Expanding the Maxpar Direct Immune Profiling Assay for CyTOF

Standardized cytometric immune profiling: 30 markers are only the beginning

Standardizing 30-plus parameter immune monitoring for flow cytometry

Maxpar® antibodies and cell labeling reagents are the most stable and robust available for use in flow cytometry, with capabilities that far outperform fluorescent-labeled antibodies. Made especially for cytometry by time-of-flight analysis (used in CyTOF® technology), the combination of these robust reagents and the precision of signal measurement provided by CyTOF is changing the game for clinical and translational researchers.

For streamlined and standardized cytometric immune monitoring studies, start with the Maxpar® Direct™ Immune Profiling Assay™ (Cat. No. 201334). This dried-down and ready-to-stain 30-marker panel provides excellent immune cell coverage for human PBMC and whole blood samples, enabling analysis of 37 immune cell populations in a single tube.

Expansion Panel key benefits

Comprehensive—Profile 37-plus immune cell populations, their functional states and cytokine profiles simultaneously.

Flexible—Create your favorite panels instantly by combining the 30-marker base panel with one or more Expansion Panels, or individual antibodies.

Efficient—Save time and sample with a simple single-tube workflow using only 300 µL of whole blood without need for data deconvolution or compensation.

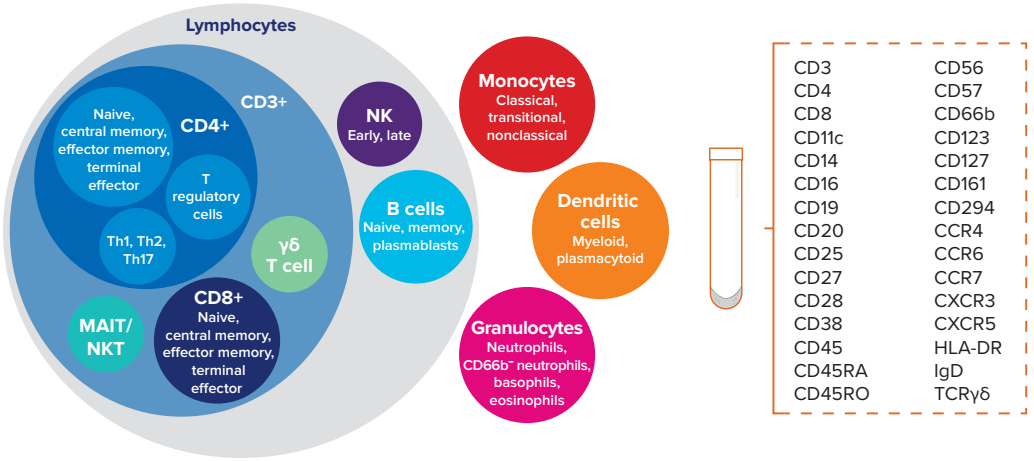


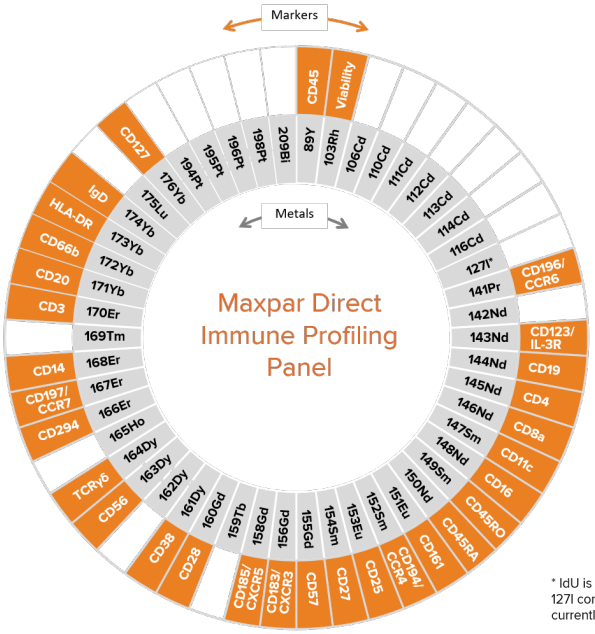
Figure 1. A comprehensive immune profile. The 37 immune cell subsets (left) identified using the 30-marker base Maxpar Direct Immune Profiling Assay (right).

Expanding the Maxpar Direct Immune Profiling Assay

While the Maxpar Direct Immune Profiling Assay provides excellent immune cell coverage, you can readily add up to 18 antibodies to achieve more insight into specific immune cell populations or functional analysis study goals. The minimal channel crosstalk of mass cytometry simplifies expansion and modification of high-parameter panels – a significant advantage over fluorescence cytometry.

Options for expanding the Maxpar Direct Assay:

- Select a preconfigured Expansion Panel containing labeled antibodies ready to drop into open channels*.
- Choose from the expansive Standard BioTools™ catalog of Maxpar metal-labeled antibodies.
- Use Custom Conjugation Services for guaranteed antibody labeling by Standard BioTools experts.
- Label your own antibodies using a Standard BioTools Maxpar labeling kit.



* IdU is the only 127I conjugate currently available.

Figure 2. The Maxpar Direct Immune Profiling Assay is made from a backbone panel of 30 antibody markers (orange slots) with space for at least 18 additional antibodies (white slots).

Simultaneously profile phenotypes and functions of 37-plus immune cell populations

Each of the 9 Maxpar Direct Expansion Panels consists of a collection of pre-selected antibodies that fit open channels of the assay to enable deeper profiling of specific immune cell subsets*.

Functional activities that can be identified for immune cell populations by using one or more of the Maxpar Direct Expansion Panels include: Activation | Exhaustion | Suppression | Migration | Regulation | Cytokine secretion | Inflammation | Cytotoxic potential

As an example of how Maxpar Direct Expansion Panels complement the Maxpar Direct Assay, the T Cell Activation Expansion Panel (Cat. No. 201409) confirms the phenotyping of T-helper (Th) Th1-like, Th2-like and Th17-like subsets defined by surface markers in the base Maxpar Direct panel (Figure 3). PMA/ionomycin-stimulated samples revealed that IL-2 and TNFα were ubiquitously expressed across all 3 Th subsets. IFNγ, IL-4 and IL-17A were predominantly expressed in, respectively, Th1, Th2, and Th17 populations, as expected. The T Cell Activation Expansion Panel could be used for purposes such as antigen recall assays and other kinds of activation-induced marker (AIM) assays.

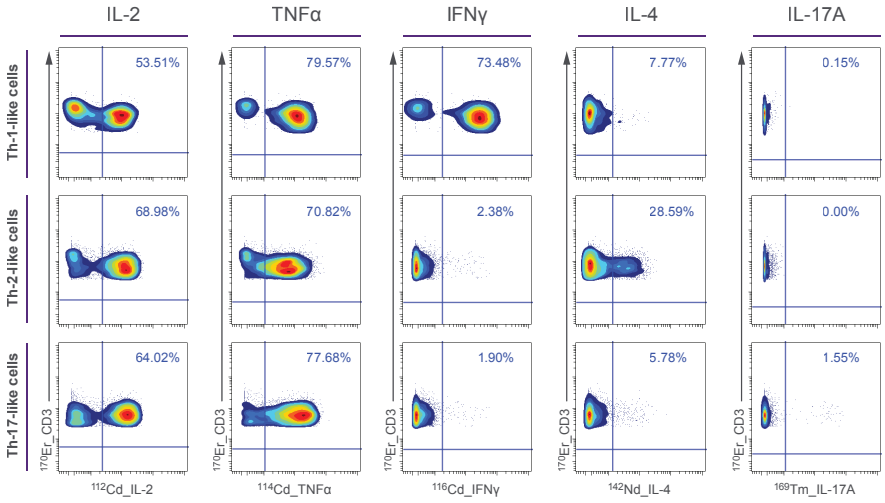


Figure 3. Cytokine profiles of CD4 T-helper subsets after PMA/ionomycin stimulation analyzed using the Maxpar Direct T Cell Activation Expansion Panel in combination with the backbone 30-marker Maxpar Direct Assay. The application note (FLDM-00120) contains complete immune profiling data on expression of cytokines, activation markers, and cytotoxic potential for multiple immune cell populations and for T cells undergoing antigen restimulation.

Details on the contents and potential application of each of the Expansion Panels can be found in the two tables at the end of this flyer.

*Preconfigured Expansion Panels consist of antibodies in individual tubes, not a pre-mixed cocktail.

Maxpar Pathsetter: Customize your personal data analysis expert

Maxpar Pathsetter™ is a fully automated reporting and data analysis solution that automatically identifies and quantifies 37 immune cell types in FCS files from samples processed with the base Maxpar Direct Immune Profiling Assay (Cat. No. 201334).

Developed using probability state modeling, a proven high-parameter analytic approach[1], the software eliminates the variability of manual gating and provides an efficient and reliable solution for longitudinal and multisite studies[2].

Maxpar Pathsetter software can be modified to analyze additional markers, cell types, and reporting metrics, enabling reproducible and rapid analysis of data generated with custom additions to the base Maxpar Direct Immune Profiling Assay.

For support customizing Maxpar Pathsetter for automatic analysis of your expanded Maxpar Direct Assays, contact your Field Application Scientist and refer to the Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151).

Configuration and application information for Maxpar Direct Expansion Panels

Isotopic metal label	Myeloid and B Cell Panel 1	Myeloid and B Cell Panel 2	NK Cell Panel 1	T Cell Panel 1	T Cell Panel 2	T Cell Panel 3	Basic Activation Panel	T Cell Activation Panel	Myeloid and Lymphoid Activation Panel
106Cd							CD107a	CD107a	IL-6
112Cd							IL-2	IL-2	
113Cd								CD69	CD69
114Cd							TNFα	TNFα	TNFα
116Cd							IFNγ	IFNγ	
142Nd	CD181	CD40	CD181		CD11a	OX40		IL-4	CD40
159Tb	CD22	CD22	NKp30	Tim-3	Tim-3	TIGIT			GM-CSF
162Dy	CD80	CD80	NKp46	CD69	CD95 (FAS)	CD69		CTLA-4	CD80
165Ho	CD163	CD163	PD-1	LAG-3	PD-1	PD-1		IL-10	IL-10
169Tm	CD33	CD24	NKG2A	NKG2A	ICOS	Tim-3		IL-17A	CD33
175Lu	PD-1	PD-1	ICOS	PD-1	CXCR4	ICOS			PD-1
196Pt							Perforin	Perforin	
198Pt							Granzyme B	Granzyme B	
209Bi	CD11b	CD11b	TIGIT	TIGIT	TIGIT	CD136/ 4-1BB			IL-1β
Cat. No.	201402	201403	201404	201405	201406	201407	201408	201409	201410

Resources

- Flyer – Maxpar Direct Immune Profiling System (FLDM-400246)
- Application Note – Maxpar Direct Immune Profiling Assay Expanded for Exploring Antigen-Specific Immune Responses (FLDM-00120)
- Bibliography – Maxpar Direct Immune Profiling Assay, March 2022

Expansion Panel	Applications
Myeloid and B Cell Panel 1	• Deeper phenotyping of myeloid and B cell subsets • Characterization of monocytic myeloid-derived suppressor cells • Monitoring of B cell regulation and activation • Monitoring of neutrophil activation and exhaustion
Myeloid and B Cell Panel 2	• Deeper phenotyping of B cell and myeloid cell subsets • Identification of transitional B cells • Monitoring of B cell regulation, activation, and exhaustion
NK Cell Panel 1	• Deeper phenotyping of NK cells • Monitoring of NK regulation, activation, and exhaustion
T Cell Panel 1	• Comprehensive T cell and NK cell exhaustion profiling with inclusion of 5 inhibitory receptor markers
T Cell Panel 2	• Investigation of T cell migration, activation, or exhaustion • In-depth assessment of circulating T follicular helper cells and T stem cell subsets
T Cell Panel 3	• Phenotyping of T cell costimulation and exhaustion • Antigen-specific T cell responses with cytokine-independent activation-induced marker (AIM) assays

Maxpar Direct Basic Activation Expansion Panel	• Contains basic markers of cell activation applicable to many cell types.
	• With Maxpar Direct Immune Profiling Assay only:
	  • Phenotyping of immune cell activation
	  • Study of antigen-specific T cell responses
	  • Measuring of cytotoxic potential in both T and NK cells
	  • Cytokine expression profiles
	• With Maxpar Direct Immune Profiling Assay and 1 of the 6 panels above:
	  • Degranulation and cytotoxic potential
	  • Cytokine profiling of cell subsets identified by the surface marker Expansion Panels
	  • Cytokine expression profiling in conjunction with AIM assays

Maxpar Direct T Cell Activation Expansion Panel	• Deep phenotyping and functional characterization of T cell activation and exhaustion
	• Includes cytokine markers used to:
	  • Study phenotype of cells responding to a particular treatment or stimulant
	  • Identify frequency of antigen-specific T cell responses after natural infection or vaccination
	  • Measure cytotoxic potential in T and NK cells
	  • Identify activated T helper cell populations

Maxpar Direct Myeloid and Lymphoid Activation Expansion Panel	• Cytokine and additional phenotypic profiling of myeloid and lymphoid cell subsets
	• Functional characterization of cell types:
	  • Inflammatory and suppressed monocytes
	  • B cell regulation and activation
	  • T cell activation and exhaustion

Learn more: fluidigm.com/immuneprofile

Contact us: fluidigm.com/about/contactus/tech-support

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References

1. Bagwell, C. Probability state modeling: a new paradigm for cytometric analysis. In: Litwin, V., Marder, P., editor. *Flow Cytometry in Drug Discovery and Development.* Hoboken NJ: John Wiley & Sons, Inc. (2010): 283.
2. Bagwell, C.B. et al. "Multi-site reproducibility of a human immunophenotyping assay in whole blood and peripheral blood mononuclear cells preparations using CyTOF® technology coupled with Maxpar® Pathsetter™, an automated data analysis system." *Cytometry Part B Clinical Cytometry* 98 (2020): 146–160.

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