

Teiko

Panel Development Report

Client: ABC Biopharma

Project Code: ABC001

Introduction

This Panel Development Report provides a detailed analysis of gated cell populations and cell state markers obtained from profiling one healthy human donor peripheral blood mononuclear cell (PBMC) sample, as well as a blended PBMC control, using ABC Biopharma's 26-Marker Human PBMC Pan-Immune Profiling, Spectral Flow Panel.

A custom marker is evaluated through a six-point titration series, with the optimal concentration determined based on a combination of factors, including stain index, arcsin ratio, separation of positive and negative populations, and spillover into other channels. Staining concentrations for each antibody are noted.

A representative gating scheme is presented from a healthy PBMC donor sample. Functional marker performance is assessed in both unstimulated and stimulated PBMC samples (e.g., PMA or PHA) to demonstrate the panel's gating performance and capabilities.

Materials & Methods

- Sample Types: Healthy human PBMCs
- Number of Samples Analyzed: 2
- Markers Analyzed: CCR7, CD45RA, IgM, CD20, CD3, CD28, CD38, CD56, PD 1, CD141, CD8, CD14, HLADR, CD25, CD4, CD16, IgD, TCRgd, CD11c, CD127, CD1c, CD19, CD123, CD45, Viability, CD27, X marker
- Cytometry Technique: Spectral Flow Cytometry
- Cytometry Instrument: Cytex Aurora, 5 Lasers
- Cytex Panel Number: Human Whole Blood Pan-Immune Profiling, Spectral Flow
- Immunoprofiling Kit: Cytex® 25-color immunoprofiling assay

Conclusion

The custom marker demonstrates positive signal detection in respective immune cell populations, including T cells, B cells, NK cells, monocytes, dendritic cells or cell lines. The optimal staining concentration has been determined to ensure an appropriate signal-to-noise ratio with minimal channel spillover for all markers.

Performance of the backbone panel is consistent with results outlined in our CLIA validation [report](#).

Results

1. Titration of custom marker: X

Teiko Lot #	Detector	Marker	Clone	Stock (mg/ml)	Optimal conc.
TEK001	R780	X	2D1	3.125	0.1875 ug/mL

a. Stain index

The stain index measures the separation between positive and negative populations. Higher values indicate stronger signal resolution and lower background. CD45 demonstrated the optimal stain index at 0.1875 ug per mL. At higher concentrations, increased background staining in the negative population was observed, which decreased the stain index.

[Staining plots for each titration concentration will be included in the finalized staining overview figure to illustrate positive and negative population resolution.]

b. Frequency of gated populations

Healthy donor PBMCs were included as a negative control to assess nonspecific staining across the titration series. Elevated false positives above 0.1 percent were detected in the negative control PBMC sample when the antibody concentration exceeded 3 ug per mL.

[Gating plots for positive and negative populations across all titration concentrations will be included in the gating overview figure.]

c. Spillover assessment

On the Cytex Aurora, fluorophore signals may spread into neighboring detectors when antibody concentrations are too high. Spillover from the fluorophore assigned to CD45 was evaluated by monitoring adjacent detectors that receive overlapping emission. Increased spreading error and higher false positive rates were observed when staining concentrations surpassed 0.375 ug per mL. These findings will be considered when applying gates to markers positioned in detectors sensitive to this spillover pattern.

[Spillover values across all titration concentrations are summarized in the accompanying spillover table and figure.]

d. FMO control for CD45

An FMO control for [marker X] was included to assess background signal and to confirm the gating boundary for the custom marker within the full panel. The [marker X] FMO shows minimal background in the R780 detector, allowing clear distinction between [marker X] positive and negative events.

[FMO plot for the custom marker will be included here to illustrate background fluorescence and the gating threshold used for custom marker(s).]

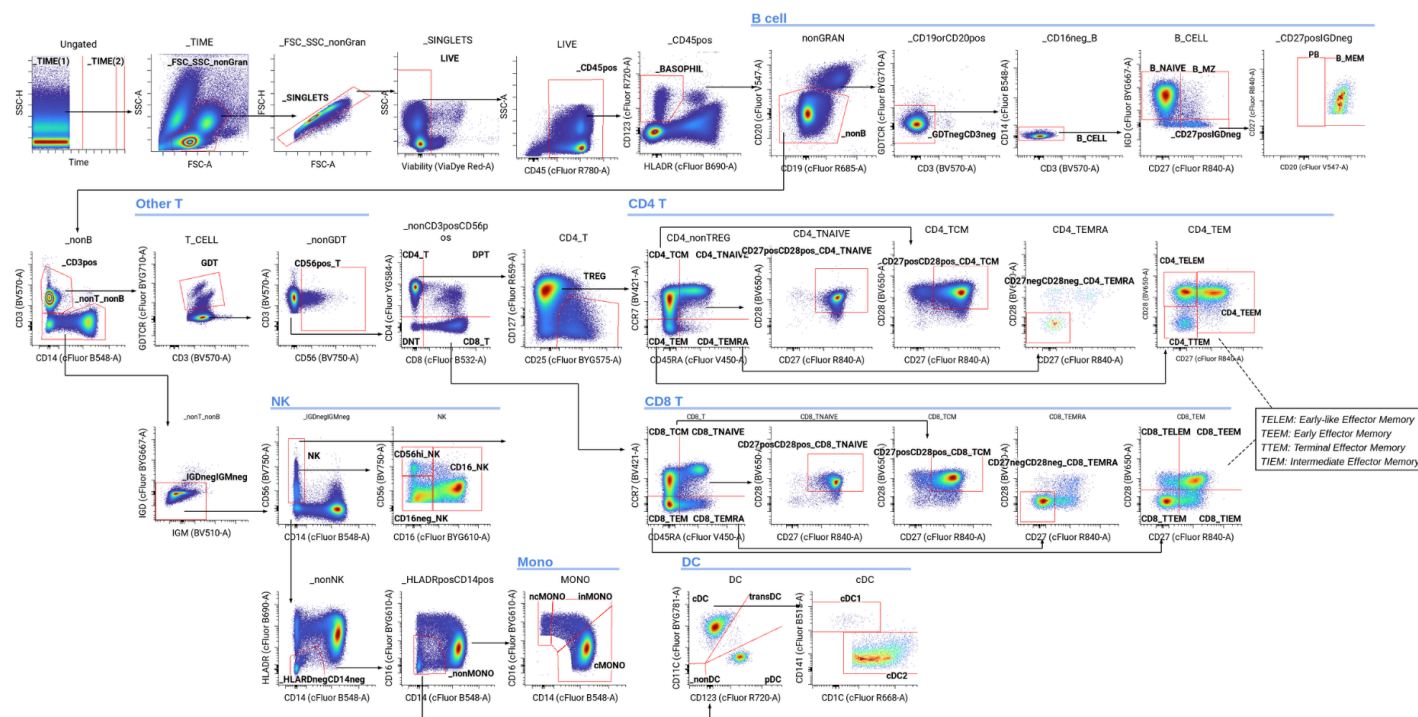
2. Panel Details

Marker	Antibody Clone	Fluorophore	Laser	Stock Concentration [ug/mL]	Staining Concentration [ng/μL]
CCR7	G043H7	Brilliant Violet 421	Violet	140	3.5
CD45RA	HI100	cFluor V450	Violet	*	N/A
IgM	MHM-88	Brilliant Violet 510	Violet	40	1
CD20	2H7	cFluor V547	Violet	*	N/A
CD3	UCHT1	Brilliant Violet 570	Violet	80	2
CD28	CD28.2	Brilliant Violet 650	Violet	50	1.25
CD38	HIT2	Brilliant Violet 711	Violet	100	2.5
CD56	5.1H11	Brilliant Violet 750	Violet	50	1.25
PD-1	EH12.2H7	Brilliant Violet 785	Violet	100	2.5
CD141	M80	cFluor B515	Blue	*	N/A
CD8	SK1	cFluor B532	Blue	*	N/A
CD14	63D3	cFluor B548	Blue	*	N/A
HLA-DR	L243	cFluor B690	Blue	*	N/A
CD25	BC96	cFluor BYG575	Yellow/Green	*	N/A
CD4	SK3	cFluor YG584	Yellow/Green	*	N/A
CD16	3G8	cFluor BYG610	Yellow/Green	*	N/A
IgD	IA6-2	cFluor BYG667	Yellow/Green	*	N/A
gdTCR	B1	cFluor BYG710	Yellow/Green	*	N/A
CD11c	3.9	cFluor BYG781	Yellow/Green	*	N/A
CD127	A019D5	cFluor R659	Red	*	N/A
CD1c	L161	cFluor R668	Red	*	N/A
CD19	HIB19	cFluor R685	Red	*	N/A
CD123	6H6	cFluor R720	Red	*	N/A
CD45	2D1	cFluor R780	Red	*	N/A
CD27	QA17A18	cFluor R840	Red	*	N/A
X Marker	xxx	xxx	xxx	xxx	xxx
Viability	Viadye	Viadye Red 615/740	Red	*	N/A

* The stock concentration is not provided by the manufacturer. 5 µL of antibody/test was used.

3. Gating Example

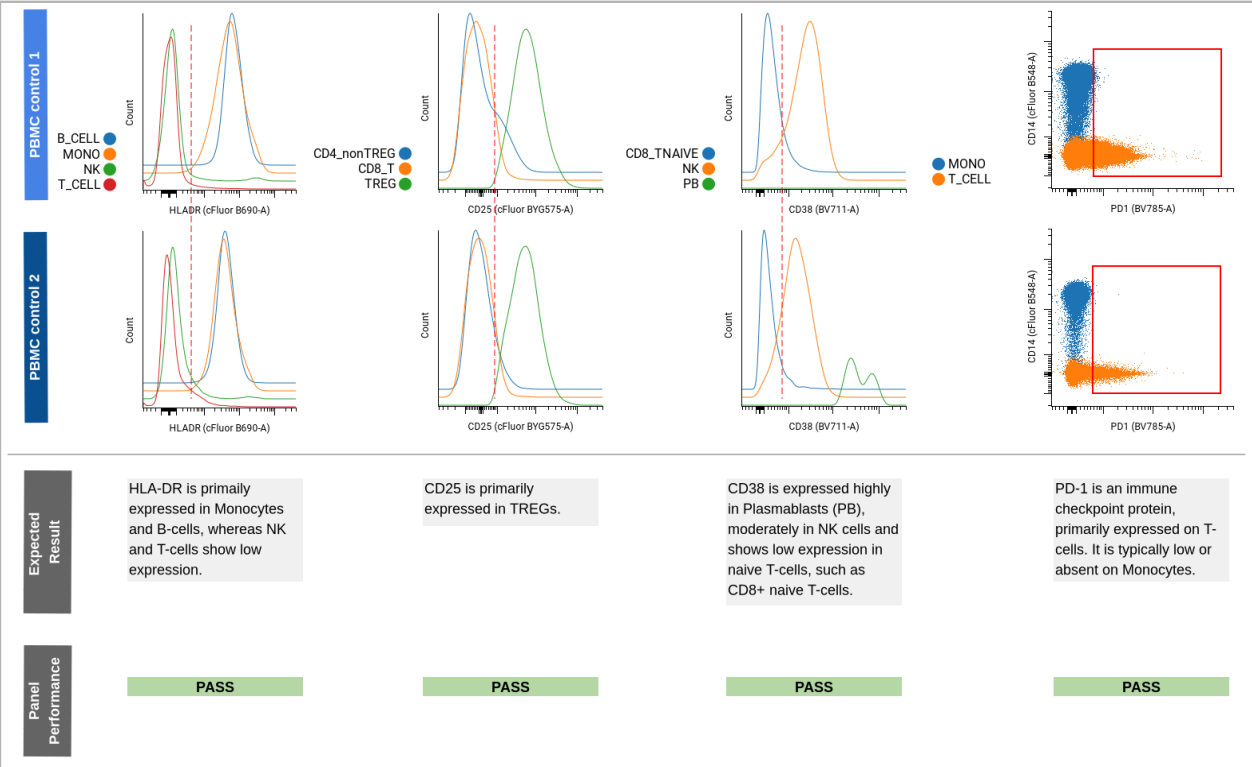
Illustration of the panel performance on PBMCs. Please note that the gates shown below are for internal panel performance assessment during verification. You will have the opportunity to review and modify the gating strategy for clinical samples after acquisition and before data analysis and delivery.



These gates are for internal panel performance assessment during verification. The gating of clinical samples will be provided in the gating QC report. Any gating changes must be communicated in writing before statistical analysis.

4. Reference Plots for State Markers

Histograms or dot plots for functional state markers are presented, with representative populations illustrating both positive and negative signals.



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