



Antibody-Dependent Cell-Mediated Cytotoxicity
and T Cell Proliferation Assays by Flow Cytometry

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Custom BiologicsTM has the facilities and expertise to develop and implement flow cytometry-based antibody-dependent cell-mediated cytotoxicity (ADCC) and T cell proliferation assays as described below.

T cell proliferation flow cytometry assay.

Custom BiologicsTM will employ a non-radioactive flow cytometry method capable of quantifying T cell proliferation by flow cytometry. Below is a summary of the assay procedure:

1. Target cells are cultured in a 96 well plate.
2. Peripheral blood mononuclear cells (PBMCs) are isolated from anti-coagulated whole blood by Ficoll and a membrane permeable fluorescent dye, CFSE is employed to stain PBMC's green. CFSE incorporates into the cytoplasm.
3. Target cells, effector cells and defined concentrations of test article are co-incubated for period of time to be determined.
4. Following this incubation period, cells are detached from the plate, stained for anti-CD3 (T cell marker) and analyzed by flow cytometry, gated on CD3 cells and the level of CFSE cytoplasmic "dilution" is assessed. One cell division dilutes CFSE by 50%, two cell divisions, by another 50% etc (see Figure 1 for example).
5. The extent of proliferation can be reported as a percent of divided cells (cells with less than 100% maximal CFSE intensity).

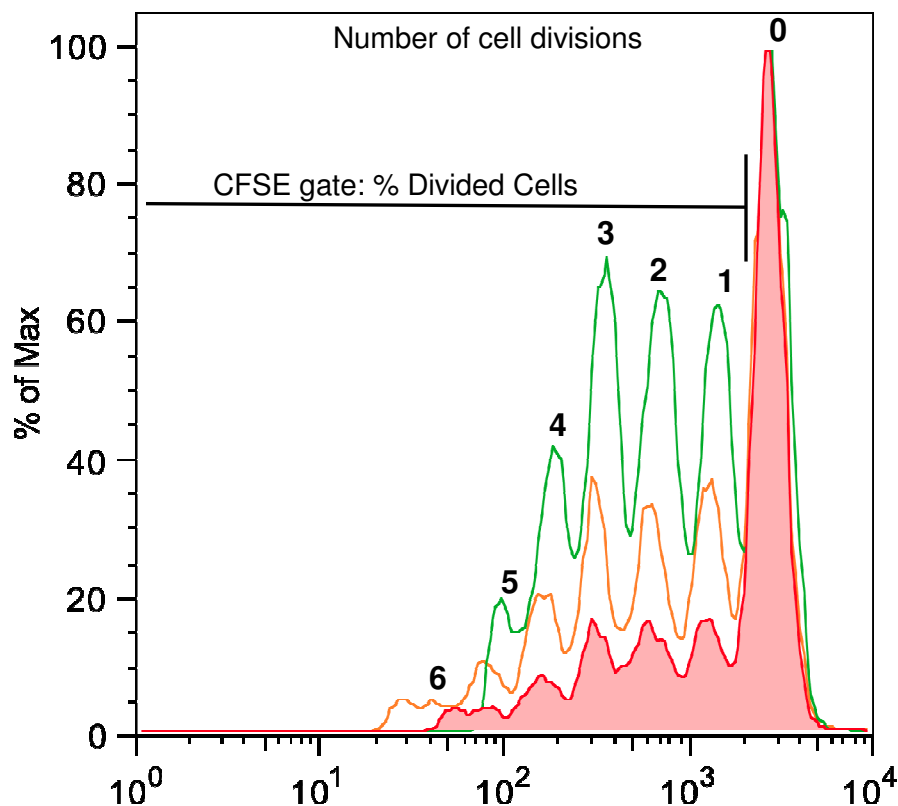


Figure 1: Example of flow cytometry analysis of T cell proliferation using CFSE. Each cell division dilutes cytoplasmic CFSE by $\frac{1}{2}$. Percent divided cells calculated as gated above. This histogram is representative of three different treatments.

ADCC flow cytometry assay.

Custom BiologicsTM will employ a non-radioactive flow cytometry method capable of quantifying T cell proliferation by flow cytometry. Below is a summary of the assay procedure:

1. Target cells are stained with CFSE in culture to differentiate the target cells from PBMC effector cells.
2. PBM cells are isolated from anti-coagulated whole blood by Ficoll.
3. Target cells, effector cells and defined concentrations of test article are co-incubated for period of time to be determined.
4. Following this incubation period, the dye 7AAD is added to the mix. This dye, known as the “live/dead” stain only enters membrane compromised cells and binds DNA thus differentiating between live and dead (permeabilized) cells.
5. Flow cytometry is then employed to gate on the target cells and measure 7AAD negative vs. positive cells (Figure 2).
6. The extent of cytotoxicity can be reported as a percent of killed cells (7ADD positive) / total cells (7ADD positive and negative).

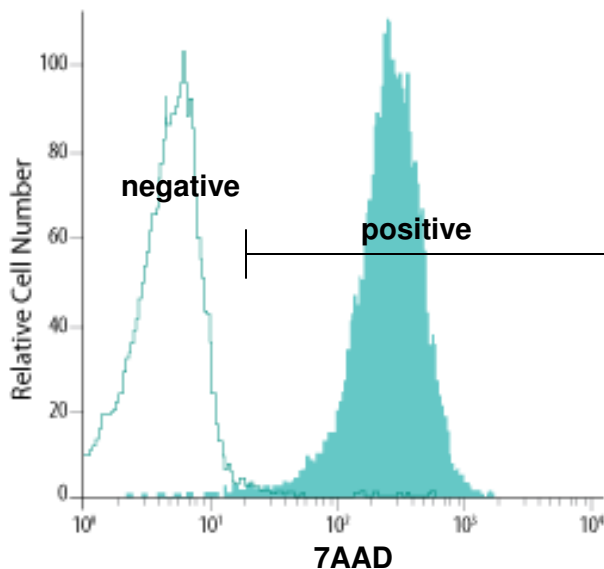


Figure 2: Example of 7AAD staining and flow cytometry analysis of cell death.