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OMIP-013: Differentiation of Human T-Cells

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Technical details may be found in Supporting Information in the online version of this article.

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Purpose and Appropriate Sample Types

The present panel was optimized to investigate the differentiation status of $CD4^+$ and $CD8^+$ T-cells in peripheral blood mononuclear cells (PBMC) from healthy individuals. It works well with cryopreserved PBMC and we have observed similar results with fresh specimens. Other tissue types have not been tested.

Background

Knowledge obtained during the optimization of reagent panels developed for previous studies was used as a starting point for the present panel. Some of these precursor panels have been published (1–3). Starting out with a backbone of $CD3^{APC\text{-}H7}$, $CD4^{QD605}$, $CD8^{QD585}$, $CD28^{PE\text{-}Cy5}$, $CD57^{QD705}$, and $CCR7^{Ax680}$, as well as AqBlu to gate out dead cells, the aim was to include more cell surface markers commonly used to investigate differentiation stages of $CD4^+$ and $CD8^+$ T-cells. In addition to being able to distinguish naïve (T_{NV}), central memory (T_{CM}), transitional memory (T_{TM}), effector memory (T_{EM}), and terminal effector cells (T_{TE}), we also wanted to identify $CD4^+$ recent thymic emigrants (RTE), as well as the recently characterized memory stem cell subset (T_{SCM}), a long-lived memory T-cell population that exhibits enhanced capacity for self-renewal and the ability to differentiate into T_{CM}, T_{EM}, and T_{TE} (4).

CD45RA, CCR7, and CD27 are sufficient for the identification of T_{CM}, T_{TM}, T_{EM}, and T_{TE}, so after AqBlu for the exclusion of dead cells, and CD3, CD4, and CD8 for the gating of $CD4^+$ and $CD8^+$ T-cells, respectively, these markers received highest priority for inclusion in the panel.

Next, CD95 was included to obtain a purer T_{NV} population, and together with CD28, CD57, and CD127 allow the identification of T_{SCM}. A stringent gating tree is employed to identify this latter population. T_{SCM} express high levels of the IL-7Rα chain, CD127, which is important for homeostatic proliferation (5), but not the senescence marker CD57 (6). They are thus phenotypically very similar to T_{NV}, the most prominent difference being the expression of the Fas receptor CD95 (4).

Finally, CD31, also known as PECAM-1, was chosen to distinguish RTE within the $CD4^+$ T_{NV} subset, as $CD31^+$ $CD45RA^+$ $CCR7^+$ cells have been shown to carry more T-cell receptor excision circles (TREC) than $CD31^-$ $CD45RA^+$ $CCR7^+$ cells (7).

Table 1. Summary table for application of OMIP-013

Purpose	T-cell differentiation
Species	Human
Cell types	PBMC
Cross-references	n.a.

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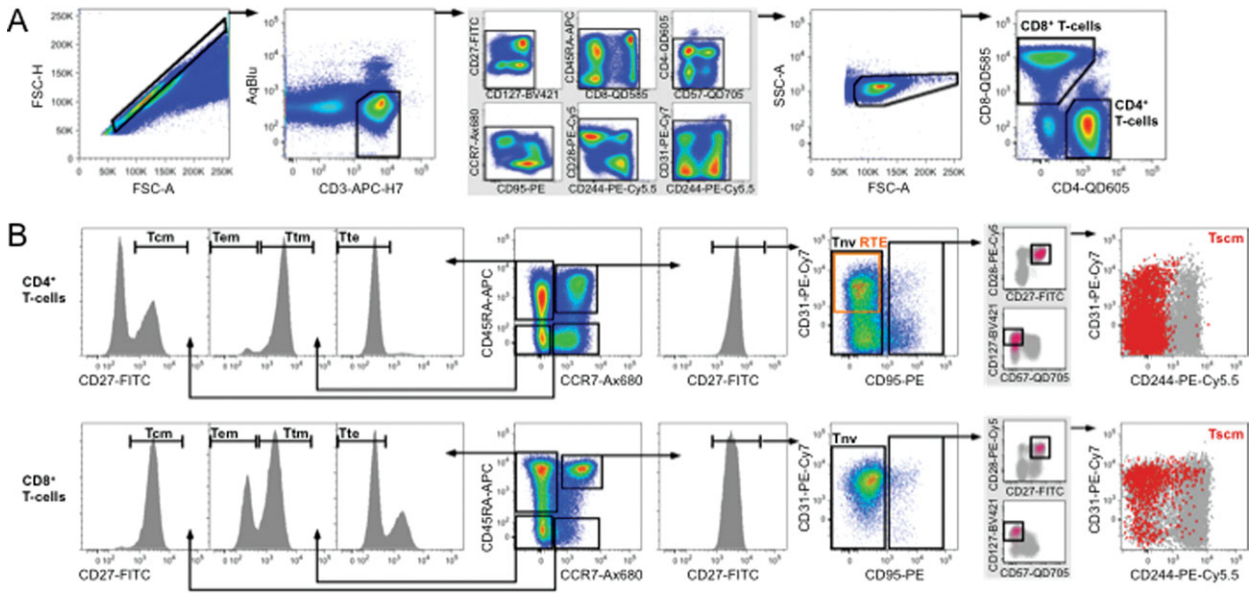

Figure 1. Example staining and gating. **A**: Identification of T-cell subsets. After selecting live $CD3^+$ single cells, eventual dye aggregates are excluded (gray box) and a lymphocyte gate set. $CD4^+$ and $CD8^+$ T-cells are then selected for further analysis. **B**: Identification of T-cell differentiation stages in both $CD4^+$ and $CD8^+$ T-cells. CD45RA, CCR7, and CD27 are used to identify T_{CM}, T_{TM}, T_{EM}, and T_{TE}. Within $CD45RA^+$ $CCR7^+$ $CD27^+$ (naïve-like) cells, $CD95^+$ $CD28^+$ $CD57^-$ $CD127^+$ cells are T_{SCM}, while $CD95^-$ cells are T_{NV}. Within these, the $CD31^+$ fraction comprises recent thymic emigrants (RTE, $CD4^+$ T-cells only). Overlay plots show total $CD4^+$ or $CD8^+$ T-cells in gray, with fuchsia ($CD45RA^+$ $CCR7^+$ $CD95^+$) or red events (T_{SCM}) gated as shown.

Table 2. Reagents used for OMIP-013

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
CD3	SK7	APC-H7	lineage
CD4	M-T477	QD605	
CD8	RPA-T8	QD585	
CCR7	150503	Ax680	memory/
CD27	O323	FITC	differentiation
CD28	CD28.2	PE-Cy5	
CD31	WM59	PE-Cy7	
CD45RA	HI100	APC	
CD57	NK-1	QD705	
CD95	DX2	PE	
CD127	A019D5	BV421	
CD244	C1.7	PE-Cy5.5	
Dead cells	–	AqBlu	dump

APC, allophycocyanin; H7, highlight 750; QD, quantum dot; Ax, Alexa; FITC, fluorescein isothiocyanate; PE, R-phycoerythrin; Cy, cyanine; BV, brilliant violet; AqBlu, live/dead fixable aqua dead cell stain.

As an additional marker we included CD244, better known as 2B4, in order to investigate its expression on individual T-cell subsets.

In order to verify the performance of each new reagent in the panel, as well as its potential effects on the detection of unrelated markers, add-in experiments were performed as previously described (8). To this end, a series of PBMC samples were incubated with subsets of the final reagent panel, with each subsequent tube containing the same reagents as the previous one, as well as one additional Ab. This process quickly identifies reagents that are not performing satisfactorily, as well as those that cause interference with unrelated detectors, thus compromising the detection of other reagents. Different reagent combinations were tested in order to identify a panel that provides the best detection (combination of reagent brightness, lack of background, and lack of spillover from other detectors) of all cell surface markers.

Similarity to Published OMIPs

None to date.

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