

Cytotoxicity Assay

The Cytotoxicity Assay in the Opto™ Cell Therapy Development 1.0 workflow enables kinetic analysis of killing activity from single T cells, followed by live cell recovery for genomic analysis. This assay avoids common problems associated with traditional killing assays, which measure average target cell lysis at fixed time points, obscuring kinetic details and ignoring the heterogeneity present in T cell subsets.

CYTOTOXICITY ASSAY OVERVIEW

T cell-mediated tumor death relies on complicated cell-cell interactions and multiple complex mechanisms including the perforin/granzyme pathway, cytokine-dependent killing, and interactions between Fas (T cell) and Fas ligand (tumor cell). To understand and define the heterogeneity of such interactions, single T cells are penned and co-cultured with fluorescently labeled target cells (**Figure 1A**). Killing activity is indicated by caspase-3 activation in target cells (**Figure 1B and 1C**). Time-lapse imaging is used to assess killing kinetics (**Figure 2**). Individual T cells of

interest can then be exported for downstream analysis, such as sequencing.

Performing this assay on a heterogenous population of single T cells provides a window into the cellular and molecular mechanisms underlying disparate cell killing behaviors, such as multiple T cells killing multiple targets and single T cells engaging in serial killing. This has direct relevance to the development of cell therapies where the ideal T cell therapeutic dose should be tailored to mediate the rapid destruction of multiple tumor cells by a small number of T cells.

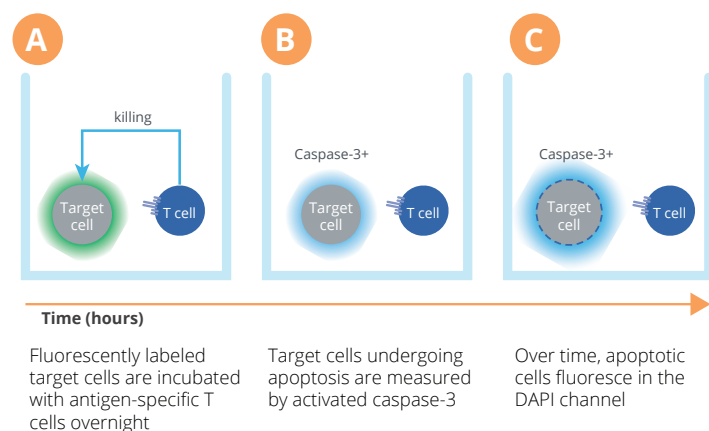


Figure 1. Assay set up to measure T cell killing activity over time.

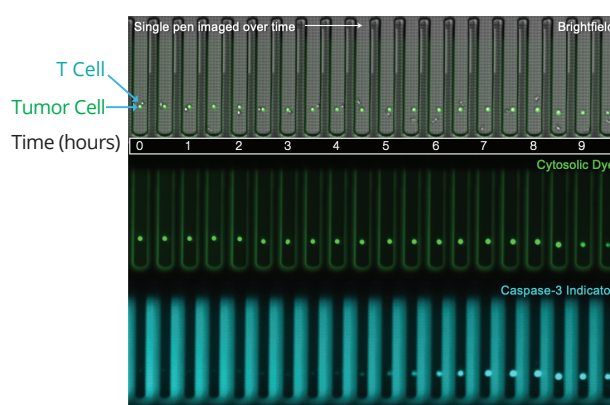


Figure 2. Single-cell readout of killing activity over time.

Opto™ Cell Therapy Development 1.0 Workflow

OPTO CELL THERAPY DEVELOPMENT 1.0 WORKFLOW

The Opto Cell Therapy Development 1.0 workflow is a collection of software capabilities, reagents and protocols that allow scientists to define and test the function of individual T cells. Cytotoxicity assays are performed on the Beacon® and Lightning™ systems using standard reagents in addition to time-lapse imaging and data analysis capabilities included in the Cell Analysis Suite software.

ORDERING INFORMATION

PART NUMBER	DESCRIPTION
110-08004	Beacon® Optofluidic System
110-02407	Lightning™ Optofluidic System
750-01000	Opto™ T Cell IFN γ Assay Kit

FOR MORE INFORMATION, VISIT
berkeleylights.com/Tcells

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