

# CASE STUDY:

## Validating Immune Cell-Mediated Cytotoxicity Assays for Research Stage Drug Discovery



### Introduction: Meeting Industry Standards with Flow Cytometry-Based Cytotoxicity Assays

At Mosaic, we specialize in executing high-quality immune cell-mediated cytotoxicity assays to support early-stage drug development. Our flow cytometry-based approach enables rapid and precise assessment of T-cell mediated cytotoxicity, Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cellular Phagocytosis (ADCP), and Complement-Dependent Cytotoxicity (CDC). While these assays are widely utilized in immuno-oncology and therapeutic antibody development, successful execution requires technical expertise, optimized protocols, and rigorous validation to ensure consistency and reliability.

This case study highlights our ability to implement and validate these methods to meet our partners' specific needs while demonstrating critical functional readouts.

#### Challenge: Ensuring Functionally Relevant Cytotoxicity Data with Custom Assay Design

A biotechnology partner developing a novel immunotherapy approached us to validate their compound's ability to elicit targeted immune cell-mediated cytotoxicity and distinguish mechanism of action. Their key requirements included:

- High sensitivity and specificity in flow cytometry-based cytotoxicity assays
- Mechanistic insights into immune effector activation and functional modulation
- Custom assay adaptation to align with their therapeutic target and cell models

Given the complexity of immune cell interactions and donor variability, ensuring assay robustness and functional relevance was critical for their go/no-go decision-making.

#### Solution: Custom Assay Development and Mechanistic Readouts

Mosaic's Cell Based Assay team optimized and validated industry accepted flow cytometry-based cytotoxicity assays while incorporating key mechanistic readouts to enhance functional characterization and assay flexibility.

- T-cell Mediated Cytotoxicity: Simultaneous measurement of T-cell activation via CD25 and CD69 expression, along with target cell death.
- ADCC & ADCP: Validation of effector function modulation using:
  - Afucosylated antibody (enhanced effector function)
  - Fc-silenced antibody (eliminated effector function)
- CDC: Evaluation of complement activation-induced target cell lysis

# Assay Customization & Flexibility

Recognizing the unique needs of each partner, Mosaic offers tailored assay development to ensure optimal performance:

- **Adaptability to Different Therapeutic Formats:** Our platform accommodates monoclonal antibodies, bispecifics, and engineered immune cell therapies, among others.
- **Customizable Effector and Target Cell Options:** Our assays can be tailored to meet any specific project needs. We optimize effector cell selection—whether PBMCs, purified primary T cells, NK cells, macrophages, or engineered effectors—and work with partner-specified tumor or primary cell lines to best replicate the intended therapeutic setting
- **Optimization of Assay Conditions:** Adjustments in effector-to-target ratios, cytokine supplementation, and incubation times are fine-tuned to maximize assay sensitivity and dynamic range.
- **Multiplexed Readouts:** Combining cytotoxicity measurements with activation markers, cytokine release profiling, and phagocytosis markers to provide mechanistic insights beyond standard endpoint measurements.

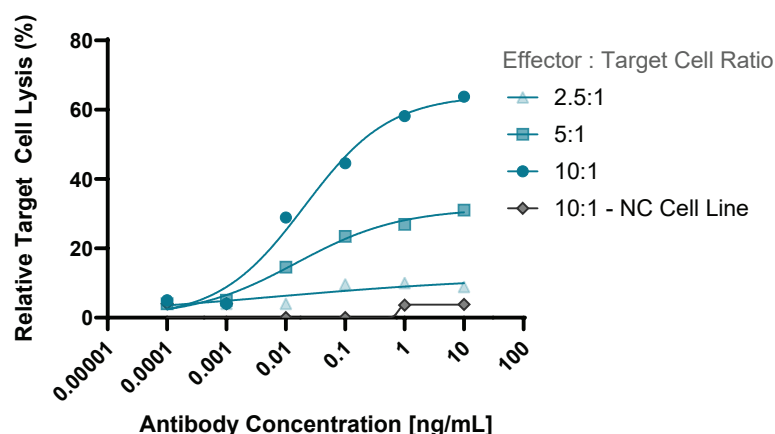


## Outcomes

### T cell mediated Cytotoxicity

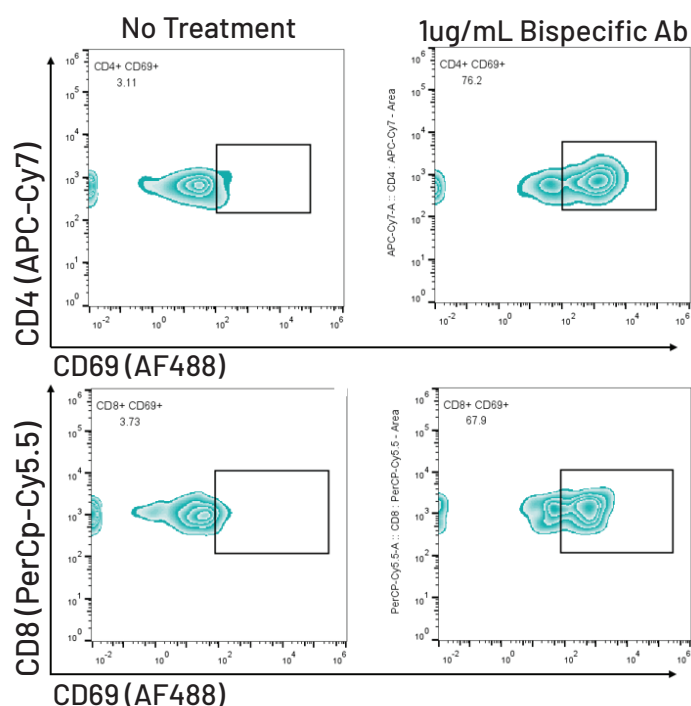
- Utilization of a **bispecific antibody** to activate T cells and drive targeted killing, ensuring effective engagement with target cells.
- Simultaneous measurement of **T-cell activation markers (CD25 and/or CD69 expression)** alongside cytotoxicity, providing insights into both T-cell engagement and killing efficiency.
- Optimization of effector-to-target (E:T) ratios and cytokine stimulation conditions to ensure maximal response detection.
- Customization to accommodate different **T-cell subsets (e.g., CD8+, CD4+)** based on the therapeutic target.

#### Target Cell Killing



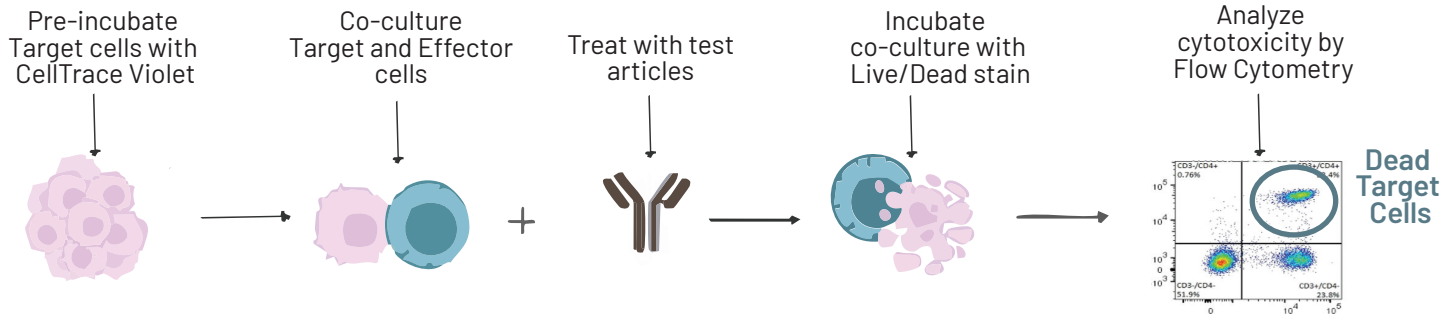
Pre-labeled CD20+ or CD20 negative control cells, were co-cultured with purified primary T cells isolated from whole human PBMCs and treated with varying concentrations of a clinically validated therapeutic antibody for 48 hours, followed by analysis of dead labeled (target) cells, and simultaneously, CD69 expression on live unlabeled (effector) cells by FACS.

#### T Cell Activation

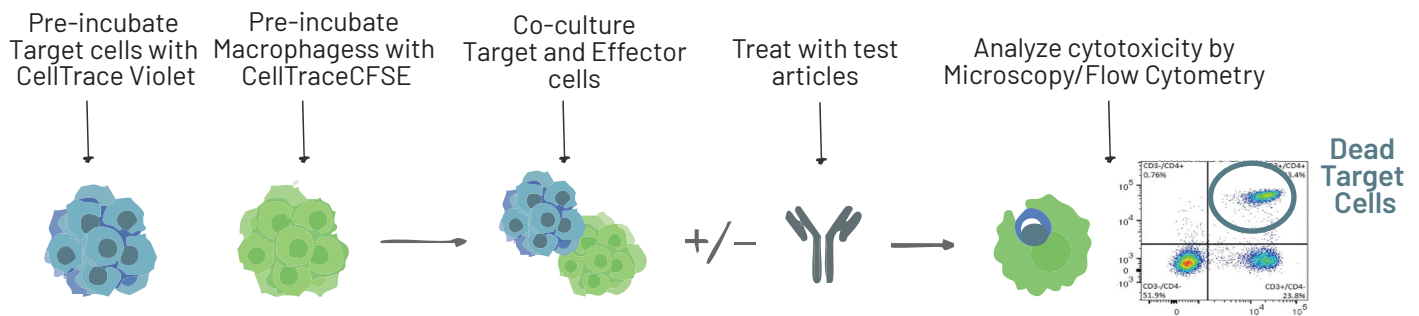


# Methods Highlight

## Antibody Directed Cell Cytotoxicity



## Antibody Directed Cell Phagocytosis

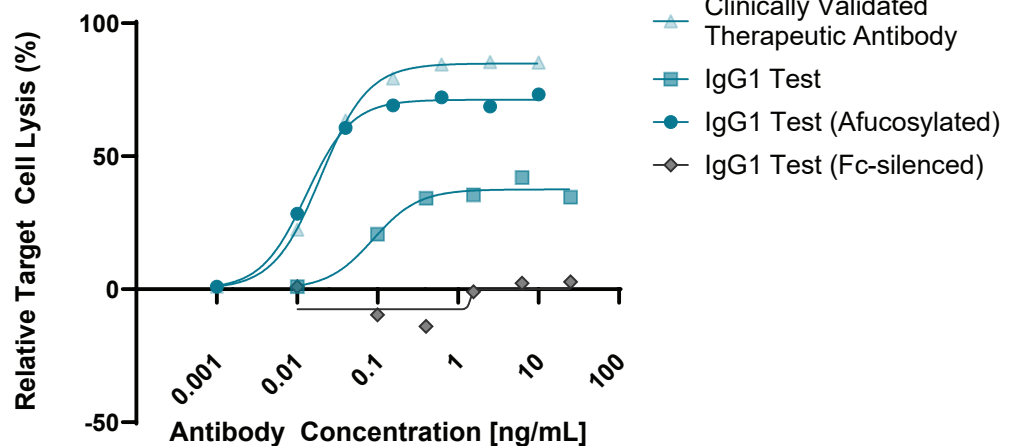


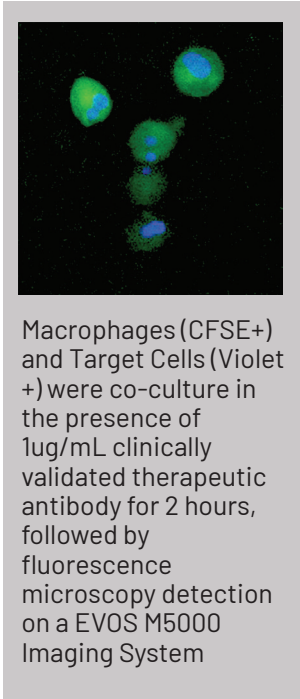
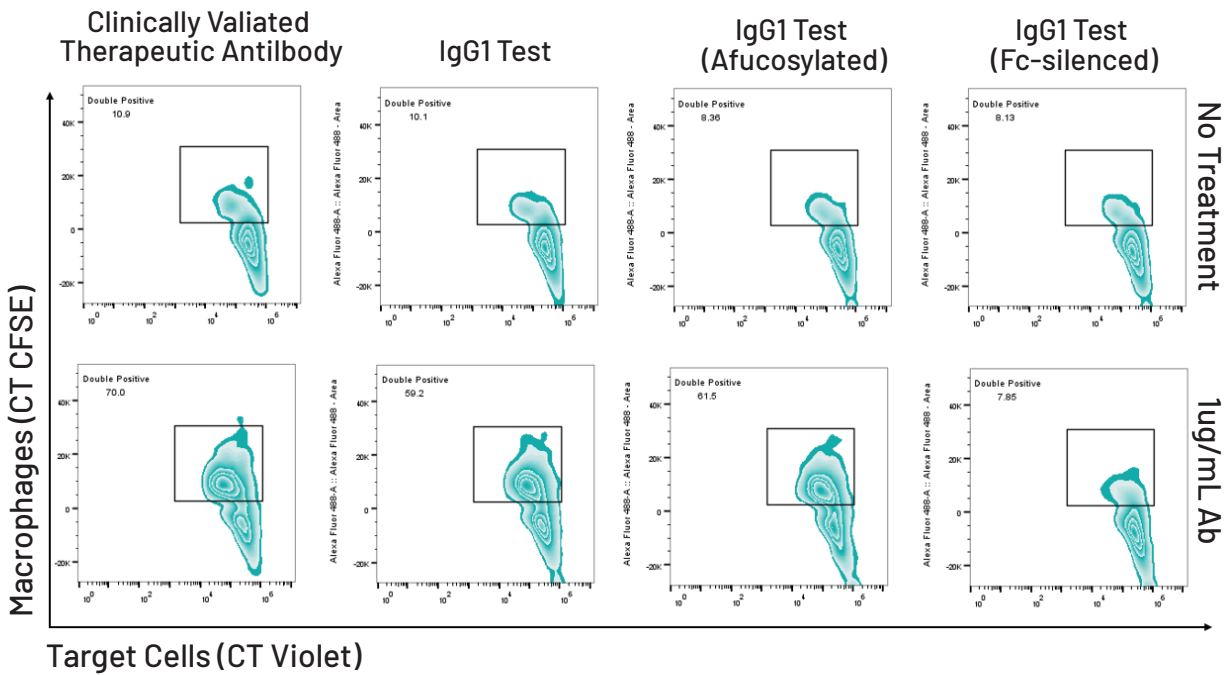
## ADCC & ADCP Functional Validation

- Assay optimization using an **afucosylated antibody**, which substantially increased effector function, demonstrating enhanced FcγRIIIa-mediated ADCC.
- Functional confirmation with an **Fc-silenced antibody**, which eliminated effector function, validating assay specificity and mechanistic accuracy.
- High-resolution flow cytometry tracking of macrophage-mediated phagocytosis in ADCP assays.
- Microscopy-based visualization of ADCP**, providing direct evidence of target engulfment and effector function.
- Flexibility to incorporate different antibody formats and effector cell types based on partner requirements.

## ADCC

Pre-labeled (Cell Trace Violet) target cells were co-cultured with activated CD16a+ NK cells and treated with varying concentrations of test articles for 4 hours, followed by analysis of dead labeled (target cells) by FACS.



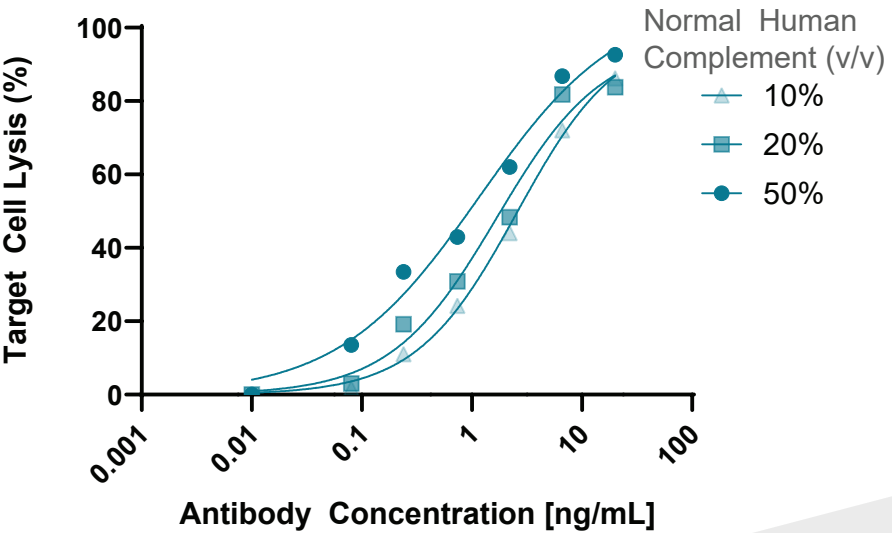


Pre-labeled (Cell Trace Violet) target cells and (Cell Trace CFSE) primary Macrophages were co-cultured and treated with varying concentrations of test articles for 2 hours. Macrophages were derived from primary monocytes, isolated from whole human PBMCs and differentiated for 7 days in the presence of mCSF and IL-10. Phagocytosis was determined via FACS, gating specifically for macrophages (CFSE-labeled) with target cell (Cell Trace Violet-labeled) engulfment (Double Positive).

CDC Assay Optimization

- Complement-mediated killing was efficiency optimized by fine-tuning **serum source selection, incubation conditions, and controls/standardization strategies.**
- Ability to assess CDC under varied conditions, including different complement sources or modified antibody structures to evaluate therapeutic modifications.

CDC



Target cells were pre-treated with varying concentrations of clinically validated therapeutic antibody followed by introduction of Normal Human Serum Complement at 10, 20, or 50% cell culture media (v/v) for 1 hour, followed by detection of dead (Zombie NIR +) cells via FACS analysis.

# Why This Matters:

## The Role of Cytotoxicity Assays in Drug Development

Immune cell-mediated cytotoxicity assays play a critical role in evaluating the efficacy and mechanism of action of immunotherapies, monoclonal antibodies, and other targeted biologics. These assays help drug developers:

- Assess whether a candidate therapy **effectively engages and activates** immune effector cells.
- Differentiate between therapeutic candidates based on **potency and specificity**.
- Generate mechanistic insights that inform **lead optimization and clinical translation**.
- **Support regulatory filings** by providing robust preclinical data on immune engagement.

Given the complexity of immune interactions, high-quality cytotoxicity assays must be carefully designed and validated to yield meaningful data. Mosaic's expertise in assay customization ensures that our partners receive actionable insights tailored to their therapeutic programs.



## Conclusion:

### A Trusted Partner for Cytotoxicity Assay Execution and Functional Validation

- This study underscores Mosaic's ability to execute and validate immune cell-mediated cytotoxicity assays with precision while providing **critical functional insights**. Our expertise extends beyond standardized protocols—we **tailor our assays to meet unique therapeutic requirements**, ensuring relevant and actionable data.
- Whether optimizing existing assays, integrating **mechanistic readouts**, or designing **fully customized assays**, Mosaic delivers technical rigor and reliable execution to accelerate immunotherapy development.

**Let's discuss how we can support your next study. Contact us today to explore tailored assay solutions.**



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