# 1. Technical Data Sheet

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| **Summary** | T Cell Memory Mimic™ is a lyophilized cell mimic control designed for use as a gating control for memory T cell populations. This product contains mimics for naive, stem central memory, central memory, effector memory, and terminal effector populations using CD45RA or CD45RO, CCR7 (CD197), and FAS (CD95) for both CD4+ and CD8+ T cell lineages at frequencies consistent with reported frequencies in healthy adults. This product is comprised of mimics with scatter coordinates that closely mimic lymphocyte populations and contains a population of mimics that stain with viability components. These synthetic mimics are intended for use as a renewable, stable alternative for FMO analysis and immunological assay standardization for memory T cells. |
| **Application** | The product is intended for use as a flow cytometry gating control and antibody qualification for the detection of memory populations using CD45RA or CD45RO, CCR7 (CD197), and FAS (CD95) for populations defined by CD3 with CD4 or CD8, along with the option of viability staining with DNA-binding or amine-reactive viability dyes.<br><br>This product is intended to provide positive signals for specified biomarkers, with established compatibility with antibody clones listed in the table below: |

| Biomarker | Compatible clones or dyes |
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| CD3 | SK7, UCHT1, REA613,OKT3, HIT3a |
| CD4 | SK3, REA623, OKT4, RPA-T4 |
| CD8 | SK1, REA734, OKT8, RPA-T8 |
| CD45RA | HI100, L48, 5H9 |
| CD45RO | UCHL1, REA611, S19021B |
| CCR7 | 150503, G043H7, 2L1-A |
| FAS | DX2, REA738 |
| DNA (dead) | Hoescht33342, 7-AAD, DRAQ-7 |
| Amine (dead) | Zombie UV, Zombie Red and Zombie NIR, e780, Viobility405 |

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

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| | **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 Memory Mimic™ from -20 °C and let it sit at room temperature for 10-15 minutes.<br>2. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial.<br>3. Add 1000 µL of staining buffer to the vial.<br>4. Note: Avoid contacting or disturbing the pellet until it has been hydrated (~1 minute).<br>5. Gently pipette up and down ten times to resuspend the cell mimics. Avoid introducing bubbles. Transfer the contents to a 5mL FACS tube.<br>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.<br>7. Centrifuge at 600 x g for 5 minutes.<br>8. Decant the supernatant being careful not to disturb the cell pellet.<br>9. The cell mimics are ready for staining. |
| **Staining Protocol A Instructions for Use (with DNA-binding viability dye)** | 1. Prepare your preferred staining antibody cocktail in flow staining buffer and add to washed cell mimics.*<br>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 .<br>3. Add 1000 µL of flow staining buffer.<br>4. Centrifuge at 600 x g for 5 minutes.<br>5. Decant the supernatant being careful not to disturb the cell pellet. |

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| | 6. Repeat the wash step one additional time.<br>7. Add 100 µL of flow staining buffer.<br>8. Add desired volume of DNA-binding viability dye and mix thoroughly by pipetting or vortex mixing.<br>9. Incubate in the dark for at least 10-15 mins.<br>10. Add additional buffer if desired.<br><br>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.<br><br>*If desired, mimics may be pre-treated with Fc block for 10-15 minutes at RT prior to initiating antibody cocktail staining.<br>For optimal resolution of biomarkers, we additionally recommend staining with CCR7 antibody first for 15-30 minutes, followed by addition of regular antibody cocktail. |
| **Staining protocol B Instructions for Use (with amine-reactive/ fixable viability dye)** | 1. Add 2000 µL of PBS.<br>2. Centrifuge at 600 x g for 5 minutes.<br>3. Decant the supernatant being careful not to disturb the cell pellet.<br>4. Repeat the wash step one additional time.<br>5. Add fixable live/dead dye diluted in PBS and mix thoroughly.<br>6. Incubate at room temperature in the dark (or according to manufacturer's instructions).<br>7. Wash with 2mL flow staining buffer.<br>8. Centrifuge at 600 x g for 5 minutes.<br>9. Decant the supernatant being careful not to disturb the cell pellet.<br>10. Repeat the wash step one additional time.<br>11. Prepare your preferred staining antibody cocktail in flow staining buffer and add to washed cell mimics.*<br>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 or 30 +/- 5 min at 4 °C .<br>13. Add 1000 µL of flow staining buffer. |

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| | 14. Centrifuge at 600 x g for 5 minutes.<br>15. Decant the supernatant being careful not to disturb the cell pellet.<br>16. Repeat the wash step one additional time.<br>17. Resuspend in 200 µL of flow staining buffer and vortex 2-3 seconds. Sample is ready for acquisition.<br><br>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.<br><br>*If desired, mimics may be pre-treated with Fc block for 10-15 minutes at RT prior to initiating antibody cocktail staining.<br>For optimal resolution of biomarkers, we additionally recommend staining with CCR7 antibody first for 15-30 minutes, followed by addition of regular antibody cocktail. |
| **QC Data** | Sample data using protocol A |

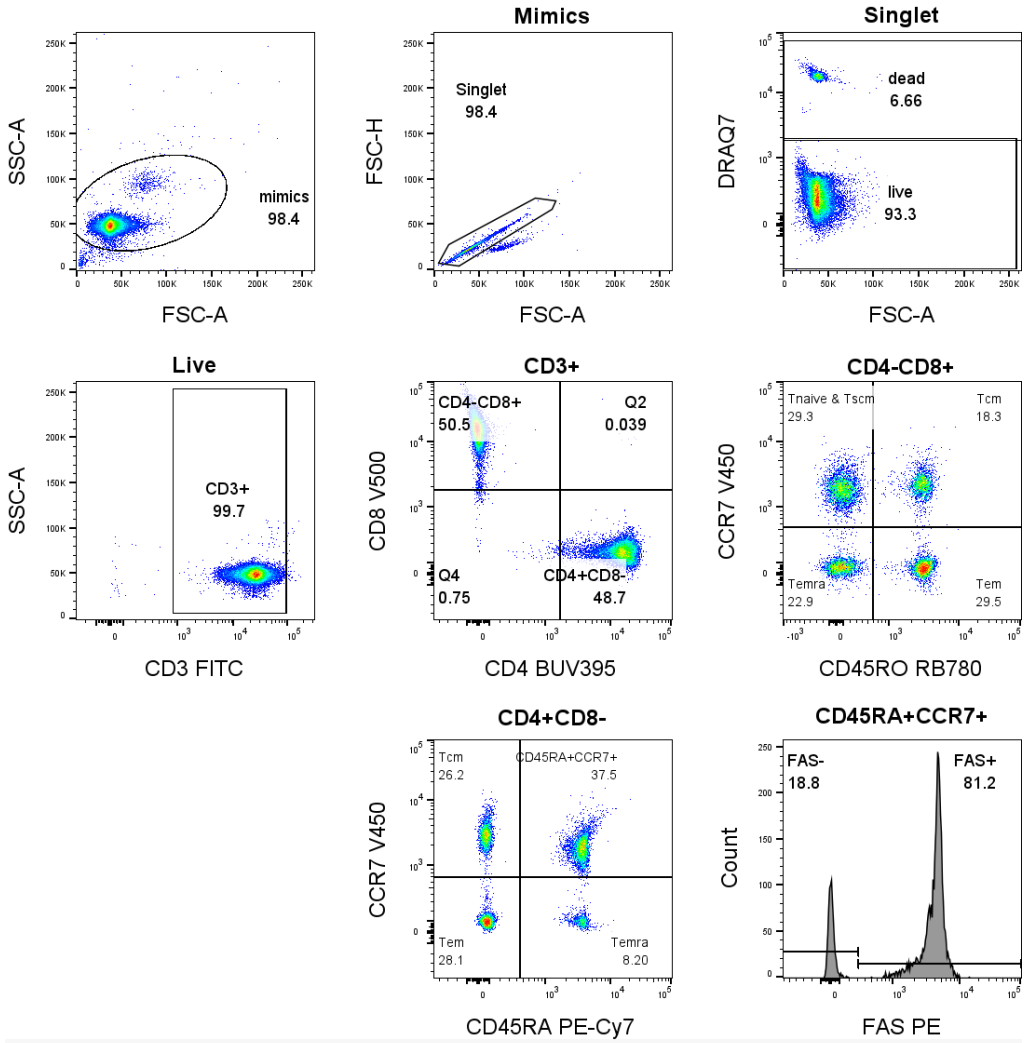


**Figure 1.** T Cell Memory MimicTM was stained with CD4 BUV395, CCR7 V450, CD8 V500, CD3 FITC, CD45RO RB780, FAS PE, CD45RA PE-Cy7, DRAQ 7 and acquired on Cytek Aurora. Compensation matrix generated using SpectraComp Flex XT and ViaComp and applied in FlowJo.

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