Cytometry Part A
Author Checklist: MIFlowCyt-Compliant Items

Requirement	Please Include Requested Information
1.1. Purpose	This panel was developed, optimized and qualified for the assessment of human antigen-specific T-cell responses by intracellular cytokine staining, including memory/differentiation markers. Multiple T cell lineages are identified including classical CD4+, CD8+, and gd, MAIT, and NK T. Additionally NK- cell responses are also evaluated.
1.2. Keywords	Cytometry, human PBMC, T cells, MAIT cells, gd T cells, NK cells, memory, intracellular cytokine staining
1.3. Experiment variables	Frequency of cytokine expressing cells of various phenotypes
1.4. Organization name and address	Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109 USA
1.5. Primary contact name and email address	Stephen De Rosa, sderosa@fredhutch.org
1.6. Date or time period of experiment	January 2017- July 2018
1.7. Conclusions	Development, optimization and cross-validation of 26-color ICS panel
1.8. Quality control measures	Fluorescence Minus One tests Unstimulated control for each sample, same cryopreserved PBMC sample used as control for each experiment Rainbow 6^{th} peak and Supra beads standardize instrument
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	Cryopreserved peripheral blood mononuclear cells
2.1.1.2. Biological sample source description	Human
2.1.1.3. Biological sample source organism description	Human adults
2.1.2.2. Environmental sample location	Not applicable
2.3. Sample treatment description	6-hour ex vivo stimulation of PBMC with DMSO (negative control), SEB (positive control) or peptide pools to various proteins (from HIV, TB)
2.4. Fluorescence reagent(s) description	See Table 2 and Online Table 2
3.1. Instrument manufacturer	Becton Dickinson (BD)
3.2. Instrument model	FACSymphony A5
3.3. Instrument configuration and settings	5 lasers, 28-colors. See Online Table 1
4.1. List-mode data files	Example of sample staining for SEB, DMSO negative control and compensation samples are available in http://flowrepository.org Repository identifier: FR-FCM-Z2DD
4.2. Compensation description	Single-stained cell and/or capture bead samples, software compensation in FlowJo
4.3. Data transformation details	Biexponential transformation (Custom transformation and Manually Specify transformation options) in FlowJo
4.4.1. Gate description	Hierarchical gating to identify lineages and then one dimensional gates on each cytokine and memory/activation marker, see Figure 1 and Online Figure

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4.4.2. Gate statistics	See Figure 1
4.4.3. Gate boundaries	See Figure 1

Notes

Feel free to use more space than allocated.

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