lymphocyte Subsets Control (P/N: SSB-31-A) Technical Data Sheet**
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1. Technical Data Sheet

<table>
<tr><td>Summary</td><td>TruCytes™ Lymphocyte Subsets are lyophilized cell mimic controls that feature T cell, B cell and NK cell markers with scatter coordinates that closely mimic lymphocyte, monocyte, and granulocyte populations. They are intended to provide positive and negative signal detection for specific surface biomarkers targeted by specific antibodies. They are formulated to work with lyse-wash and lyse-no wash conditions.</td></tr>
<tr><td>Application</td><td>This product is intended to provide positive signals for specified biomarkers and their antibodies listed in the table below:
<table>
<tr><th>Biomarker</th><th colspan="3">Tested Antibody Clone</th></tr>
<tr><td>CD45</td><td>2D1</td><td>MEM-28</td><td>HI30</td></tr>
<tr><td>CD3*</td><td>SK7</td><td>UCHT1</td><td>OKT3/HIT-3α*</td></tr>
<tr><td>CD4</td><td>SK3</td><td>RPA-T4</td><td>OKT4</td></tr>
<tr><td>CD8</td><td>SK1</td><td>RPA-T8</td><td>HIT-8α</td></tr>
<tr><td>CD19</td><td>SJ25C1</td><td>HIB19</td><td>4G7</td></tr>
<tr><td>CD16</td><td>B73.1</td><td>CB16</td><td>3G8</td></tr>
<tr><td>CD56</td><td>NCAM16.2</td><td>MEM-188</td><td>MY-31</td></tr>
<tr><td>CD14</td><td>M5E2</td><td>61D3</td><td>HCD-14</td></tr>
<tr><td colspan="4">*Clones OKT3/HIT-3α resulted in low signal</td></tr>
</table>
NOTE: Antibody clones not listed above need to be to tested independently to determine compatibility.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.</td></tr>
<tr><td>Materials</td><td>This product is lyophilized for stability and ease of use. Each vial contains $2.5x10^5$ cell mimics.</td></tr>
<tr><td>Handling and Safety</td><td>No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at slingshotbio.com.</td></tr>
<tr><td>Storage</td><td>Store lyophilized products at -20 °C upon receipt.</td></tr>
<tr><td>Expiration</td><td>18 months from the date of manufacturing when stored at -20 °C.
Use the entire vial immediately upon reconstitution of lyophilized product.</td></tr>
<tr><td>Instructions for Use</td><td>This product can be used in a FACS tube format (Procedure A) or a 96 well V bottom plate format (Procedure B). You may select the one needed for your application.</td></tr>
</table>

Procedure A : Running in a FACS tube

Sample Preparation:

1. Remove the vial of TruCytes™ Lymphocyte Subsets Control from the -20 °C and let it sit at room temperature for 15 minutes.
2. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial.
3. Add 250 µL of staining buffer to the vial.
4. Note: Avoid contacting or disturbing the pellet until it has been hydrated (~1 minute).
5. Gently pipette up and down ten times to resuspend the cell mimics. Avoid introducing bubbles. Transfer the contents to a 5mL FACS tube.
6. Add 1000 µL of flow staining buffer to the original vial, mix by gentle pipetting, and transfer the remaining of the mimics to the FACS tube prepared in the previous step for a final volume of 1250 µL.
7. Centrifuge at 600 x g for 5 minutes.
8. Decant the supernatant being careful not to disturb the cell pellet.
9. The cell mimics are ready for staining.

Staining Procedure:

1. Prepare your preferred staining antibody cocktail in flow staining buffer and then add the mixed solution to the FACS tube. Mix well by vortexing.
2. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 30 +/- 2 min.
3. Add 400 µL of flow staining buffer.
4. Centrifuge at 600 x g for 5 minutes.
5. Decant the supernatant being careful not to disturb the cell pellet.
6. Add 500 µL of flow staining buffer.
7. Centrifuge at 600 x g for 5 minutes.
8. Decant the supernatant being careful not to disturb the cell pellet.
9. Add desired volume of flow staining buffer and mix thoroughly by pipette mixing**.**
10. The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as leukocytes. For best results, we recommend acquiring your sample immediately.

We recommend using Slingshot Biosciences Compensation Controls for unmixing or compensating your TruCytes™ Lymphocyte Subsets flow cytometry data. See product selection at slingshotbio.com

Procedure B : Running in a 96 Well V Bottom Plate

Sample Preparation:

1. Remove the vial of TruCytes™ Lymphocyte Subsets Control from the -20 °C and let it sit at room temperature for 15 minutes.
2. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial.
3. Add 250 µL of staining buffer to the vial.
   Note: Avoid contacting or disturbing the pellet until it has been hydrated (~1 minute).
4. Gently pipette up and down ten times to resuspend the cell mimics. Avoid introducing bubbles. Transfer the contents to a 5mL FACS tube.
5. Add 1000 µL of flow staining buffer to the original vial, mix by gentle pipetting, and transfer the remaining of the mimics to the FACS tube prepared in the previous step for a final volume of 1250 µL.
6. Centrifuge at 600 x g for 5 minutes.
7. Decant the supernatant being careful not to disturb the cell pellet.
8. Add 200 µL of flow staining buffer and mix by gentle pipetting.
9. Transfer the entire sample volume from the FACS tube to the desired well of the 96 well V bottom plate.
10. Add 200 µL of flow staining buffer and centrifuge at 600 x g for 2 minutes.
11. Decant the supernatant being careful not to disturb the cell pellet.
12. The cell mimics are ready for staining.

Staining Procedure:

1. Prepare your preferred staining antibody cocktail in flow staining buffer and then add the mixed solution to the appropriate well of the 96 well V bottom plate. Mix well by vortexing.
2. Prepare your preferred staining antibody cocktail in flow staining buffer such that a total of 100 µL of cocktail diluted in flow staining buffer is added to 96 well V bottom plate. Mix well by pipetting.
3. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 30 +/- 2 min.

4. Add 100 µL of flow staining buffer.
5. Centrifuge at 600 x g for 2 minutes.
6. Decant the supernatant being careful not to disturb the cell pellet.
7. Add 200 µL of flow staining buffer.
8. Centrifuge at 600 x g for 2 minutes.
9. Add 200 µL of flow staining buffer and mix thoroughly by pipette mixing.
10. The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as leukocytes. For best results, we recommend acquiring your sample immediately.

We recommend using Slingshot Biosciences Compensation Controls for unmixing or compensating your TruCytes™ Lymphocyte Subsets flow cytometry data. See product selection at slingshotbio.com

QC Data

Figure 1. Scatter plot and gating. The following shows a representative scatter plot of the gating strategy used for detection of the lymphocyte subsets.