Development of a human T cell backbone flow cytometry panel enabling flexibility in reagent choice while minimizing panel design challenges

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Abstract

Expansion of existing flow cytometry panels with new markers of interest can result in suboptimal resolution and, in some cases, the need to design a new panel, impacting cost and increasing time to insight. To minimize these challenges and to provide increased flexibility, we have developed a human T cell backbone panel strategically designed to be complemented with 4–5 drop-in fluorochromes and markers of choice, depending on an instrument configuration, with minimal panel design effort. The backbone panel contains five T cell markers (CD3, CD4, CD8, CD45RA, CD45RO) conventionally used to identify different T cell subsets. The backbone panel was complemented with viability dye and four markers conventionally used in a viability dye and four markers conventionally used in T cell analysis.

Methods

Sample handling and staining

Normal donor samples included lyzed whole blood, freshly prepared PBMCs, and cultured activated PBMCs.

• BD Horizon™ Brilliant Stain Buffer Plus is used at 10 µl/sample and included in all compensation controls, single-color controls and panels.

• Backbone panel reagents were used at 5 µl/test.

• Cells are pre-stained with CD197 (CD27) BV711 reagent at 37 °C for 10 minutes. The same protocol was followed for the staining of any chemokine receptor in the CD4+ Th subset panel.

• Cells are stained with the full cocktail for 30 minutes.

• The BD Pharmingen™ Transcription Factor Buffer Set was used for the intracellular staining of FoxP3, according to manufacturer’s instructions. Briefer cells were stained with surface markers prior to fixation and permeabilization and then intracellular markers are added for an additional 30 minute incubation at 4 °C.

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• BD Pharmingen™ T-AND was added 10 minutes prior to acquisition, whereas BD Horizon™ Flexible Viability Stain (FVX) 620 was added during panel incubation in protein-free PBS.

• T cells were acquired with Dyneamids™ Human T-Activator CD3/CD28 for 2 days.

Instrument set up and acquisition

• BD FACSuite™ CS/SM Research Beads were used to check QC of instrument performance.

• Instruments were set up with optimal voltage application settings or lys/wash settings for BD FACSuite™ Cell Analysis software. Lys/wash settings were adjusted to keep PE-Cy7 on scale on compensation.

• Compensation was calculated with single-color control cells.

Data Analysis

• FlowJo™ 10.1. Software was used for data analysis and Total Spreading Matrix (TSM) table generation.

• Gates were drawn based on FMO controls.

• GraphPad Prism was used for graph generation.

Optimal resolution of T cell subsets

Strategically designed to enable marker addition with minimal panel design and risk of resolution loss

The backbone does not impact resolution of the drop-ins

The recommended drop-ins do not impact each other

Dive deeper into T cell biology through more comprehensive immunophenotypic and functional analyses

The human T cell backbone panel was tested on different flow cytometers

• Good resolution of major human T cell subsets

• The backbone does not impact resolution of the recommended drop-in fluorochromes

• The recommended drop-in fluorochromes do not impact the backbone resolution

• The recommended drop-in fluorochromes do not impact each other’s resolution

Comprehensive T cell differentiation analysis

• The human T cell backbone panel is compatible with:
  - Several human sample types (lyzed whole blood and fresh, cultured and activated PBMCs)
  - Surface and intracellular stains
  - Detailed staining and viability Flowcharts
  - Live and non-live staining
  - With flowJo™, a flexible and customizable platform for data analysis

Conclusions

• The human T cell backbone panel meets the criteria for a truly flexible and easy-to-expand flow cytometry panel:
  - Good resolution of major human T cell subsets
  - The backbone does not impact resolution of the recommended drop-in fluorochromes
  - The recommended drop-in fluorochromes do not impact the backbone resolution
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• Up to five markers plus a viability dye can be added with minimal panel design effort without impact to resolution and quantifications depending on instrument configuration

• No reagents of the backbone system in order to add new markers

• No concerns about spread, compensation and co-expressionwhen adding the recommended drop-ins

• Fluorochromes with appropriate brightness still need to be paired with wash buffer to ensure optimal brightness and signal-to-noise ratios

• Fluorochromes with different brightness can be chosen for a given detector (e.g., dim FITC to bright BD Horizon™ Brilliant™ Blue 515 [BB515], or dim APC or bright BD Horizon™ Brilliant™ Violet 450 [BV421] to improve flexibly in panel design)

• The human T cell backbone panel is strategically and prospectively designed to simplify the transition from four to eleven color flow cytometry panels leading to increased efficiency and biologic insight

• Double the number of markers and it is still a single tube

• Dive deeper into T cell biology through more comprehensive immunophenotypic and functional analyses

Identification of FoxP3+ T reg subsets

Upregulation of inhibitory receptors

Comprehensive T cell differentiation analysis

Dissection of CD4+ T cell developmental pathways

Total Spread

Recommended drop-ins

Not recommended

Recommended drop-ins

Not recommended

PBMCs from N=2 healthy donors were stained with the full cocktail and analyzed on the BD FACSymphony™ A1 Flow Cytometer. Similar results were obtained on a 5-laser BD LSRFortessa™ X-20 Cell Analyzer equipped with a 355-nm UV laser.