DATASHEET

Single Cell Cytokine Assays

Cytokine assays in the Opto™ Cell Therapy Development 1.0 workflow allow simultaneous functional interrogation of thousands of individual T cells as they interact with antigen-presenting cells, granting insight into the heterogenous mechanisms that regulate cytokine secretion. The automated assays also allow live recovery of individual clones for downstream analysis. Cytokine assays and cell surface markers can be customized.

FUNCTIONAL ASSAY OVERVIEW

To demonstrate the principle workflow of Berkeley Lights' cytokine assays, the Opto™ T Cell IFNγ Assay is used as an example. IFNγ capture beads (Figure 1A) and single antigen-specific CD8+ T cells (Figure 1B) are precisely loaded into thousands of nanoliter-size NanoPen™ chambers across an OptoSelect™ microfluidic chip on the Lightning™ system using opto-electropositioning (OEP™). Target tumor cells are loaded next (Figure 1C), allowing precise timing of cell-cell interactions. After overnight incubation (Figure 1D), fluorescently labeled antibodies are flowed through the chip to detect IFNγ captured on the capture bead, the T cell lineage marker CD8, and CD137, a surface marker of T cell activation. Antigen-specific T cell activation is assessed by quantifying fluorescence signal in three of four fluorescence channels (Figure 1E). By evaluating data using Berkeley Lights' Assay Analyzer software, cells of interest are selected for optional live-cell export and recovery.

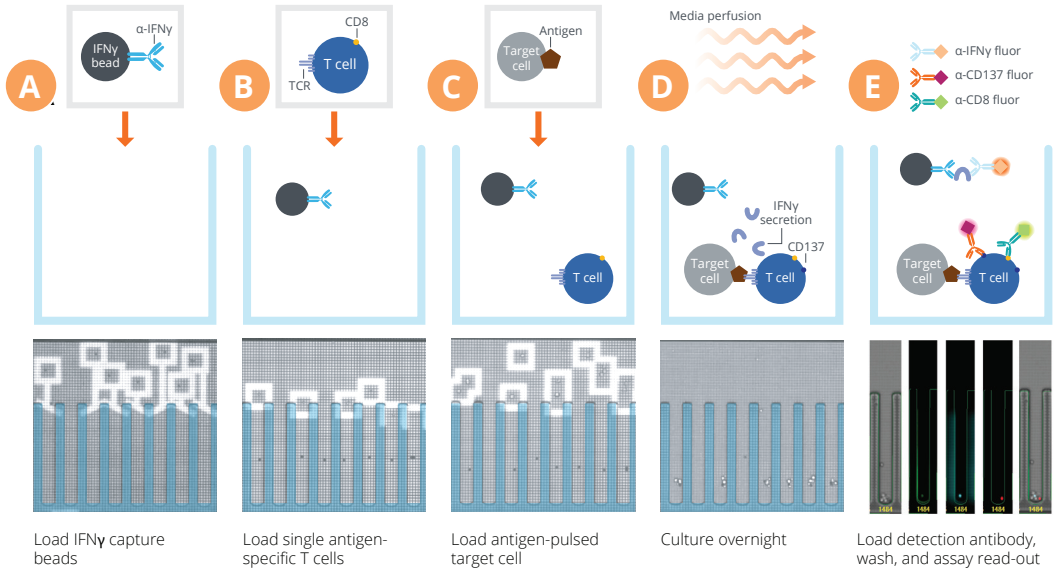

Figure 1. Precisely create and assay individual cell-cell interactions by moving single cells and beads with light.

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FUNCTIONAL ASSAY RESULTS

IFNγ secretion was detected in NanoPen chambers with T cells stimulated with target cells (T2 cells) presenting target peptide, while significantly lower IFNγ secretion was observed in response to irrelevant peptide-pulsed T2 cells. Across three donors, 20–40% of pens were IFNγ+ in the presence of target antigen and 1–12% in pens loaded with control T2 cells (Figure 2A). When investigating the simultaneous presence of secreted IFNγ and CD137 upregulation, we observed that the majority of cells that were secreting IFNγ had also upregulated CD137 upon antigenic stimulation. However, we also identified a subset of cells, ranging from 1% to 31%, which upregulated CD137 without secreting IFNγ, while another subset of cells, ranging from 8% to 20%, was positive for IFNγ secretion in the absence of CD137 expression (Figure 2B–C). This finding indicates that, counter to traditional methods, antigen-specific T cells can be isolated without selecting for cells that upregulate CD137. This enrichment strategy may yield both IFNγ-secreting and non-secreting cells, while excluding cells that secrete IFNγ without CD137 upregulation. Cells of interest were then unloaded and exported for further analysis (Figure 2D).

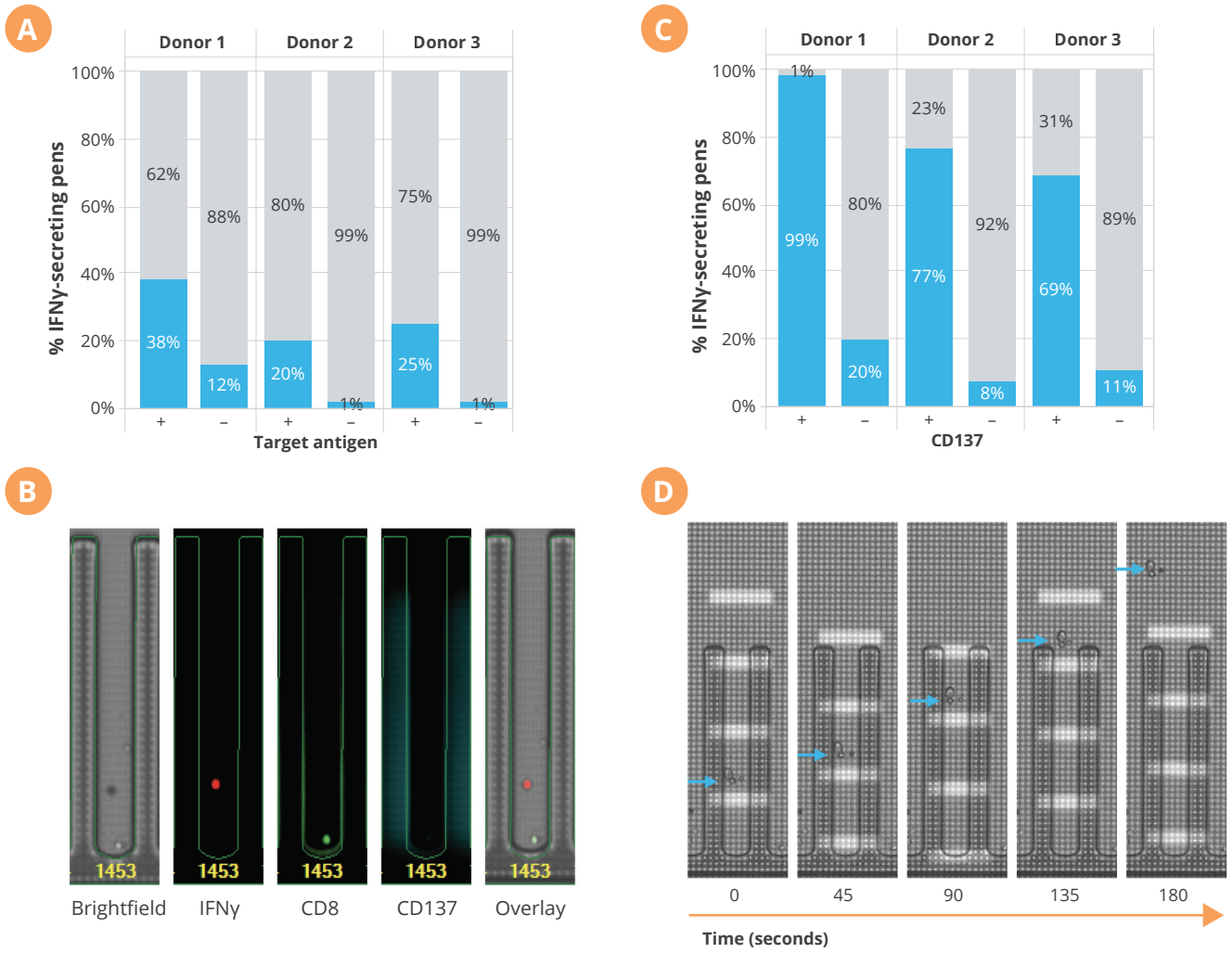

Figure 2. Assessing CD137 upregulation and IFNγ secretion upon antigen stimulation. A. Quantification of IFNγ secretion measured as the fraction of pens loaded with a single T cell, antigen-presenting T2 cells and an IFNγ-capture bead that shows a fluorescent signal in the PE channel, indicating the presence of IFNγ on the surface of the bead (■). **B.** Representative image of single NanoPen chambers after detection of IFNγ secretion and CD137 expression showing CD137 upregulation is not detected in the presence of IFNγ secretion. **C.** Quantification of CD137 upregulation in NanoPen chambers loaded with T2 cells pulsed with target antigen, that are positive (■) or negative (■) for IFNγ secretion. **D.** Following assay analysis, OEP is used to select and recover activated T cells for downstream expansion or genomic profiling. Blue arrows highlight cell being unloaded from a NanoPen chamber for subsequent export into a well plate. Each panel represents a time point during OEP unloading. IFNγ+ (■), IFNγ- (■).

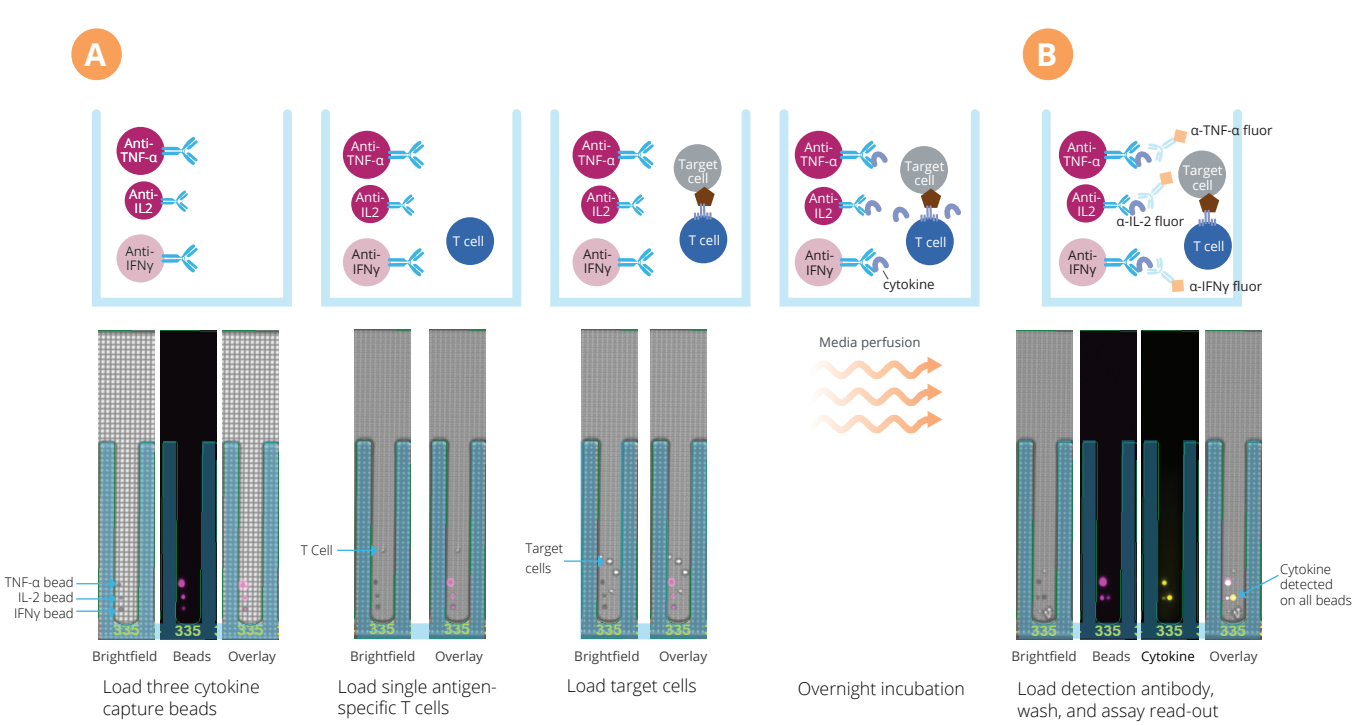

Figure 3. Measure secretion of three cytokines from single T cell after stimulation.

MULTIPLEXING THE CYTOKINE ASSAY

By loading three cytokine capture beads per pen, users can measure secretion of multiple combinations of three cytokines. Shown in the example, three cytokine capture beads (specific for IFNγ, IL-2, and TNF-α) are co-incubated with T cells and target cells. Beads are differentiated by size and internal fluorescence intensity in the CY5 channel (Figure 3A). Cytokine-bound beads are visualized by incubating with fluorescent secondary antibodies conjugated to PE (Figure 3B).

CONCLUSIONS

Viable T cells can be visualized and analyzed in a single day, and then recovered, enabling downstream genomic analysis. This has particular relevance to the study of polyfunctional T cells that simultaneously produce multiple cytokines and play a key role in effective anti-tumor immunity. Analyzing the cytokine secretion from individual T cells on Berkeley Lights systems will enable discoveries into the mechanisms underlying T cell polyfunctionality.

USE CASES

With the Opto Cell Therapy Development 1.0 workflow, user-specific cell-cell interactions can be rapidly and precisely assembled. Hundreds to thousands of experiments with single T cells can be run in parallel. By adding multiplexed functional assays, a rich and robust visual record of T cell phenotype and function can be generated. This enables use cases such as:

- CAR-T construct screening and validation
- Antigen or TCR discovery and validation
- Investigating helper or regulatory T cell function

HANDLING

Please refer to the Berkeley Lights Opto™ T Cell IFNγ Workflow User Manual before handling the reagents.

SAFETY

Use standard laboratory safety protocols. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, please email techsupport@berkeleylights.com.

Opto™ Cell Therapy Development 1.0 Workflow

OPTO™ T CELL IFNγ KIT CONTENTS

PART NUMBER	COMPONENT	UNIT QUALITY	VOLUME/UNIT	USES/UNIT	STORAGE CONDITIONS
520-08016	Wetting Additive	1	1.5 mL	4	4°C
520-08007	Loading Reagent	4	1 mL	1	-20°C
520-08106	Basal Media	1	129 mL	4	-20°C
520-08107	T cell Media Supplement	1	15 mL	4	4°C
520-70003	Media Additive A2	1	150 µL	4	4°C
520-08100	IFNγ Capture Beads	1	50 µL	4	4°C
520-08101	IFNγ Detection Antibody	1	30 µL	4	4°C
520-08103	CD137 Detection Antibody	1	30 µL	4	4°C
520-08102	CD8 Detection Antibody	1	30 µL	4	4°C
520-08104	Detection Reagent	1	50 µL	4	4°C
520-08105	Buffer P-	1	1 mL	4	4°C

ORDERING INFORMATION

PART NUMBER	DESCRIPTION
110-02407	Lightning™ Optofluidic System
750-01000	Opto™ T Cell IFNγ Assay Kit
750-00060	OptoSelect™ 1500 Chip
500-00045	Lightning™ Plastic Flush Chip
110-08004	Beacon® Optofluidic System

Lightning Optofluidic System

OptoSelect 1500 Chip

Opto T Cell IFNγ Assay Kit

FOR MORE INFORMATION, VISIT
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