STANDARD BIOTOOLS™

A 47-Marker Immune Profiling Flow Cytometry Assay to Enable Comprehensive Antigen-Specific Immune Analysis

Michael Cohen, Huihui Yao, Stephen Li, Lauren Tracey, Christina Loh

IMMUNE PROFILING

A 47-parameter CyTOF® flow cytometry assay was developed to **enable deep immunophenotyping and functional profiling** of activated immune cells. The assay and accompanying software solution provides insight into the adaptive immune response to foreign targets relevant in infection, vaccine development and immunotherapy research.
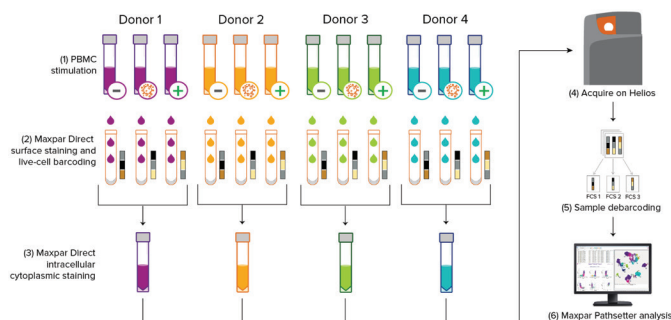
KEY TAKEAWAYS

- A novel intracellular staining workflow was introduced by easily combining preconfigured panels.
- Automated analysis classified new populations such as antigen-specific T cells and cytokine expression.
- The expanded panel enabled comprehensive analysis of immune cell activation and antigen-specific responses without requiring complicated panel design or signal deconvolution.

Background

The complex nature of the immune system requires deep interrogation to understand the response to therapy or exposure to pathogens. This poster presents a simplified flow cytometry workflow using CyTOF technology and preconfigured reagents to enable highly multiparametric phenotypic and functional characterization at the single-cell level.
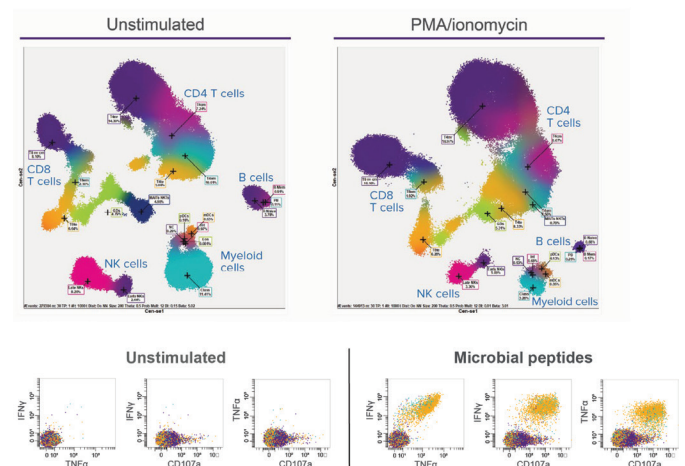
Study Design

A validated 30-marker Maxpar® Direct™ Immune Profiling Assay™ for resolving 37 immune populations was customized by adding an array of 17 surface and intracellular parameters with antibodies from the Maxpar® Direct T Cell Activation Expansion Panel and for live-cell barcoding (LCB).

Results

- High-dimensional Cen-se′™ analysis with Maxpar Pathsetter™ software illustrated expansions or reductions in specific cellular islands corresponding to immune activation when stimulated by PMA/ionomycin.
- Investigation of cytokine profiles confirmed definitions of T helper cell subsets.
- Polyfunctional antigen-specific CD8 T cells were identified using Maxpar Pathsetter.

Workflow depicting cell stimulation, surface staining, barcoding, intracellular staining and acquisition and analysis. Cell stimulation: Human peripheral blood mononuclear cells from four different donors either left untreated (-) or stimulated with PMA/ionomycin (+) or a mix of immunodominant microbial peptides (⚙). LCB enabled intracellular staining for all three conditions per donor in a single tube, thereby reducing staining variability.

Top: Visualization of immune cell activation via high-dimensional Cen-se′ analysis. Maxpar Pathsetter generated Cen-se′ maps of unstimulated and PMA/ionomycin stimulated samples from PBMC Donor 2 are shown. Clusters are colored by cell types and subsets. **Bottom: Antigen-specific CD8 T cells identified using Maxpar Pathsetter.** Bivariate plots from CD8 T cells showing IFNγ vs. TNFα, IFNγ vs. CD107a and TNFα vs. CD107a for unstimulated and peptide-stimulated samples from PBMC Donor 2.

Immune Profiling

Panel Information

Maxpar Direct Immune Profiling Assay					
30-marker single-tube backbone panel					
CD45	CD8	CD161	CD183/CXCR3	TCRγδ	CD20
CD196/CCR6	CD11c	CD194/CCR4	CD185/CSCR5	CD294	CD66b
CD123/IR-3R	CD16	CD25	CD28	CD197/CCR7	HLA-DR
CD19	CD45RO	CD27	CD38	CD14	IgD
CD4	CD45RA	CD57	CD56	CD3	CD127

T Cell Activation Panel					
11-marker Expansion Panel					
CD107a	CD69	IFNγ	CTLA-4	IL-17A	Granzyme B
Il-2	TNFα	IL-4	IL-10	Perforin	

Ordering Information

Product Name	Product Number
Maxpar® Direct™ Immune Profiling Assay™	201334
Maxpar Direct T Cell Activation Expansion Panel	201409
Anti-CD45 (30-F11) antibodies	3110001B 3111001B 3194001B 3195001B

Product Name	Product Number
Maxpar Pathsetter™ v3.0	401019
Cell-ID™ Intercalator-Ir	201192A (125 µM) 201192B (500 µM)

Scan the QR code to see the full digital version of the poster.