FLUIDIGM® | Application Note

FLDM-00043 Rev 01

Seven Cadmium Labeling Kits Increase Flexibility in Panel Design

Introduction

Mass cytometry, powered by CyTOF® technology, leads the field of high-parameter cytometric analysis with 135 independent channels (75–209 Da) available for signal detection. With the release of seven cadmium (Cd) labeling kits (106, 110, 111, 112, 113, 114, 116 Da), there are now 56 commercially available tags for use in panel building and cell identification. In addition to increasing the breadth and depth of profiling at a single-cell resolution, the newly added metals increase flexibility of panel design for users.

This application note presents an example showing reconfiguration of an established 29-marker human immune monitoring panel in order to open the more sensitive lanthanide (Ln) channels for detection of additional markers. Figure 1 depicts how the original 29-marker panel (top) was rearranged to include the cadmium tags (bottom), thereby increasing the number of additional available detection channels from 8 to 15.

Summary

- Cadmium labeling kits expand the maximum panel size with commercially available reagents for mass cytometry to more than 50 parameters.
- Cadmium-conjugated lineage marker antibodies provide clear separation between positive and negative populations.
- Designing a panel with high-expression major lineage markers in cadmium channels increases flexibility of panel design and reserves the high sensitivity lanthanide channels for additional markers.

Tips for Success

Cadmium isotopes are on the lower mass range of detection and are therefore detected with lower sensitivity relative to lanthanide metal isotopes on the mass cytometer. For optimal performance, cadmium tags should be conjugated to mid-to-high-intensity markers. Major lineage markers, such as CD3 for T cells and CD19 for B cells, are prime candidates for cadmium conjugation.

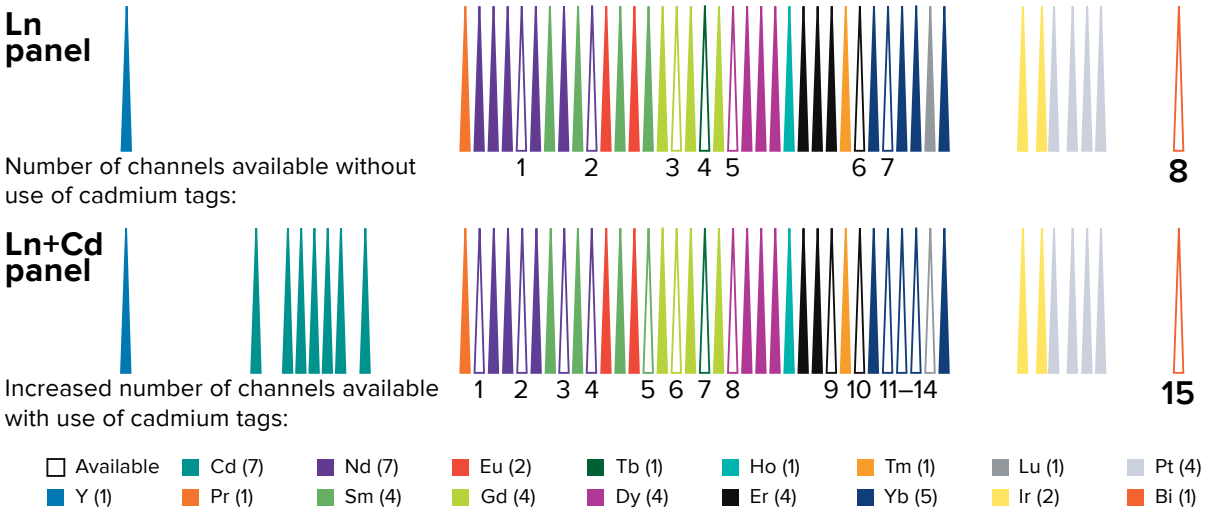

Figure 1. The use of cadmium tags increases lanthanide channel availability in panel design. Staining PBMC with a 29-marker panel containing only lanthanides (Ln) provides 8 available channels (top panel). Conjugating seven major lineage markers from the panel to cadmium expands available channels to 15 for custom markers of interest (bottom panel).

Study Design

Seven mid-to-high-intensity major lineage markers from a conventional 29-marker human immune monitoring panel were selected for metal reassignment. Subsequently, antibodies were conjugated to cadmium metal tags that were designed with minimal signal crosstalk. Human peripheral blood mononuclear cells (PBMC) were stained with the following panels:

1. **Ln panel:** 29 markers as shown in Table 1 with the seven highlighted major lineage markers in lanthanide (Ln) channels
2. **Ln+Cd panel:** 29 markers as shown in Table 1 with the seven highlighted major lineage markers in cadmium (Cd) channels

Table 1. A 29-marker panel was reconfigured by moving seven major lineage markers to cadmium to increase available lanthanide channels.

Markers highlighted in orange were used in either the Ln panel or the Ln+Cd panel, as appropriate. All other markers were used in both panels.

Marker (clone)	Metal Tags	Marker (clone)	Metal Tags
CD45 (HI30)	^{89}Y	CD28 (CD28.2)	^{160}Gd
CCR6 (G034E3)	^{141}Pr	CD66b (80H3)	^{162}Dy
CD19 (HIB19)	^{142}Nd	CXCR3 (G025H7)	^{163}Dy
	^{111}Cd	CD161 (HP-3G10)	^{164}Dy
CD127 (A019D5)	^{143}Nd	CD45RO (UCHL1)	^{165}Ho
CD38 (HIT2)	^{144}Nd	CD24 (ML5)	^{166}Er
IgD (IA6-2)	^{146}Nd	CCR7 (G043H7)	^{167}Er
CD11c (Bu15)	^{147}Sm	CD8 (SK1)	^{168}Er
CD16 (3G8)	^{148}Nd		^{112}Cd
	^{113}Cd	CD25 (2A3)	^{169}Tm
CCR4 (L291H4)	^{149}Sm	CD20 (2H7)	^{171}Yb
CD123 (6H6)	^{151}Eu	HLA-DR (L243)	^{173}Yb
TCRγδ (11F2)	^{152}Sm		^{106}Cd
CXCR5 (RF8B2)	^{153}Eu	CD4 (SK3)	^{174}Yb
CD3 (UCHT1)	^{154}Sm		^{116}Cd
	^{114}Cd	CD14 (M5E2)	^{175}Lu
CD45RA (HI100)	^{155}Gd		^{110}Cd
CD27 (L128)	^{158}Gd	CD56 (NCAM16.2)	^{176}Yb

Experimental Results

Cadmium isotopes fall within the lower detection range of the mass cytometer. Therefore, a cadmium-labeled antibody may be detected at a lower intensity than the same antibody labeled with a lanthanide metal. To evaluate data quality when using cadmium-tagged antibodies, population separation and frequencies were compared between antibody panels that utilized only lanthanide-labeled antibodies (Ln panel) or lanthanide- and cadmium-labeled antibodies (Ln+Cd panel) when PBMC were stained according to the study design. Live, singlet, CD45+ CD66b– events were gated. Biaxial plots of the reassigned markers (HLA-DR, CD19, CD14, CD8, CD16, CD3, and CD4) were compared between the two panels.

Population separation and frequencies were similar between Ln and Ln+Cd panels for the reassigned markers. Populations were both qualitatively and quantitatively comparable among the gated populations (Figure 2).

In addition to population analysis by manual gating, further investigations explored population identification using the dimensionality reduction clustering algorithm t-distributed stochastic neighbor embedding (t-SNE). To assess distinct as well as overlapping population islands, t-SNE plots for HLA-DR (expressed on B cells and monocytes) and CD4 (expressed on CD4 T cells and monocytes) were selected for analysis to compare major population islands between Ln and Ln+Cd panel staining (Figure 3).

HLA-DR and CD4 in the Ln and Ln+Cd panels exhibited analogous intensity patterns on corresponding population islands. Similar patterns were observed for all other Cd-labeled antibodies tested (data not shown). In turn, Cd-labeled antibodies provided adequate signal for comparable t-SNE islands to their Ln-tagged counterparts.

Population islands were gated on the t-SNE plots and major lineages were identified using phenotyping markers. Population frequencies of islands were in agreement between the two panels (Table 2), confirming that the distribution from the seven Cd tagged antibodies paralleled that of Ln-tagged counterparts within high-dimensional analysis.

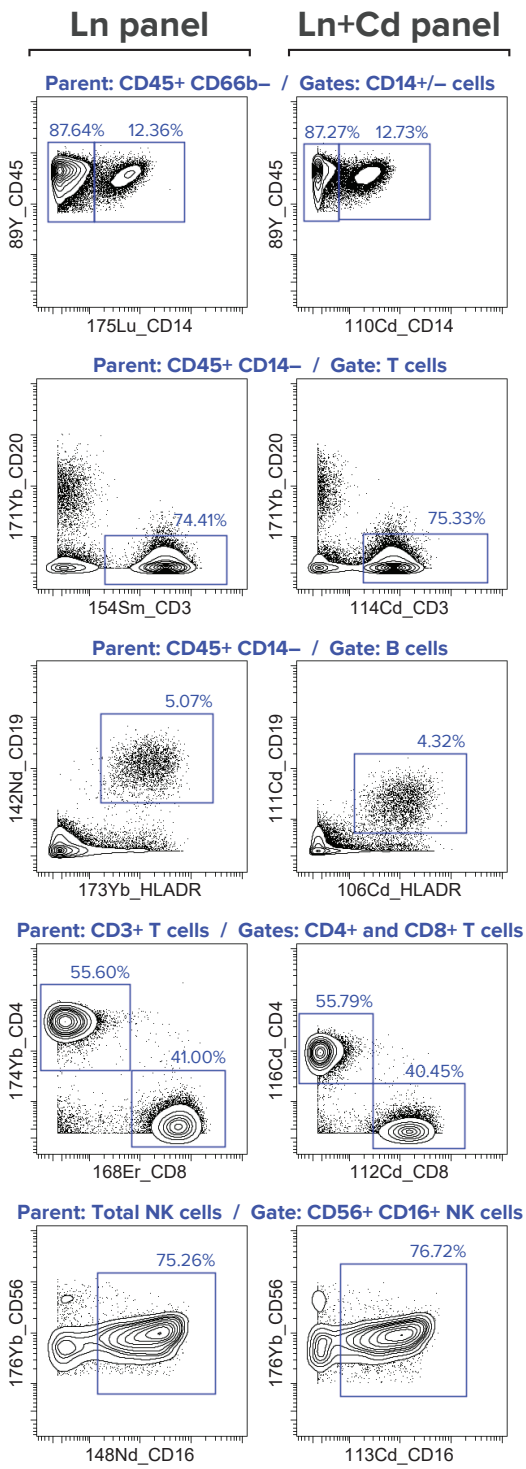

Figure 2. Reassignment of major lineage markers to cadmium channels provides matching population separation and frequencies to lanthanide channels. PBMC were stained with either the Ln or Ln+Cd panel, according to the study design. Cells were gated for live, singlet, CD45+ CD66b– events prior to further gating. Biaxial plots using the reassigned markers (HLA-DR, CD19, CD14, CD8, CD16, CD3, CD4) display parent populations, downstream gates, and associated frequencies in blue.

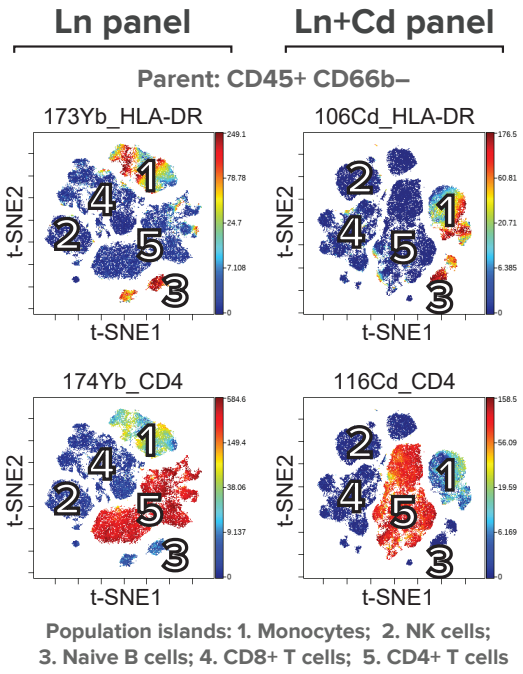

Figure 3. Population islands identified with Ln or Ln+Cd panels using t-SNE dimensionality reduction analysis are comparable. PBMC were stained with either Ln (left) or Ln+Cd (right) panels, according to the study design. Cells were gated for live, singlet, CD45+ CD66b– events. Upon t SNE dimensionality reduction analysis of CD45+ CD66b– cells, plots were generated for intensity of HLA-DR and CD4 tagged with either lanthanide metals (left) or cadmium metals (right). Major population islands are numerically labeled.

Table 2. Frequencies of events in major t-SNE population islands from the Ln panel and Ln+Cd panel.

Population	Ln Panel (% pop freq)	Ln+Cd Panel (% pop freq)
NK cells	12.74	11.80
Monocytes	15.87	15.45
Naive B cells	2.74	2.60
T cells	65.87	67.80
CD4+ T cells	36.74	37.64
CD8+ T cells	27.05	28.41

Both manual gating and the t-SNE automatic clustering algorithm confirmed that Cd-tagged major lineage marker antibodies resulted in data comparable to their Ln-tagged counterparts.

Discussion and Conclusion

The metal tag reassignment experiment demonstrated successful use of cadmium labeling kits for increased channel availability. Staining with cadmium resulted in effective population separation for comparable frequencies to the benchmark Ln panel. We recommend assigning high-intensity markers to cadmium metal tags when redesigning panels for the most effective use of the mass cytometer detection range.

Upon metal reassignment, seven valuable lanthanide channels were made available. For these seven channels, the number of preconjugated antibody clones from the Fluidigm catalog are listed below, with a common marker suggested:

Table 3. The number of preconjugated antibody (Ab) clones that correspond to available channels in the Ln+Cd panel design.

Available Channels	No. of Abs in Catalog	Example Ab (clone)	Catalog Number
142Nd	6	CD11a (HI111)	3142006B
148Nd	9	CD274/PD-L1 (29E.2A3)	3148017B
154Sm	9	TIM-3 (F38-2E2)	3154010B
168Er	13	CD278/ICOS (C398.4A)	3168024B
173Yb	10	CD137/4-1BB (4B4-1)	3173015B
174Yb	8	CD279/PD-1 (EH12.2H7)	3174020B
175Yb	12	CD223/LAG-3 (11C3C65)	3175033B

The power of mass cytometry resides in the high number of independent channels available for signal detection. The new cadmium labeling kits enable over 50 surface and intracellular parameters to be interrogated at once, opening new possibilities to study single cells with greater breadth and depth.

Protocols

All antibodies were titrated prior to use in this application note. The Gaussian parameter cleanup method and gating hierarchies followed the Cytobank Premium experiment (No. 151947).

For detailed instructions on conjugation and staining procedures, instrument and software operation, see Maxpar® Antibody Labeling User Guide (PRD002), Maxpar Cell Surface Staining with Fresh Fix Protocol (400276) and Helios™, a CyTOF System User Guide (400250).

Appendix: Ordering Information

Fluidigm antibodies	
Name	**Catalog Number**
^{89}Y-CD45 (HI30)	3089003B
^{141}Pr-CCR6 (G034E3)	3141003A
^{142}Nd-CD19 (HIB19)	3142001B
^{143}Nd-CD127 (A019D5)	3143012B
^{144}Nd-CD38 (HIT2)	3144014B
^{146}Nd-IgD (IA6-2)	3146005B
^{147}Sm-CD11c (Bu15)	3147008B
^{148}Nd-CD16 (3G8)	3148004B
^{149}Sm-CCR4 (L291H4)	3149029A
^{151}Eu-CD123 (6H6)	3151001B
^{152}Sm-TCRγδ (11F2)	3152008B
^{153}Eu-CXCR5 (RF8B2)	3153020B
^{154}Sm-CD3 (UCHT1)	3154003B
^{155}Gd-CD45RA (HI100)	3155011B
^{158}Gd-CD27 (L128)	3158010B
^{160}Gd-CD28 (CD28.2)	3160003B
^{162}Dy-CD66b (80H3)	3162023B
^{163}Dy-CXCR3 (G025H7)	3163004B
^{164}Dy-CD161 (HP-3G10)	3164009B
^{165}Ho-CD45RO (UCHL1)	3165011B
^{166}Er-CD24 (ML5)	3166007B
^{167}Er-CCR7 (G043H7)	3167009A
^{168}Er-CD8 (SK1)	3168002B
^{169}Tm-CD25 (2A3)	3169003B
^{171}Yb-CD20 (2H7)	3171012B
^{173}Yb-HLA-DR (L243)	3173005B
^{174}Yb-CD4 (SK3)	3174004B
^{175}Lu-CD14 (M5E2)	3175015B
^{176}Yb-CD56 (NCAM16.2)	3176008B

Fluidigm reagents	
Name	**Part Number**
Maxpar® MCP9 Antibody Labeling Kit, 106Cd—4 Rxn	201106A
110Cd—4 Rxn	201110A
111Cd—4 Rxn	201111A
112Cd—4 Rxn	201112A
113Cd—4 Rxn	201113A
114Cd—4 Rxn	201114A
116Cd—4 Rxn	201116A
Cell-ID™ Cisplatin	201064
Maxpar Cell Staining Buffer	201068
Maxpar Fix and Perm Buffer	201067
Maxpar PBS	201058
Cell-ID Intercalator-Ir—125 µM	201192A
Maxpar Cell Acquisition Solution	201240
EQ™ Four Element Calibration Beads	201078

Third-party reagents		
Vendor	**Name**	**Part Number**
BioLegend®	Anti-HLA-DR (L243)	307651
BioLegend	Anti-CD14 (M5E2)	301843
BioLegend	Anti-CD19 (HIB19)	302247
BioLegend	Anti-CD8 (SK1)	344727
BioLegend	Anti-CD16 (3G8)	302051
BioLegend	Anti-CD3 (UCHT1)	300443
BioLegend	Anti-CD4 (SK3)	344625
Thermo Fisher Scientific	Pierce 16% Formaldehyde	28906
Millipore® Sigma	TCEP Solution	646547
CANDOR Bioscience	HRP-Protector Peroxidase	222 050
CTL	PBMC vials	n/a

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