1. Technical Data Sheet

Summary	T Cell Activation/Exhaustion Mimic™ (SSB-33-A) consists of lymphocyte-like cell mimics that represent different stages of the activation and exhaustion spectrum in CD8+ T cells. In addition, this product includes a synthetic "dead" population capable of binding to either amine-reactive or DNA-intercalating viability dyes. Non-dead populations can be gated with CD45, CD3, and CD8, and can be further gated for CD69, CD25, PD-1, LAG3, TIM3, TIGIT and CTLA4 to serve as positive reference controls for activation and exhaustion markers. Robust detection of transient activation markers reduces the need for *in vitro* activation controls. Reliable presence of exhaustion markers rarely found in healthy donor PBMC enables confidence in panel and template development. These synthetic mimics are intended for use as stable, reproducible controls to provide a baseline comparison for longitudinal studies.

Application

The product is intended for use as a flow cytometry gating control and antibody qualification for the detection of activation and exhaustion markers CD69, CD25, PD-1, LAG3, TIM3, TIGIT, and CTLA4 for populations defined by CD45, CD3, and CD8, along with the option of viability staining with DNA-binding or amine-reactive viability dyes.

This product is intended to provide positive signals for specified biomarkers, with established compatibility with the antibody clones listed in the table below:

Biomarker	Compatible clones or dyes
CD45	2D1, HI30, J33
CD3	OKT3, UCHT1, SK7, REA613
CD8	SK1, REA734,HIT8a, RPA-T8
CD69	FN50, CH/4, L78, REA824
CD25	Blocking: 2A3,BC96, Functional: M-A251, CD25-4E3
PD-1	EH12.2H7, EH12.1, MIH4, J105
LAG3	T47-530, 11C3C65, 3DS5223H
TIM3	7D3, F38-2E2,344823
TIGIT	A15153G,TgMab-2,741182, MBSA43
CTLA4	BNI3, 14D3, 20A-A3
Viability: DNA	7-AAD, DRAQ7, PI, Vybrant Dye Cycle Violet
Viability: amine	e780, Live/Dead Blue, Zombie Red

Note: Antibody clones and viability reagents not listed above need to be tested independently, and may require titration for optimal staining.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Materials

This product is lyophilized for stability and ease of use. Each vial contains $5x10^5$ mimics.

Handling and Safety

No special handling or safety precautions are necessary.

Storage

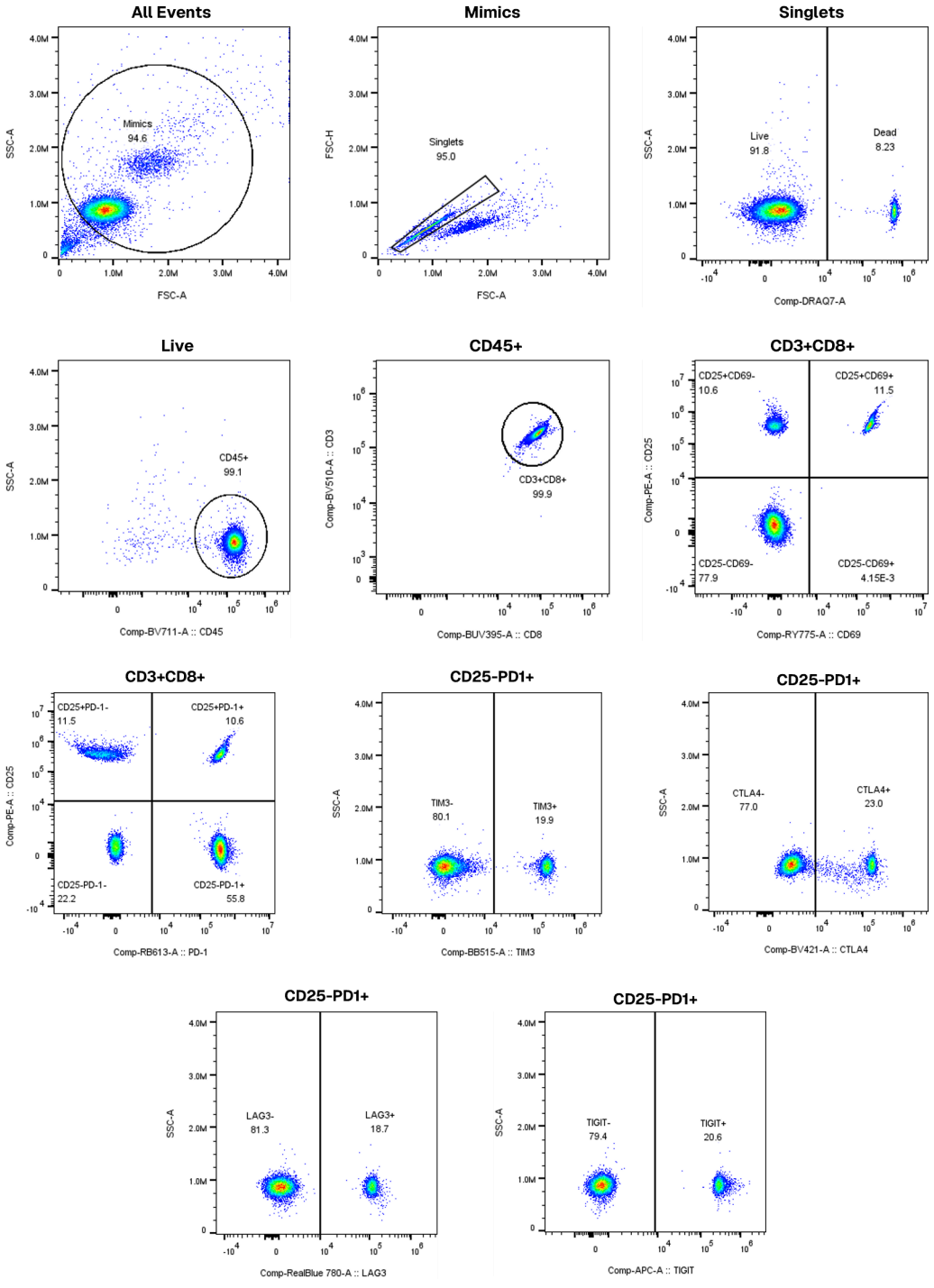
Store lyophilized products at -20°C upon receipt.

Expiration	24 months from the date of manufacturing when stored at -20°C. Use the entire vial immediately upon reconstitution of lyophilized product.
Instructions for Use: Sample Preparation	1. Remove the vial of T Cell Exhaustion Mimic™ from -20 °C and let it sit at room temperature for 10-15 minutes. 2. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial. 3. Add 1000 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 2000 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 (see steps below).

Instructions for Use: Staining with DNA-binding viability dyes	1. Prepare your preferred staining antibody cocktail in flow staining buffer and add appropriate volume to washed cell mimics from protocol A. * 2. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 15 +/- 3 min at RT or 30 +/-5 min at 4 °C . 3. Add 1000 µL of flow staining buffer. 4. Centrifuge at 600 x g for 5 minutes. 5. Decant the supernatant being careful not to disturb the pellet. 6. Repeat the wash step one additional time. 7. Add 100 µL of flow staining buffer. 8. Add desired volume of DNA-binding viability dye and mix thoroughly by pipetting or vortex mixing. 9. Incubate in the dark for at least 10-15 mins. 10. Add additional volume of buffer if desired. 11. The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as your biological samples. For best results, we recommend acquiring your sample within 30 minutes of adding viability dye using low or medium flow rate. * If desired, mimics may be pre-treated with Fc block for 10-15 minutes at RT prior to initiating antibody cocktail staining.

Staining protocol B Instructions for Use: Staining with amine-reactive/ fixable viability dyes	1. Add 2000 µL of PBS to washed cell mimics from protocol A. 2. Centrifuge at 600 x g for 5 minutes. 3. Decant the supernatant being careful not to disturb the pellet. 4. Repeat the wash step one additional time. 5. Add fixable live/dead dye diluted in PBS at desired volume and mix thoroughly. 6. Incubate at room temperature in the dark (or according to manufacturer's instructions). 7. Wash with 2mL flow staining buffer. 8. Centrifuge at 600 x g for 5 minutes. 9. Decant the supernatant being careful not to disturb the cell pellet. 10. Repeat the wash step one additional time. 11. Prepare your preferred staining antibody cocktail in flow staining buffer and add the appropriate volume to washed cell mimics.* 12. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 15 +/- 3 min at RT or 30 +/-5 min at 4 °C .. 13. Add 1000 µL of flow staining buffer. 14. Centrifuge at 600 x g for 5 minutes. 15. Decant the supernatant being careful not to disturb the cell pellet. 16. Repeat the wash step one additional time. 17. Resuspend in 200 µL of flow staining buffer and vortex 2-3 seconds. 18. The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as your biological samples. For best results, we recommend acquiring your sample using low or medium flow rate. * If desired, mimics may be pre-treated with Fc block for 10-15 minutes at RT prior to initiating antibody cocktail staining.

Sample Data using DNA viability dye	**Figure 1. Scatter plot and gating.** The following shows a representative scatter plot of the gating strategy used for detection of the T Cell Activation/Exhaustion Mimic™. Text on the top of the plot indicates the parent gate.



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