# T Cell Health Annexin V Assay (Non-Lyse) INSTRUCTIONS FOR ANALYSIS

## INTENDED USE

These instructions are intended for analyzing data obtained with the Accellix T Cell Health Annexin V Assay (Non-Lyse) run on the Accellix Platform. The data can be analyzed with FlowJo™ Software (BD Biosciences) using the **gating template** Accellix T Cell Health Annexin V Assay (NL) Gating Template.wsp provided with the assay or accessible in the Customer Resources section of accellix.com. For additional FlowJo Software instructions, refer to Accellix Data Analysis Guide for FlowJo Software, also available in the Customer Resources section on accellix.com. Equivalent software can also be used for data analysis.

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## ANALYSIS PROTOCOL

1. Retrieve the **events.fcs** files for analysis in FlowJo Software or an equivalent analysis software.
2. Download the **gating template** and use it to pull each FMO/FS pair into the workspace for analysis.
3. See the recommended gating strategy in **figure 1**.
    a. Use this gating strategy to identify the early-stage apoptosis cells (Q3: Annexin-V+, Viability-) and necrotic cells (Q2: Annexin-V+, Viability+).
    b. Identify T cell subsets, including viable CD45+ leukocytes (viable CD45+), CD3+ T cells, CD4+ T helper cells, and CD8+ cytotoxic T cells.
4. Example FlowJo plots for the T Cell Health Annexin V (NL) assay using the recommended gating strategy are provided in **figure 2**. Use a conservative cells (tight) gate to identify the cells in the CD45/FSC plot. Be sure to exclude debris (low forward scatter events).

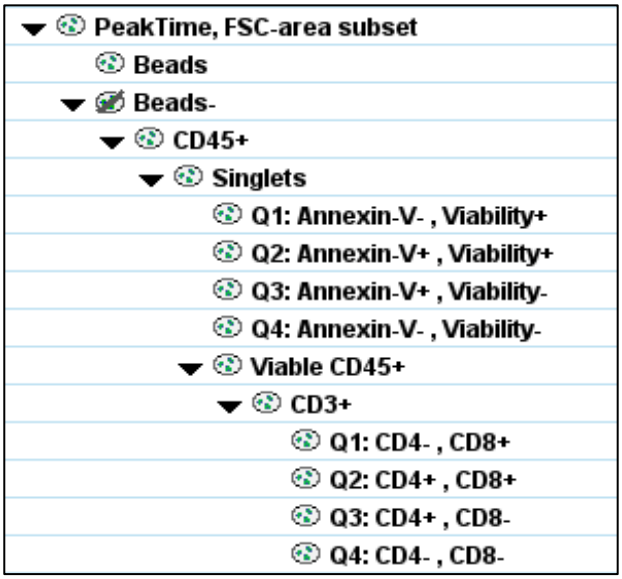


**Figure 1.** Proposed gating strategy for Accellix T Cell Health Annexin V (Non-Lyse) Assay.

5. Apply the FMO for the Viability/Annexin V plot to each FS in the pair.
    a. Use a 5% contour setting to place the vertical and horizontal gates for the Viability/Annexin V plots.
    b. Place the horizontal gate at the density minimum in the FMO (**figure 3)**.
    c. Apply the FMO (full stain minus one) gates to the FS (full stain) run (**figure 3**).
6. Place the gates at the density minimum for the Viability/FSC plot (**figure 4**).

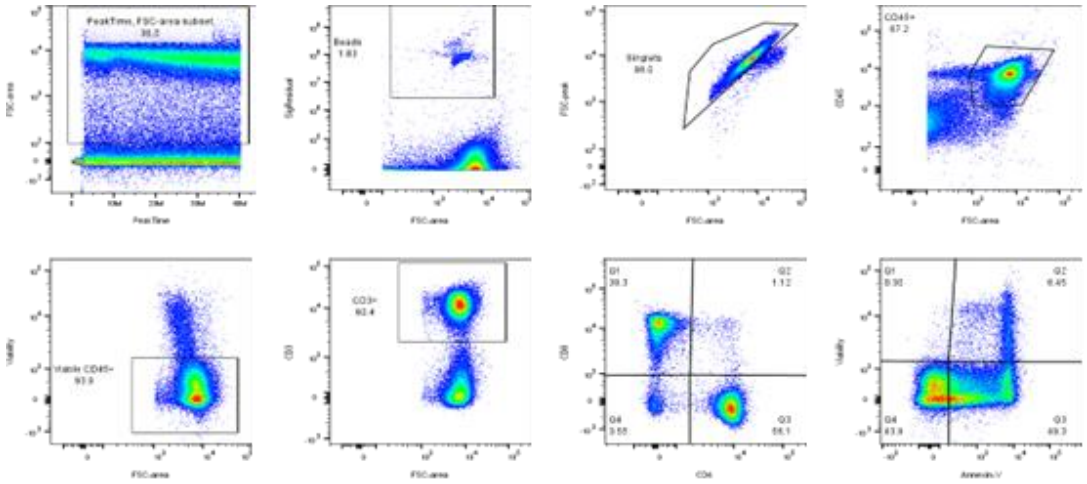

**Figure 2.** Cryopreserved PBMCs thawed and stained with the T Cell Health Annexin (V) NL assay

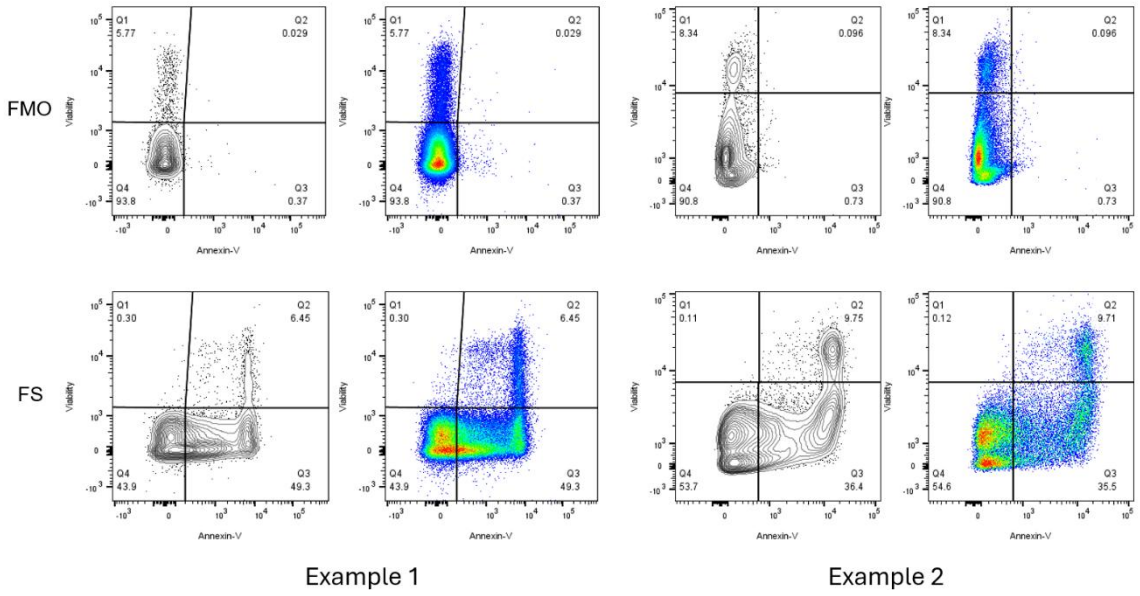


**Figure 3.** Examples of FMO and FS gate placement.

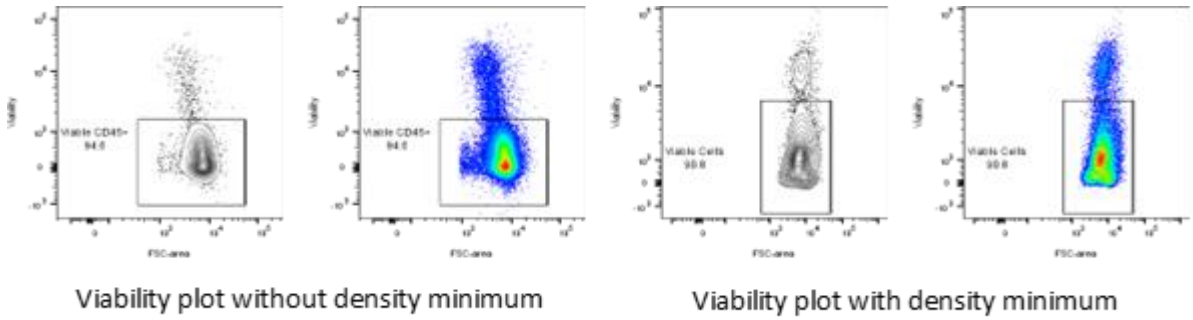


**Figure 4.** Examples of viability plot gate placement.