

1. Technical Data Sheet

<p>Summary</p>	<p>TBNK Mimic™ 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.</p>																																							
<p>Application</p>	<p>This product is intended to provide positive signals for specified biomarkers and their antibodies listed in the table below:</p> <table border="1" data-bbox="405 725 1430 1283"> <thead> <tr> <th data-bbox="405 725 660 786">Biomarker</th> <th colspan="3" data-bbox="665 725 1430 786">Tested Antibody Clone</th> </tr> </thead> <tbody> <tr> <td data-bbox="405 792 660 846">CD45</td> <td data-bbox="665 792 916 846">2D1</td> <td data-bbox="920 792 1171 846">MEM-28</td> <td data-bbox="1176 792 1430 846">HI30</td> </tr> <tr> <td data-bbox="405 853 660 907">CD3*</td> <td data-bbox="665 853 916 907">SK7</td> <td data-bbox="920 853 1171 907">UCHT1</td> <td data-bbox="1176 853 1430 907">OKT3/HIT-3α*</td> </tr> <tr> <td data-bbox="405 913 660 967">CD4</td> <td data-bbox="665 913 916 967">SK3</td> <td data-bbox="920 913 1171 967">RPA-T4</td> <td data-bbox="1176 913 1430 967">OKT4</td> </tr> <tr> <td data-bbox="405 974 660 1028">CD8</td> <td data-bbox="665 974 916 1028">SK1</td> <td data-bbox="920 974 1171 1028">RPA-T8</td> <td data-bbox="1176 974 1430 1028">HIT-8α</td> </tr> <tr> <td data-bbox="405 1034 660 1088">CD19</td> <td data-bbox="665 1034 916 1088">SJ25C1</td> <td data-bbox="920 1034 1171 1088">HIB19</td> <td data-bbox="1176 1034 1430 1088">4G7</td> </tr> <tr> <td data-bbox="405 1095 660 1149">CD16</td> <td data-bbox="665 1095 916 1149">B73.1</td> <td data-bbox="920 1095 1171 1149">CB16</td> <td data-bbox="1176 1095 1430 1149">3G8</td> </tr> <tr> <td data-bbox="405 1155 660 1209">CD56</td> <td data-bbox="665 1155 916 1209">NCAM16.2</td> <td data-bbox="920 1155 1171 1209">MEM-188</td> <td data-bbox="1176 1155 1430 1209">MY-31</td> </tr> <tr> <td data-bbox="405 1216 660 1270">CD14</td> <td data-bbox="665 1216 916 1270">M5E2</td> <td data-bbox="920 1216 1171 1270">61D3</td> <td data-bbox="1176 1216 1430 1270">HCD-14</td> </tr> </tbody> </table> <p data-bbox="405 1290 1430 1339">*Clones OKT3/HIT-3α resulted in low signal</p> <p data-bbox="405 1375 1430 1447">NOTE: Antibody clones not listed above need to be tested independently to determine compatibility.</p> <p data-bbox="405 1456 1430 1487">For Research Use Only. Not for use in diagnostic or therapeutic procedures.</p>				Biomarker	Tested Antibody Clone			CD45	2D1	MEM-28	HI30	CD3*	SK7	UCHT1	OKT3/HIT-3α*	CD4	SK3	RPA-T4	OKT4	CD8	SK1	RPA-T8	HIT-8α	CD19	SJ25C1	HIB19	4G7	CD16	B73.1	CB16	3G8	CD56	NCAM16.2	MEM-188	MY-31	CD14	M5E2	61D3	HCD-14
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<p>Materials</p>	<p>This product is lyophilized for stability and ease of use. Each vial contains 2.5x10⁵ cell mimics.</p>																																							
<p>Handling and Safety</p>	<p>No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at slingshotbio.com.</p>																																							
<p>Storage</p>	<p>Store lyophilized products at -20 °C upon receipt.</p>																																							
<p>Expiration</p>	<p>36 months from the date of manufacturing when stored at -20 °C. Use the entire vial immediately upon reconstitution of lyophilized product.</p>																																							
<p>Instructions for Use</p>	<p>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.</p> <p data-bbox="405 2007 1430 2038"><u>Procedure A : Running in a FACS tube</u></p>																																							

Sample Preparation:

1. Remove the vial of TBNK Mimic™ 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 TBNK Mimic™ 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 TBNK Mimic™ 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:

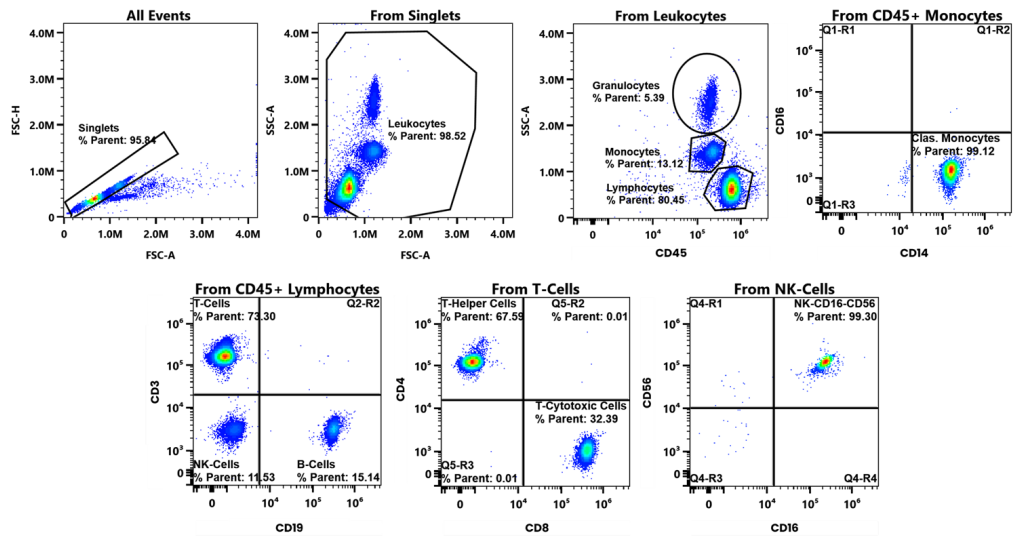
1. Prepare your preferred staining antibody cocktail in flow staining buffer and then add the mixed solution to the appropriate well of the 96 well V bottom plate. Mix well by vortexing.
2. Prepare your preferred staining antibody cocktail in flow staining buffer such that a total of 100 µL of cocktail 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](https://www.slingshotbio.com) for unmixing or compensating your TBNK Mimic™ flow cytometry data. See product selection at [slingshotbio.com](https://www.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 TBNK Mimic™.



Technical Support

For technical support regarding this product please contact: support@slingshotbio.com