#P201 2020 CYTO®

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Expanding the Maxpar Direct Immune Profiling Assay panel with additional cadmium-labeled antibodies and functional markers

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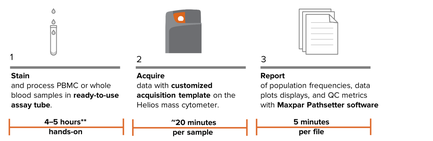
Introduction

CyTOF® technology, based on cytometry by time-of-flight, utilizes metal-tagged antibodies for single-cell detection by mass cytometry. A major advantage of mass cytometry is the ability to conduct comprehensive deep immune profiling studies using highly multiplexed panels comprising upwards of 50 markers[1] without the signal spillover and compensation limitations of flow cytometry.

The **Maxpar® Direct™ Immune Profiling Assay**™ (Cat. No. 201325) utilizes a **ready-to-use dry-format 30-antibody staining panel** for human whole blood and PBMC immunophenotyping by mass cytometry (Figure 1). Paired with **Maxpar Pathsetter™ software,** users can **automatically resolve** this core 30 marker panel into **37 immune cell populations** with reproducible results[2]. This assay is ideal for use in longitudinal studies of immune response in the context of immune-mediated diseases and is already in use in COVID-19 research[3,4].



**Staining includes an overnight incubation

Target (clone)	Isotope
CD45 (HI30)	89Y
Cell-ID Intercalator-103Rh	103Rh
Available open channel	**106Cd**
Available open channel	**110Cd**
Available open channel	**111Cd**
Available open channel	**112Cd**
Available open channel	**113Cd**
Available open channel	**114Cd**
Available open channel	**116Cd**
CD196 /CCR6 (G034E3)	141Pr
Available open channel	**142Nd**
CD123 (6H6)	143Nd
CD19 (HIB19)	144Nd
CD4 (RPA-T4)	145Nd
CD8a (RPA-T8)	146Nd

Target (clone)	Isotope
CD1c (Bu15)	147Sm
CD16 (3G8)	148Nd
CD45RO (UCHL1)	149Sm
CD45RA (HI100)	150Nd
CD161 (HP-3G10)	151Eu
CD194/CCR4 (L291H4)	152Sm
CD25 (BC96)	153Eu
CD27 (O323)	154Sm
CD57 (HCD57)	155Gd
CD183/CXCR3 (G025H7)	156Gd
CD185/CXCR5 (J252D4)	158Gd
Available open channel	**159Tb**
CD28 (CD28.2)	160Gd
CD38 (HB-7)	161Dy
Available open channel	**162Dy**

Target (clone)	Isotope
CD56/NCAM (NCAM16.2)	163Dy
TCRgd (B1)	164Dy
Available open channel	**165Ho**
CD294 (BM16)	166Er
CD197/CCR7 (G043H7)	167Er
CD14 (63D3)	168Er
Available open channel	**169Tm**
CD3 (UCHT1)	170Er
CD20 (2H7)	171Yb
CD66b (G10F5)	172Yb
HLA-DR (LN3)	173Yb
IgD (IA6-2)	174Yb
Available open channel	**175Lu**
CD127 (A019D5)	176Yb
Available open channel	**209Bi**

Figure 1. Maxpar Direct Immune Profiling Assay workflow (top) and panel with open channels.

The Maxpar Direct Immune Profiling Assay **is highly customizable** with the availability of **more than 14 additional channels to provide panel flexibility** (Figure 1)**.** As shown below, background signal in these 14 channels is minimal, resulting in high-resolution data for analysis.

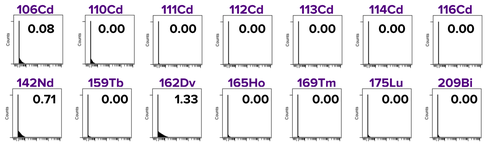


Figure 2. Low background signal in available channels, indicating high degree of flexibility. Histograms depict signal intensity and median dual counts (inset) of open channels on total live singlets in human PBMC.

Additional markers may be obtained from the **Fluidigm catalog of reagents** or may be **self-custom-conjugated**, for example, with the Fluidigm Maxpar MCP9 Antibody Labeling Kits (7 cadmium isotope labeling kits; see technical data sheet FLDM-00018) as demonstrated in this poster or by Fluidigm custom conjugation services (3999999-1).

Furthermore, the Maxpar Direct Immune Profiling Assay model in Pathsetter software can incorporate additional markers to profile expression changes, update cell type definitions, or identify new immune cell types. A custom report can be updated to reflect the changes in the model, such as reporting new cell type frequencies.

Collectively, we demonstrate that the Maxpar Direct Immune Profiling Assay consists of a streamlined workflow in an easy-to-use format that can be further expanded to accommodate specific needs of users.

Materials and Methods

Expansion of the Maxpar Direct Immune Profiling Assay to a 44-marker panel

Additional antibodies were selected in open channels to expand the 30-marker Maxpar Direct Immune Profiling Assay to a 44-marker panel. Markers were selected to further resolve monocyte subsets and to classify monocytic myeloid-derived suppressor cells (Mo-MDSCs). In addition, high-value functional markers were added to the panel to investigate their expression on cells in a disease model. Antibodies used to expand the panel consisted of custom conjugates using the MCP9 Cd labeling kits and Fluidigm catalog reagents. Purified antibodies used for MCP9 Cd labeling were purchased from BioLegend®.
Antibodies were titrated on PBMC to determine optimal concentration in the panel.

Fluidigm Cat. No.	Antibody (clone)	Isotope
Custom	CD33 (WM53)*	106Cd
Custom	CD99 (HCD99)*	110Cd
Custom	CD11b (ICRF44)*	111Cd
Custom	CD2 (TS1/8)*	112Cd
Custom	CD9 (SN4 C3-3A2)*	113Cd
Custom	CD64 (10.1)*	114Cd
Custom	CD7 (CD7-6B7)*	116Cd
3142018B	CD134/OX40 (ACT35)	142Nd
3159037B	CD366/Tim-3 (F38-2E2)	159Tb
3162038B	CD95/Fas (DX2)	162Dy
3165042B	CD279/PD-1 (EH12.2H7)	165Ho
3169029B	CD274/PD-L1 (MIH1)	169Tm
3175039B	CD278/ICOS (C398.4A)	175Lu
3209013B	TIGIT (MBSA43)	209Bi

*Custom conjugation using MCP9 Cd labeling kit (Fluidigm catalog numbers 201106A, 201110A, 201111A, 201112A, 201113A, 201114A, 201116A)

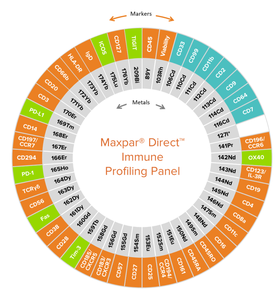


Figure 3. Additional antibodies tabulated (left) and shown in the integrated 44-antibody panel in the wheel

Sample Preparation, Staining, and Analysis

- The expanded 44-marker Maxpar Direct Immune Profiling Assay panel was tested on frozen human PBMC (STEMCELL™ Technologies) from healthy donors and donors diagnosed with rheumatoid arthritis.
- Thawed PBMC were resuspended in complete Gibco™ Advanced RPMI-1640 media (Thermo Fischer Scientific™) and left untreated or treated with 1 µg/mL PHA (Sigma-Aldrich®) for 18 hr in a 6-well plate.
- 3 x 10^6 cells were stained per Maxpar Direct Immune Profiling Assay tube. A master mix comprising the 14 additional antibodies was aliquoted into the tube immediately following the addition of sample to the dried antibody pellet. Staining and acquisition proceeded as outlined in the Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition User Guide (PN 400286).
- Samples were acquired on a Helios™ instrument running CyTOF Software v7.0.5189.
- Normalized FCS files were analyzed using Maxpar Pathsetter software v2.0.45.
- The default Immune Profiling Assay model template in Maxpar Pathsetter software was modified to incorporate the additional markers by following the Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151). For more information, contact your local Fluidigm field application specialist.

Figure 4. Maxpar Direct Immune Profiling Assay stained samples are acquired on a Helios cytometer. Normalized data may be analyzed by Maxpar Pathsetter software for automated analysis by default or customized models.

Results

Validation of marker expression using Maxpar Pathsetter Cen-se′ plots

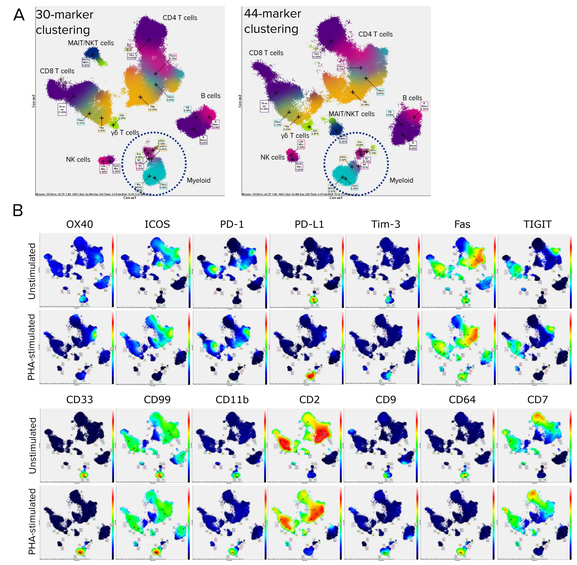


Figure 5. Cen-se′[5] (Cauchy enhanced nearest-neighbor stochastic embedding) clustering, a dimensionality reduction tool (GemStone™), performed in Maxpar Pathsetter software allows visualization of expression levels of the additional markers in immune cell clusters. **(A)** Cen-se′ clustering on the 30 markers in the Maxpar Direct Immune Profiling Assay in the panel compared to the clustering on the 44 markers. The additional markers further resolve the myeloid cell populations into more distinct clusters (dashed circle). **(B)** The Cen-se′ plot's color scheme was set to display intensity heat map, and the relative intensity is visualized for each of the additional markers. Increased functional marker expression are observed in PHA-stimulated cells. Plots shown are a representative of 1 of 3 donor PBMC samples with rheumatoid arthritis.

Reduced PD-L1 and OX40 up-regulation upon PHA stimulation in RA PBMC

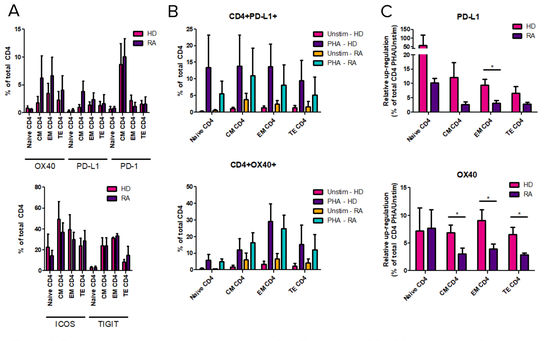


Figure 6. Expression levels of functional markers on CD4 T cells within PBMC from healthy (HD) and rheumatoid arthritis (RA) donors were measured using Maxpar Pathsetter and compared. **(A)** Basal expression of OX40, PD-L1, PD-1, ICOS, and TIGIT on unstimulated HD and RA CD4 T cells. **(B)** PBMC samples were either treated with PHA overnight or cultured unstimulated. Population frequencies of PD-L1 expressing cells within naive, central memory (CM), effector memory (EM), and terminal effector (TE) CD4 T cell subsets are shown. **(C)** The relative up-regulation (ratio of PHA/unstimulated) of PD-L1 and OX40 are shown for CD4 T cell subsets. For A–C, data shown is mean and SD of 3 independent HD and RA donors.
* P value <0.05 as calculated using Student's *t*-test.

Updating the Maxpar Pathsetter report and model to include additional markers

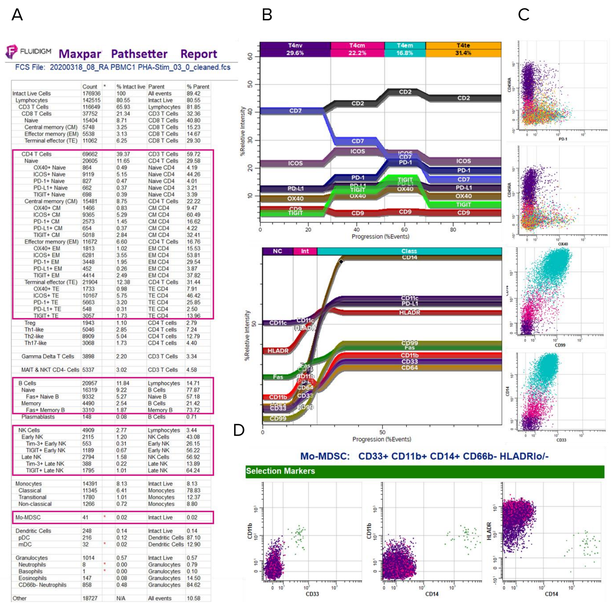


Figure 7. The Immune Profiling Assay model and report template provided in Maxpar Pathsetter were updated to incorporate the additional markers. **(A)** The summary report page was updated to report the event count, % of intact live cells, and % of parent for OX40, ICOS, PD-1, PD-L1, and TIGIT expressing cells on CD4 T cell subsets, Fas positive events on B cells, and Tim-3 and TIGIT expressing NK cells, and to report monocytic MDSCs. **(B)** The additional markers were included in cell progression plots for CD4 T cells (staged into naive, central memory, effector memory, and terminal effector) and monocytes (staged into nonclassical, intermediate, and classical), allowing comparison of marker expression in each cell stage. For example, CD33 and CD99 expression is lowest in nonclassical monocytes and highest in classical monocytes. **(C)** Examples of dot plots using the additional markers are shown. The colors of the dots correspond to the cell stages shown in B. **(D)** The additional markers were used to classify monocytic MDSC ($CD33^+CD11b^+CD14^+HLA\text{-}DR^{lo/-}$). The MDSC events are green dots. Purple and magenta dots are B cells.

Conclusions

- The Maxpar Direct Immune Profiling Assay panel was expanded by adding 14 markers to create a 44-marker panel.
- The additional markers were incorporated into the Immune Profiling Assay model and report template in Maxpar Pathsetter to include 27 additional frequency measurements on various cell subsets.
- Relative expression levels of these 14 new markers can be visualized in Cen-se′ plots and cell progression plots generated in Maxpar Pathsetter.
- Frequencies of populations can be rapidly quantified using Maxpar Pathsetter.
  - Increased frequencies of OX40-expressing CD4 T cells in PBMC from RA patients were observed, as previously reported by Jiang et al.[5]
  - Reduced OX40 and PD-L1 up-regulation was observed on CD4 T cell subsets upon PHA stimulation in PBMC from rheumatoid arthritis donors.
- We demonstrated the flexibility of the Maxpar Direct Immune Profiling Assay (and Maxpar Pathsetter software) to accommodate expanded panels in order to further facilitate deep immune profiling studies.

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