STANDARD BIOTOOLS™ | Application Note

Maxpar Direct Immune Profiling Assay Expanded to Explore Antigen-Specific Immune Responses

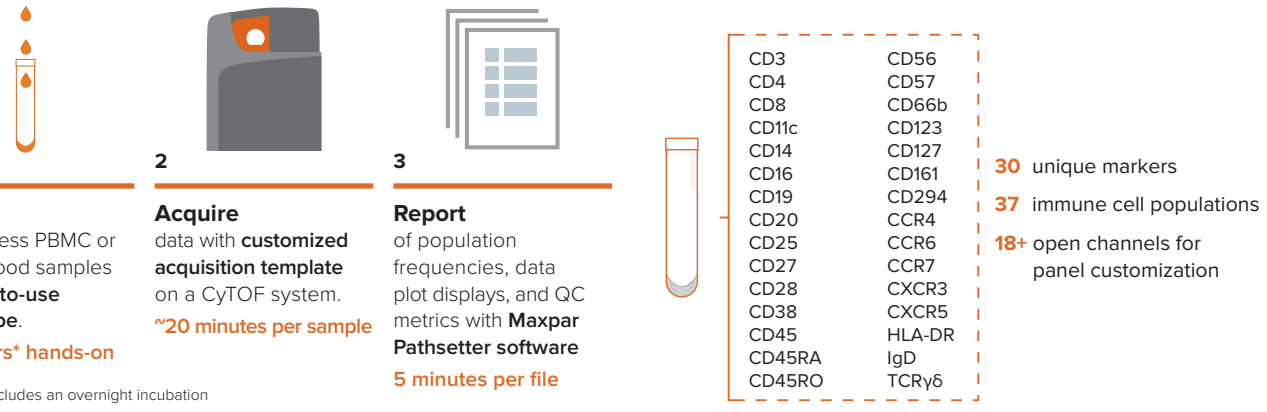
Introduction

Monitoring the immune response to disease and assessing clinical recovery are critical steps towards identifying effective targets for therapies. The complex and heterogeneous nature of the immune system requires deep interrogation at the single-cell level.[1] Mass cytometry, which uses CyTOF® technology, utilizes antibodies tagged with monoisotopic metals, resulting in discrete signals that enable highly multiplexed characterization on a single-cell level. CyTOF can currently enable cellular phenotyping of over 50 single-cell parameters simultaneously, including phenotypic and functional markers, thereby significantly increasing the ability to comprehensively evaluate immune responses.[2] The Maxpar® Direct™ Immune Profiling Assay™ is a pre-titrated, dried-down, 30-marker antibody cocktail for immune profiling of human whole blood and peripheral blood mononuclear cells (PBMC) by CyTOF (Figure 1). When coupled with Maxpar Pathsetter™ software for post-acquisition data analysis, single-cell events can be automatically identified as one of 37 immune populations including major lineages and their subsets by using probability state modeling. The software eliminates the variability of manual gating and provides an automated report including population statistics, stain assessments, and relevant data plots. The Maxpar Direct Assay has been applied in numerous **publications and clinical trials**.

With 18 open channels for panel expansion, the Maxpar Direct Assay was designed with customization in mind. This application note describes the use of the Maxpar Direct T Cell Activation Expansion Panel and sample multiplexing with live-cell barcoding to add 15 parameters to the backbone Maxpar Direct panel. However, other specific markers can be chosen to address a variety of research applications.

The array of key surface and intracellular functional parameters in this expanded panel enables comprehensive analysis of immune cell activation and antigen-specific recall responses to cells stimulated with a pool of immunodominant microbial peptides. Furthermore, the existing Maxpar Pathsetter model was modified to automate analysis of the expanded panel and report on new populations and additional functional parameters such as cytokine production.

Taken together, this powerful technique unlocked deeper immunophenotyping and functional profiling of activated immune cells and provided insight into the cell-mediated adaptive immune response to foreign targets through detection and characterization of antigen-specific T cells. Such phenomena are hallmarks for research on infection, vaccine development, and immunotherapy.



* Staining includes an overnight incubation

Figure 1. The Maxpar Direct Immune Profiling System. The Maxpar Direct Immune Profiling System is a complete sample-to-answer solution for high-dimensional immune profiling of human PBMC and whole blood. Designed as a simple single-tube workflow, the system brings together CyTOF technology, a dry 30-marker antibody panel, and automated analysis with Maxpar Pathsetter software for quantification of 37 immune cell populations. Flexible by design, the assay allows for the addition of 18-plus additional antibodies for further panel customization.

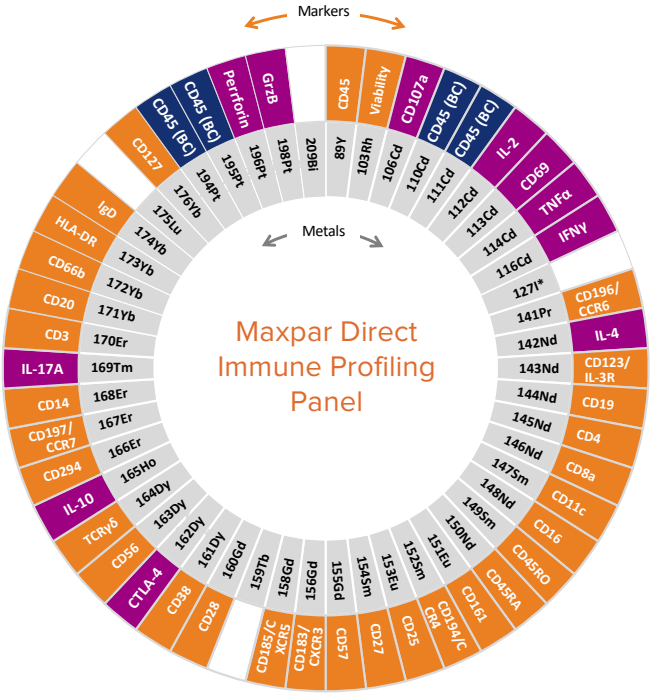
Objectives

- Demonstrate the flexibility of the Maxpar Direct Assay to incorporate additional functional markers and automatically report on new populations using a customized Maxpar Pathsetter model template
- Showcase combined applications in a single CyTOF experiment, including immunophenotyping with Maxpar Direct, intracellular cytokine profiling, and sample multiplexing with live-cell barcoding
- Illustrate the utility of the Maxpar Direct T Cell Activation Expansion Panel to identify antigen-specific immune responses in the context of infectious disease

Study Design

A 47-parameter panel was designed for this application note (Figure 2). Specifically, the Maxpar Direct Immune Profiling Panel, consisting of 30 markers, was used as the backbone for immune cell phenotyping. An 11-marker panel comprised of both surface and intracellular markers, the Maxpar Direct T Cell Activation Expansion Panel (Cat. No. 201409), was selected for deeper phenotyping and functional characterization of activated immune cells. This panel enables the measurement of immune cell activation, frequency of antigen-specific T cells after natural infection or vaccination, cytotoxic potential in T and natural killer (NK) cells, and identification of activated helper T cell populations. The diverse phenotypic and functional features of this panel allow for in-depth immune cell characterization in one tube.

A schematic of the stimulation and multi-step staining experiment carried out in this application note is shown in Figure 3. Human PBMC from 4 different donors were rested overnight and either left untreated or stimulated with phorbol myristate acetate (PMA) and ionomycin or a mix of immunodominant microbial peptides (Step 1 in Figure 3). Brefeldin A and monensin transport inhibitors were added to all samples 1 hr post-stimulation and incubated for an additional 4 hr.



Target (clone)	Isotope	Staining
CD107a (H4A3)	106Cd	Surface
CD45 (HI30)	110Cd	Surface (barcode)
CD45 (HI30)	111Cd	Surface (barcode)
IL-2 (MQ1-17H12)	112Cd	Intracellular
CD69 (FN50)	113Cd	Surface
TNFα (Mab11)	114Cd	Intracellular
IFNγ (B27)	116Cd	Intracellular
IL-4 (MP4-25D2)	142Nd	Intracellular
CTLA-4 (14D3)	162Dy	Intracellular
IL-10 (JES3-9D7)	165Ho	Intracellular
IL-17A (BL-168)	169Tm	Intracellular
CD45 (HI30)	194Pt	Surface (barcode)
CD45 (HI30)	195Pt	Surface (barcode)
Perforin (B-D48)	196Pt	Intracellular
Granzyme B (Gb11)	198Pt	Intracellular

Figure 2. Building a 47-parameter panel for functional profiling of activated immune cells. (Left) Panel wheel illustrating cellular markers and their respective isotope channels. The base Maxpar Direct Immune Profiling Panel (Cat. No. 201334) is depicted by the orange boxes. The Maxpar Direct T Cell Activation Expansion Panel (Cat. No. 201409) is depicted by the purple boxes. Cadmium- and platinum-labeled anti-CD45 antibodies used for live-cell barcoding (Cat. Nos. 3110001B, 3111001B, 3194001B, 3195001B) are depicted by the blue boxes. Empty boxes indicate open channels. (*IdU is the only 127I conjugate currently available.) (Right) Table detailing expansion panel antibody clones, live-cell barcoding reagents, and staining method required.

After stimulation, cells were added to the Maxpar Direct dry antibody pellet and concurrently stained with additional surface markers from the Maxpar Direct T Cell Activation Expansion Panel (Step 2 in Figure 3). Furthermore, a unique 4-choose-3 anti-CD45 barcode mix for each stimulation condition was added to corresponding tubes during this surface staining step.

Multiplexed samples from each PBMC donor were combined into a single tube, fixed, permeabilized, and stained with the intracellular antibodies from the Maxpar Direct T Cell Activation Expansion Panel (Step 3 in Figure 3). Samples were then incubated with Cell-ID™ Intercalator-Ir overnight for total cell identification.

Barcoded samples were acquired on a Helios™ instrument (Step 4 in Figure 3).* Sample debarcoding was carried out (Step 5 in Figure 3) and individual FCS files were uploaded into Maxpar Pathsetter software for automatic analysis using a customized model template (Step 6 in Figure 3; see section on **Updating the Maxpar Pathsetter Model** in this application note).

Additional details on this experiment and ordering information for the reagents used in this study can be found below in the **Methods** section and **Appendix A**, respectively.

* This assay can also be acquired on a CyTOF XT system.

Results

Optimizing Workflow and Data Quality with Sample Multiplexing and Automatic Analysis

Live-cell barcoding

Live-cell barcoding (LCB) using Maxpar anti-human CD45 antibodies allowed for all stimulation conditions from a single PBMC donor to be stained with intracellular antibodies simultaneously in a single tube. LCB effectively eliminates staining variability between samples, reduces reagent use, and streamlines the acquisition workflow.

Figure 4 shows a representative sample debarcoding analysis from PBMC Donor 3 and illustrates efficient sample separation between the positive and negative barcode channels used. A clear visualization of barcode channel intensities can be observed in the scatter plot and resulted in a barcode yield of 81% (Figure 4A).

After sample debarcoding, FCS files were further evaluated for data quality and consistency. Specifically, cells were gated on live, singlet populations, and CD45 signal intensity was analyzed. All debarcoded files exhibited a positive signal for the expected barcode channels (yellow histograms) and had minimal signal overlap into negative barcode channels (black histograms) (Figure 4B). This was also visualized in a heat map depicting median signal intensities for each barcode channel.

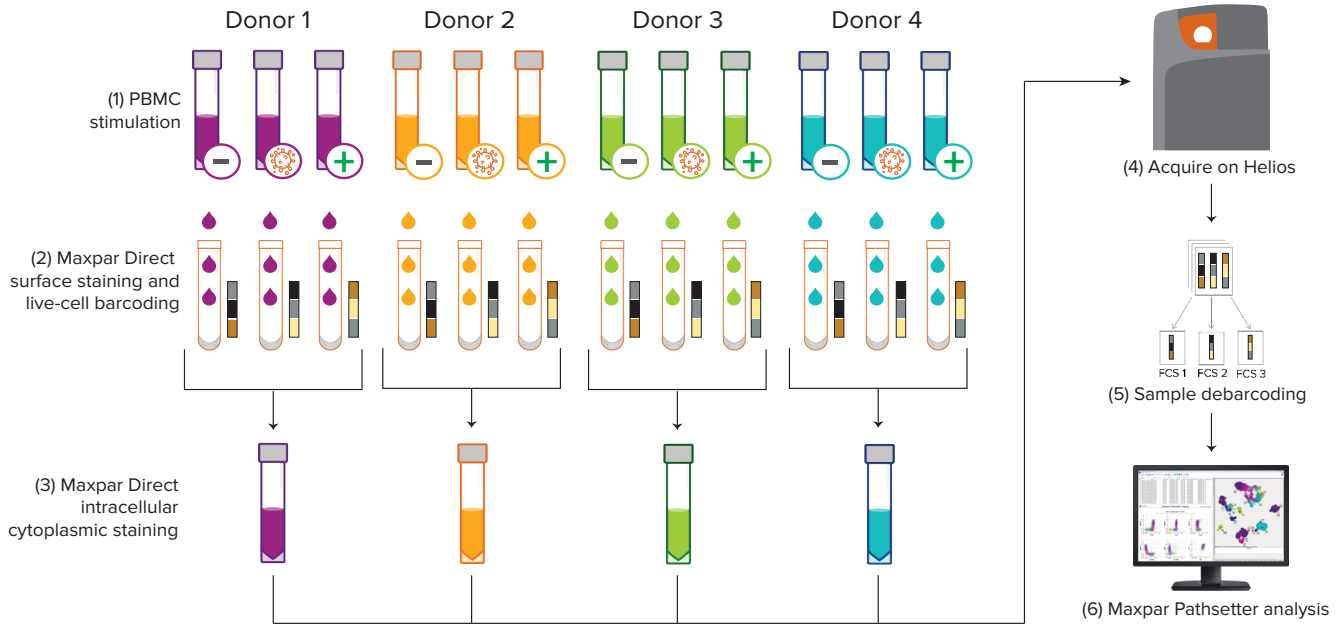


Figure 3. Study design. Experimental workflow illustrating the multi-step stimulation and staining procedures. Sample tubes are color-coded by human PBMC donor. Stimulations depicted by (–) for unstimulated, (⚙) for microbial peptide pool, and (+) for PMA/ionomycin. Live-cell barcoding is illustrated by gray (110Cd-CD45), black (111Cd-CD45), gold (194Pt-CD45), and yellow (195Pt-CD45) bars.

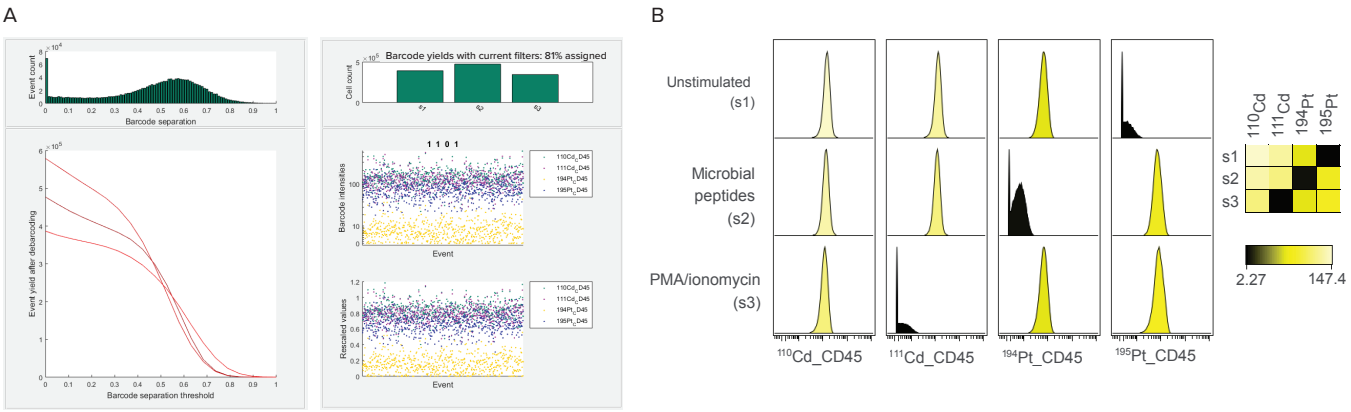


Figure 4. Debarcoding results. A) Single-cell Debarcoder[3] software screen capture of sample deconvolution. Green histogram bars and barcode separation curves are displayed for all 3 barcoded samples from PBMC Donor 3 as a representative donor (left panel). Barcode event counts illustrate sample yields and event dot plots depict barcode channel intensities (right panel). Barcode 2 (s2) is shown as a representative sample. B) Histograms and heat map depicting median signal intensities for 110Cd-CD45, 111Cd-CD45, 194Pt-CD45, and 195Pt-CD45 barcode channels. Both plots display data from PBMC Donor 3 gated on the viable, singlet PBMC population.

Updating the Maxpar Pathsetter model

Maxpar Pathsetter is a fast, reliable, and flexible data analysis solution. The default Pathsetter model was specifically designed to automatically identify 37 immune cell types in samples processed with Maxpar Direct. For automated analysis of the 11 additional markers introduced in the Maxpar Direct T Cell Activation Expansion Panel, a customized model template was created following the Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151).

The Maxpar T Cell Activation Pathsetter model template incorporates new functional information based on the activation markers and cytokines in this panel and results in >100 additional measurements within specified immune cell subsets. All samples in this study were processed using the updated model template and a summary of the results are shown in the following sections.

Mapping the Functional Landscape of Activated Immune Cells

Broad immune cell activation via PMA/ionomycin stimulation typically results in a marked upregulation in the frequency of CD4 and/or CD8 T cells expressing functional activation markers and induces significant cytokine production. PMA/ionomycin stimulated samples were used in this study to showcase that when used in tandem, the base Maxpar Direct panel and T Cell Activation Expansion Panel robustly capture activated immune subsets.

Maxpar Pathsetter analysis of activated immune cells

Population frequency reports, automatically generated by the Maxpar T Cell Activation Pathsetter model template, effectively identified the anticipated upregulation of functional markers across all PBMC samples tested when compared to unstimulated controls (Figure 5). This included activation markers CD107a and CD69, intracellular cytokines IL-2, IFNγ, and TNFα, as well as the exhaustion marker CTLA-4.

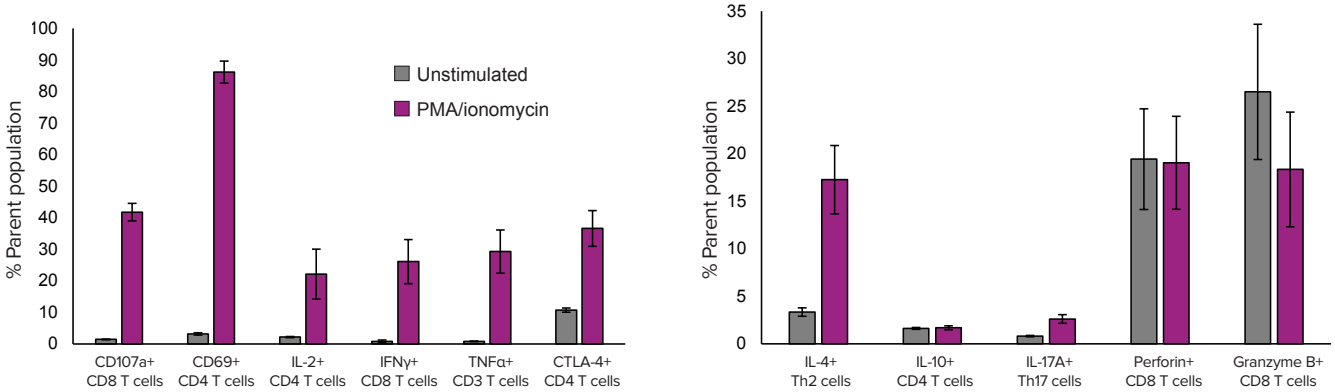


Figure 5. Frequency of T cells expressing functional markers post stimulation. CD4 or CD8 T cells expressing markers from the Maxpar Direct T Cell Activation Expansion Panel between unstimulated and PMA/ionomycin-stimulated samples were measured and reported by Maxpar Pathsetter software. Bar graphs depict mean % parent population frequencies ± standard error of the mean (SEM) from all 4 human PBMC donors.

Cauchy enhanced nearest-neighbor stochastic embedding (Cen-se™)[4] is a powerful dimensionality reduction tool built into Pathsetter. This tool can be used to visualize the phenotypic and functional landscape across various immune cell populations on a single plot. This high-dimensional modeling correctly identified various expansions or reductions in the cellular islands corresponding to CD4 and CD8 T cells, NK cells, B cells, and myeloid populations when stimulated with PMA/ionomycin (Figure 6A). Additionally, the relative signal intensity for each of the 11 expansion panel markers was overlaid as heat maps to monitor expression on immune cell population clusters between unstimulated and PMA/ionomycin pairs from the same donor (Figure 6B). These heat maps revealed more intricacies of the cellular response to stimulation in the Cen-se′ plot such as ubiquitously high expression of CD69 across the T cell islands, exhausted CTLA-4+ CD4 T cells, and increased production of cytokines such as IFNγ in specific T cell clusters. Upregulation of CD107a across numerous activated populations was also observed. This included NK and CD8 T cell islands, indicating cytotoxic potential of these cell subsets.

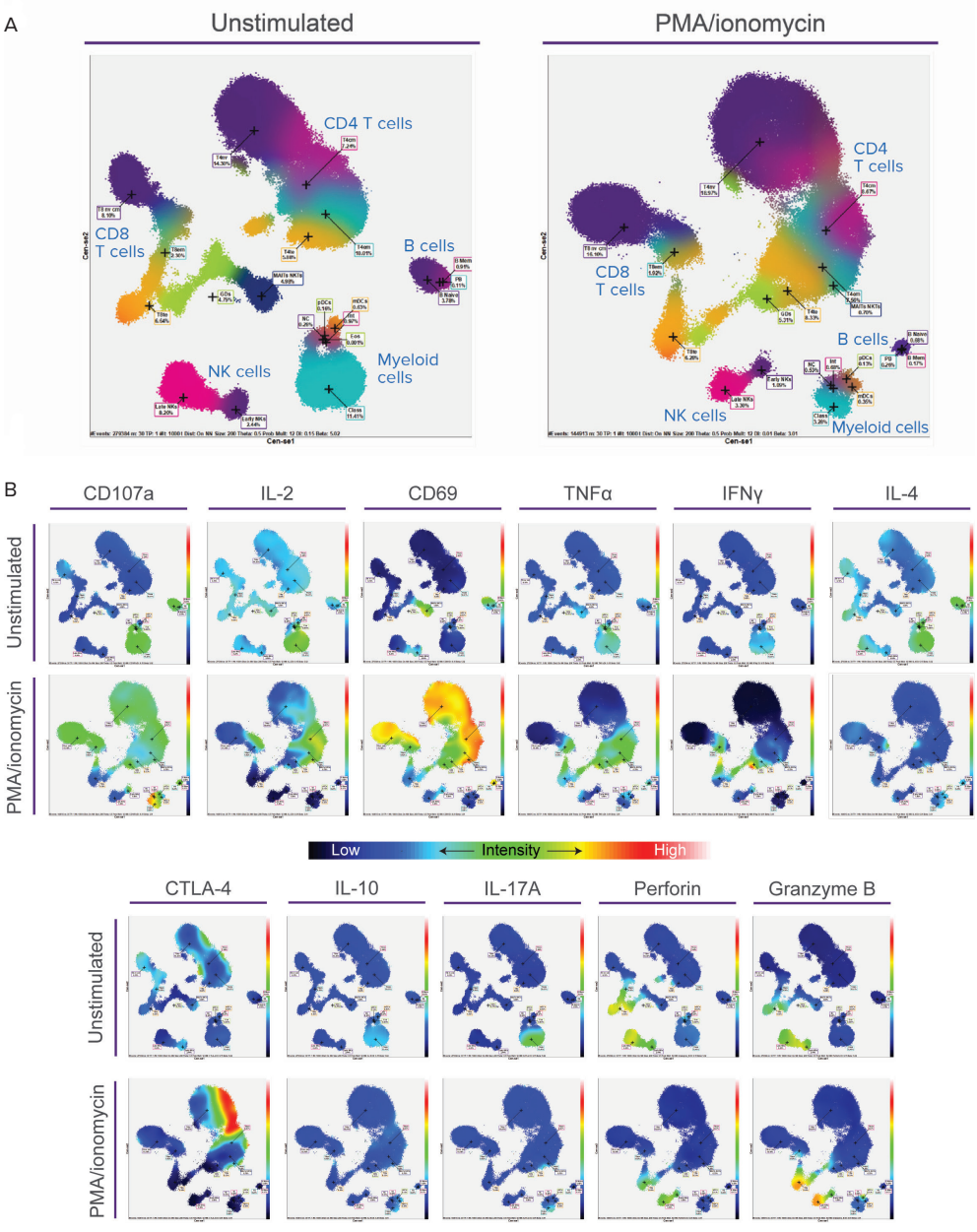


Figure 6. Visualization of immune cell activation via high-dimensional Cen-se′ analysis. Maxpar Pathsetter generated Cen-se′ maps of unstimulated and PMA/ionomycin-stimulated samples from PBMC Donor 2 are shown. A) Clusters are colored by cell types and subsets. CD8 T cells, CD4 T cells, B cells, NK cells, and myeloid cells are labeled. B) Heat maps of signal intensities illustrating relative expression across immune cell populations for each of the 11 functional markers from the Maxpar Direct T Cell Activation Expansion Panel are shown for both unstimulated and PMA/ionomycin stimulated samples from the same donor.

Cytokine profiling of CD4 T cell subsets

The addition of specific activation and intracellular markers can also complement the phenotyping carried out by the base Maxpar Direct panel. For example, the helper T cell (Th) subsets Th1, Th2, and Th17 are defined by expression of specific chemokine receptors in the base Maxpar Direct assay. Investigation of cytokine profiles in this work confirmed the definition of these subsets (Figure 7). As expected, PMA/ionomycin-stimulated samples revealed that while IL-2 and TNFα were ubiquitously expressed across all 3 Th subsets, IFNγ, IL-4, and IL-17A were predominantly expressed in Th1, Th2, and Th17 populations, respectively.[5]

Taken together, these results demonstrate the ability of the Maxpar Direct Assay combined with the T Cell Activation Expansion Panel to effectively and reliably identify and phenotypically profile activated immune cells.

Exploring T Cell-Mediated Host Immune Responses to Foreign Antigens

Quantification of antigen-specific T cell responses is critical for understanding immunity and pathogenesis to infectious disease. Furthermore, clinical research applications, such as vaccine development, aim to recapitulate the response to natural infection, and the enumeration of vaccine-induced T cells is frequently used to assess immunogenicity. Importantly, the Maxpar Direct T Cell Activation Expansion Panel, combined with Maxpar Pathsetter, allows for the robust assessment of these antigen-specific responses through measurement of cytokines and markers of activation and cytotoxicity.

In this study, cells stimulated with a pool of immunodominant microbial peptides were used as an *in vitro* model of infectious disease and were processed with the Maxpar Direct Assay and Maxpar Direct T Cell Activation Expansion Panel. A detailed report page focusing on antigen-specific CD4 and CD8 T cell recall and functionality is provided following Pathsetter analysis, as depicted in Figure 8 and Figure 9A below.

Investigating the spectrum of antigen-specific CD8 memory subsets

Representative bivariate plots portraying the expression of IFNγ, TNFα, and CD107a in CD8 T cells depict an antigen-specific immune response, as shown by a clear upregulation and positive correlation of these functional markers in peptide-stimulated samples when compared to paired unstimulated samples from the same donor (Figure 8, top panel). These data plots are conveniently color-coded based on CD8 T cell memory subsets: naive/central memory (Nv/Cm), effector memory (Em), and terminal effector (TEff). The majority of the antigen-specific recall responses were found in CD8 effector cells, which are poised to protect the host from invading pathogens. Expression profile plots, which are unique to Pathsetter, visualize individual functional marker expression across the CD8 T cell memory subsets for additional granularity on reactive T cell populations. A clear antigen-specific upregulation of cytokines and degranulation markers in CD8 Em and TEff cells was observed here (Figure 8, bottom panel). IFNγ, TNFα, CD107a, and IL-2 are shown as representative plots. However, all 8 functional markers within CD8 T cells are available in the full customized Pathsetter report.

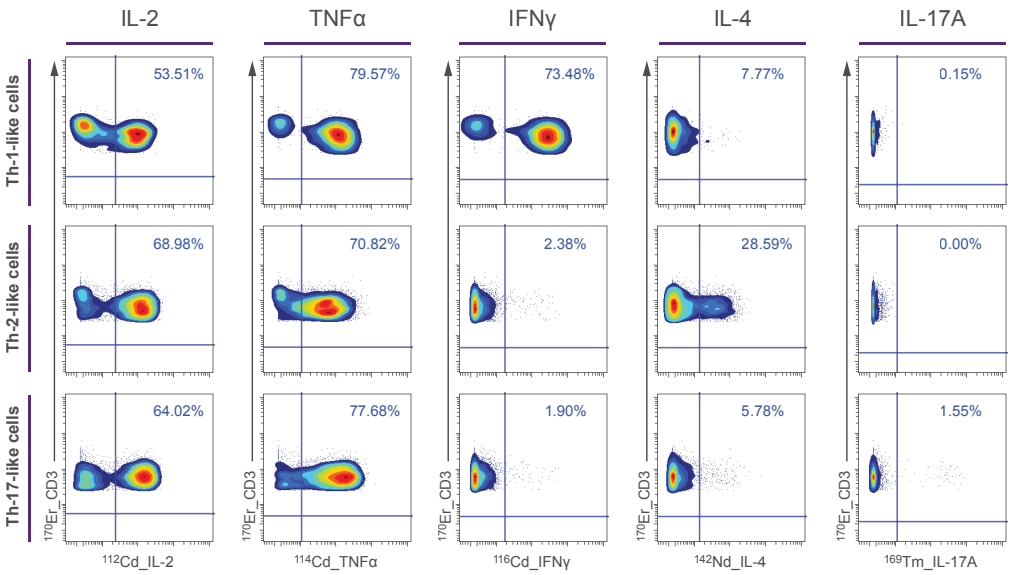


Figure 7. Cytokine profile of CD4 T-helper subsets after PMA/ionomycin stimulation. CD3 vs. IL-2, TNFα, IFNγ, IL-4, or IL-17A bivariate plots are displayed for Th1-like, Th2-like, and Th17-like populations from PBMC Donor 3. Gate labels display % of cells positive for the corresponding cytokine expression. Th1-like cells were gated on CD4+CXCR5-CCR4-CD45RA-CD45RO+CXCR3+CCR6- populations. Th2-like cells were gated on CD4+CXCR5-CD45RA-CCR4+CXCR3-CCR6- populations. Th17-like cells were gated on CD4+CXCR5-CD45RA-CCR4+CXCR3-CCR6+ populations.

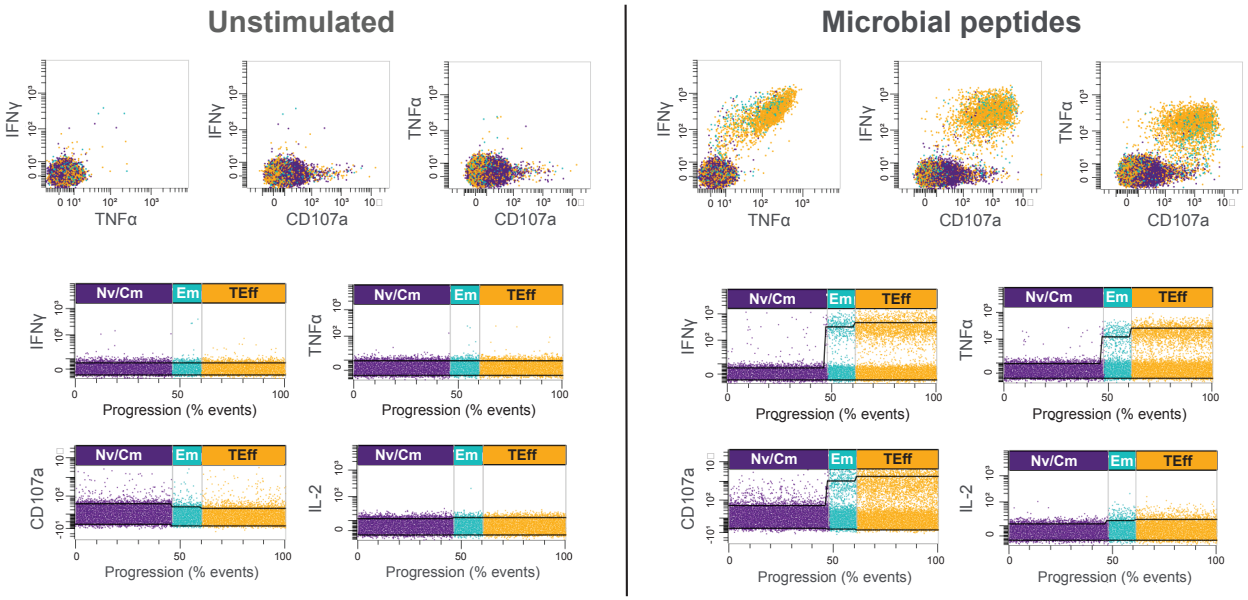


Figure 8. Antigen-specific CD8 T cells identified using Maxpar Pathsetter. Bivariate plots from CD8 T cells showing IFNγ vs. TNFα, IFNγ vs. CD107a, and TNFα vs. CD107a for unstimulated and peptide stimulated samples from PBMC Donor 2 (top panel). Upregulation and positive correlation of functional markers highlight antigen-specific T cells identified in microbial peptide-stimulated samples. Expression profile plots of IFNγ, TNFα, CD107a, and IL-2 across naive/central memory (Nv/Cm), effector memory (Em), and terminal effector (TEff) CD8 T cell memory subsets (bottom panel). All plots were generated by Maxpar Pathsetter automatic analysis and are color-coded by CD8 T cell memory subsets.

Quantification of polyfunctional antigen-specific CD8 T cell populations

The frequency of functional marker expression within antigen-specific T cells, defined as IFNγ+ CD8+ T cells, was also automatically reported in a summary table for additional quantification and phenotypic analysis (Figure 9A). As expected, greater frequencies of activation markers and cytokine production can be observed in antigen-specific CD8 T cells when compared to global CD8 T cell populations across all PBMC donors tested (Figure 9B). These pathogen-specific T cells were polyfunctional, as assessed by co-expression of numerous activation and cytolytic markers, a correlate of T cell efficacy.[6]

Overall, endogenous antigen-specific CD8 T cells across various PBMC donors were successfully identified and characterized in response to microbial peptide stimulation using Maxpar Direct panels and automated Pathsetter analysis.

A

Maxpar Pathsetter Report

FCS File: Donor2_s2_cleaned.fcs

	Count	% Intact live	Parent	% Parent
CD8 T Cells	51705	17.23	CD3 T Cells	26.39
CD107a+	3196	1.07	CD8 T Cells	6.18
CD69+	4561	1.52	CD8 T Cells	8.82
CTLA-4+	202	0.07	CD8 T Cells	0.39
Granzyme B+	16613	5.54	CD8 T Cells	32.13
Perforin+	15596	5.20	CD8 T Cells	30.16
TNFa+	3389	1.13	CD8 T Cells	6.55
IL-2+	1340	0.45	CD8 T Cells	2.59
IFNg+	3364	1.12	CD8 T Cells	6.51
IFNg+CD107a+	2876	0.96	IFNg+ CD8 T Cells	85.49
IFNg+CD69+	2082	0.69	IFNg+ CD8 T Cells	61.89
IFNg+CTLA-4+	49	0.02	IFNg+ CD8 T Cells	1.46
IFNg+Granzyme B+	1981	0.66	IFNg+ CD8 T Cells	58.89
IFNg+Perforin+	1336	0.45	IFNg+ CD8 T Cells	39.71
IFNg+TNFa+	3029	1.01	IFNg+ CD8 T Cells	90.04
IFNg+IL-2+	868	0.29	IFNg+ CD8 T Cells	25.80

B

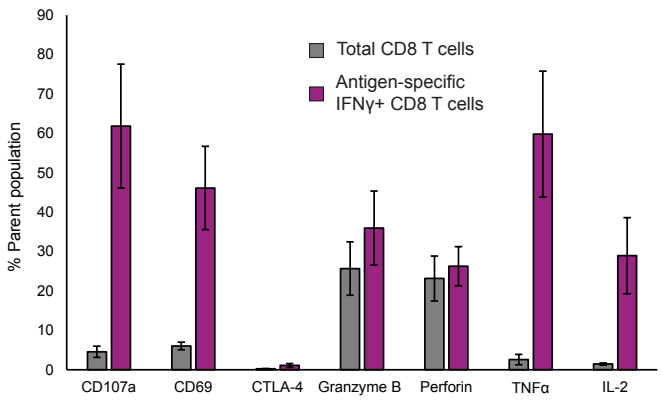


Figure 9. Profiling antigen-specific CD8 T cells. A) Screen capture of the customized Maxpar Pathsetter report page for peptide-stimulated CD8 T cells from PBMC Donor 2. Frequency of CD8 T cells expressing CD107a, CD69, CTLA-4, Granzyme B, Perforin, TNFα, and IFNγ is shown. Additional breakdown of these frequencies on antigen-specific CD8 T cells, defined as IFNγ+, is reported. B) Bar graphs depicting frequency of functional markers expressed on total CD8 T cells compared to antigen-specific IFNγ+ CD8 T cells across all four PBMC donors (mean % parent population frequency ± SEM).

Summary

This application note highlights the flexibility of the Maxpar Direct Immune Profiling Assay to accommodate expansion panels and further facilitate comprehensive immune profiling and functional analysis of cellular responses. Several key takeaways from this study are listed below:

- A 47-parameter CyTOF panel was developed by combining the base Maxpar Direct Immune Profiling Panel, the Maxpar Direct T Cell Activation Expansion Panel, and Maxpar anti-CD45 LCB reagents.
- The preconfigured expansion panel included 9 antibodies targeting cytoplasmic markers, thereby introducing a novel workflow of intracellular staining to the Maxpar Direct Immune Profiling Assay.
- LCB enhanced efficiency of intracellular antibody staining and sample acquisition thereby streamlining the Maxpar Direct workflow.
- The existing Pathsetter model was adapted to incorporate new functional immune markers resulting in >100 additional measurements on various cell subsets
- Automatic analysis using Pathsetter successfully classified new populations such as antigen-specific T cells in response to microbial peptide pools.
- Immunophenotyping and functional profiling of these populations were enabled by assessing expression of key activation, cytokine, and cytotoxic markers, thereby providing insight into potential cell-mediated immune protection from foreign pathogens.

Conclusions

The Maxpar Direct Immune Profiling System is a complete and flexible sample-to-answer solution for single-cell high-dimensional immune profiling. This study demonstrated how to leverage the power of Maxpar Direct Expansion Panels for clinically-relevant research to interrogate antigen-specific T cells in an *in vitro* model of infectious disease.

Although this application note focused on recall responses in CD8 T cell populations, antigen-specific CD4+ T cells can also be identified and characterized automatically with this method. Furthermore, experimental workflows and antibody panels similar to the one described herein can easily be applied to other infectious disease models such as SARS-CoV-2 infection[7], response to vaccination[8], and immunotherapy research[9].

Additional preconfigured Maxpar Direct Expansion Panels are available, encompassing a broad selection of surface and intracellular targets for studies requiring more in-depth characterization of specific immune cell populations or functional states in both human PBMC and whole blood.

With robust high-parameter coverage, efficient and reliable workflows, and even more flexibility for panel expansion, the Maxpar Direct Immune Profiling System powered by CyTOF technology is an invaluable tool that empowers users to carry out deep interrogation of the immune system and unravel its complexities in a single tube.

Tips for Success

- Expression of functional markers may vary depending on stimulation or disease conditions. We recommend titrating the Maxpar Direct Expansion Panel antibodies with representative test samples.
- Heparin blocking of permeabilized cells prior to intracellular staining is recommended to reduce background staining in select immune cell populations. Refer to the Maxpar Cytoplasmic/ Secreted Antigen Staining with Fresh Fix Protocol (400279) for additional details.
- Titration of each metal-labeled anti-CD45 antibody is essential prior to carrying out LCB to ensure sufficient signal intensities for sample debarcoding. Concentrations often vary between sample types. For example, whole blood samples may require higher concentrations of anti-CD45 antibodies compared to PBMC. Furthermore, downregulation of CD45 may occur under some stimulation or diseased sample conditions.
- After pooling barcoded samples, scale-up buffers and antibody volumes according to cell number as described in the Cell-ID 20-Plex Barcoding Kit User Guide (PRD023).
- More information on how to customize the Maxpar Pathsetter model can be found in the Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151). For a copy of the model template used in this study, contact your local Field Applications Specialist or Technical Support Specialist, who can connect you with a regional Field Applications Specialist.
- Contact your local Field Applications Specialist for further guidance on any details described in this application note.

References

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Methods

Human PBMC from 4 donors were obtained from STEMCELL Technologies™ and thawed in complete RPMI 1640 (cRPMI) medium (Thermo Fisher Scientific™) containing Anti-Aggregate Wash™ Supplement (Cellular Technology Ltd.), following manufacturer instructions, and rested overnight at 37 °C and 5% CO_2.

Cells were seeded in 24-well plates at a density of 6 x 10^6 cells/mL and were either left untreated or cultured in the presence of PMA/ionomycin (Cell Activation Cocktail; BioLegend®) or a mix of immunodominant microbial peptides (CEFX peptide pool; JPT Peptide Technologies) according to manufacturer guidelines. Cells were also stained with ^{106}Cd-CD107a antibodies during the stimulation. Brefeldin A (BioLegend) and monensin (BioLegend) were added to all wells 1 hr into the culture and were subsequently incubated for an additional 4 hr prior to harvesting.

Cells were washed with Maxpar Cell Staining Buffer (CSB), blocked with Human TruStain FcX™ (BioLegend) and stained with the Maxpar Direct Immune Profiling Panel according to the Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286). ^{113}Cd-CD69 and corresponding CD45 antibody-based barcode mixtures were added as drop-in antibodies during surface staining (unstimulated: ^{110}Cd-CD45, ^{111}Cd-CD45, ^{194}Pt-CD45; microbial peptides: ^{110}Cd-CD45, ^{111}Cd-CD45, ^{195}Pt-CD45; PMA/ionomycin: ^{110}Cd-CD45, ^{194}Pt-CD45, ^{195}Pt-CD45).

After surface and barcode staining, cells were washed with CSB and fixed with 0.1X methanol-free Pierce™ 16% Formaldehyde (w/v) (Thermo Fisher Scientific) diluted in Maxpar PBS. Cells were washed with CSB and barcoded samples corresponding to each individual donor were combined into a single 5 mL round bottom tube.

Combined samples were permeabilized with Maxpar Perm-S Buffer and blocked with 100 U/mL sodium heparin salt (Sigma Aldrich™) in Perm-S Buffer. Samples were then stained with the following intracellular antibodies according to the Maxpar Cytoplasmic/ Secreted Antigen Staining with Fresh Fix Protocol (400279): ^{112}Cd-IL-2, ^{114}Cd-TNFα, ^{116}Cd-IFNγ, ^{142}Nd-IL-4, ^{162}Dy-CTLA-4, ^{165}Ho-IL-10, ^{169}Tm-IL-17A, ^{196}Pt-Perforin, and ^{198}Pt-Granzyme B. After intracellular staining, cells were washed with CSB and fixed a second time. Finally, samples were incubated with Cell-ID Intercalator-Ir (31.25 nM final concentration) in Maxpar Fix and Perm Buffer overnight at 4°C.

The Helios instrument with CyTOF Software version 7.0.8493 was tuned using Tuning Solution. Note that this assay can also be acquired on a CyTOF XT™ system. Immediately prior to acquisition, the instrument was conditioned with Maxpar Cell Acquisition Solution (CAS) Plus. Samples were washed and resuspended to 1 x 10^6 cells/mL in 0.1X EQ™ Four Element Calibration Beads as an internal standard diluted in CAS Plus. Approximately 1.5 x 10^6 cellular events were acquired per pooled sample at 250–350 events/sec on the Helios instrument with the WB Injector.

Original FCS files were normalized using CyTOF Software version 7.0.8493 and were subsequently debarcoded using the Zunder Lab Single Cell Debarcoder.[3] For more information on LCB, refer to the application note: Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry (FLDM-00488).

The processed and debarcoded FCS files from each experiment were analyzed using Maxpar Pathsetter software version 3.0.15 and Cytobank (Cytobank.org).

Related Documents

The following related documents can be found at standardbiotools.com:

- Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (400286)
- Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay Technical Note (400248)
- Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151)
- Maxpar Cytoplasmic/Secreted Antigen Staining with Fresh Fix Protocol (400279)
- Cell-ID 20-Plex Pd Barcoding Kit User Guide (PRD023)
- The Benefits of Palladium Barcoding on Data Quality and Workflow Application Note (FLDM-00012)
- Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry Application Note (FLDM-00488)
- Helios, a CyTOF System User Guide (400250)
- CyTOF XT User Guide (FLDM-00254)

Appendix A: Ordering Information

Standard BioTools™ Reagents

Name	Catalog Number
Maxpar® Direct™ Immune Profiling Assay™, 30 Marker—25 Tests	201334
Maxpar Direct T Cell Activation Expansion Panel, 11 Marker—25 Tests	201409
Anti-Human CD45 (HI30)-^{110}Cd—100 or 25 Tests	3110001B/C
Anti-Human CD45 (HI30)-^{111}Cd—100 or 25 Tests	3111001B/C
Anti-Human CD45 (HI30)-^{194}Pt—100 or 25 Tests	3194001B/C
Anti-Human CD45 (HI30)-^{195}Pt—100 or 25 Tests	3195001B/C
Maxpar Cell Staining Buffer	201068
Maxpar PBS	201058
Maxpar Perm S Buffer	201066
Maxpar Fix and Perm Buffer	201067
EQ™ Four Element Calibration Beads	201078
Tuning Solution	201072

Reagents from Other Suppliers

Vendor	Name	Part Number
STEMCELL Technologies	Human PBMC	70025
Cellular Technology Limited (CTL)	CTL Anti-Aggregate Wash 20x Solution	CTL-AA-010
Thermo Fisher Scientific	Pierce 16% Formaldehyde (w/v), Methanol-free	28906
BioLegend	Human TruStain FcX	422302
BioLegend	Cell Activation Cocktail (without Brefeldin A)	423301
BioLegend	Brefeldin A Solution	420601
BioLegend	Monensin Solution	420601
JPT Peptide Technologies	CEFX Ultra SuperStim Pool	PM-CEFX-1
Sigma Aldrich	Heparin Sodium Salt	H3149-10KU

Appendix B: Customer Support

For access to the CyTOF Software acquisition template and custom Maxpar Pathsetter model template used in this application note, contact your local Field Applications Specialist.

Learn more: **fluidigm.com/immuneprofile**

Or contact: **tech.support@fluidigm.com**

CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
standardbiotools.com

SALES
North America | +1 650 266 6170 | info-us@fluidigm.com
Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com
Latin America | +1 650 266 6170 | info-latinamerica@fluidigm.com
Japan | +81 3 3662 2150 | info-japan@fluidigm.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | info-china@fluidigm.com
All other Asia-Pacific countries | +1 650 266 6170 | info-asia@fluidigm.com

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