Streamlining Antibody Panel Creation with the iQue® Cytometry Panel Builder

Technical Note

Developing complex antibody panels for phenotyping experiments can be challenging. This document provides a practical guide for generation of antibody panels using the iQue® Cytometry Panel Builder, plus an example of such a panel in action on the iQue® 5 HTS by Cytometry Platform. The Panel Builder is a simple, easy to use, and powerful companion for all of your antibody panel experiments.

Introduction

Designing antibody panels for complex characterization of cells is time consuming and complicated, requiring experience and in-depth knowledge of the fluorochrome spectra and the capabilities of the cytometry system used. The iQue® Cytometry Panel Builder streamlines the antibody panel development process, enabling fast and simple creation of complex antibody panels for your workflows.

This technical note outlines the use of this tool to develop complex antibody panels, specifically for T-cell characterization.

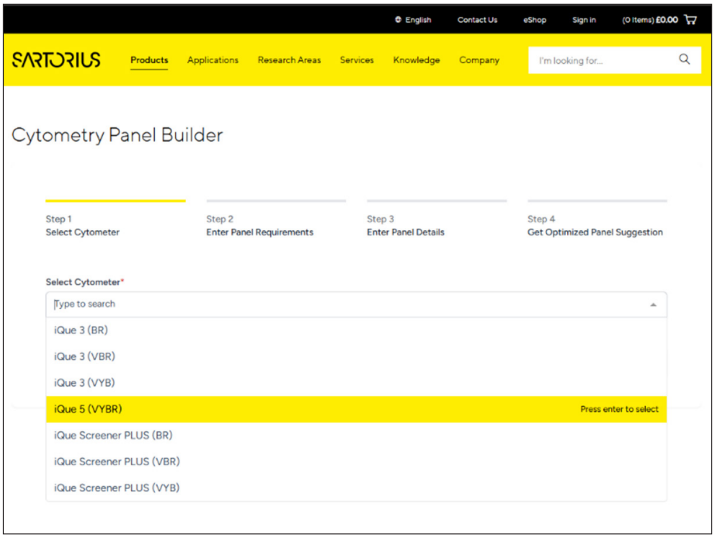
Process

The iQue® Cytometry Panel Builder (powered by EasyPanel) provides a simple solution for intelligent design of complex antibody panels suitable for running on the iQue® High Throughput Screening (HTS) by Cytometry Platform. The proprietary algorithm considers your instrument configuration, antigen expression levels, co-expression profiles, fluorochrome brightness, and spillover spread matrix to rapidly suggest panels with up to 25 colors. As an example, using the online tool, a T-cell panel of antibodies was developed to characterize multiple stages of T-cell activation suitable for the iQue®5 (VYBR), 4 laser platform.

Below is a step-by-step guide to using the tool (iQue® Cytometry Panel Builder | Sartorius).

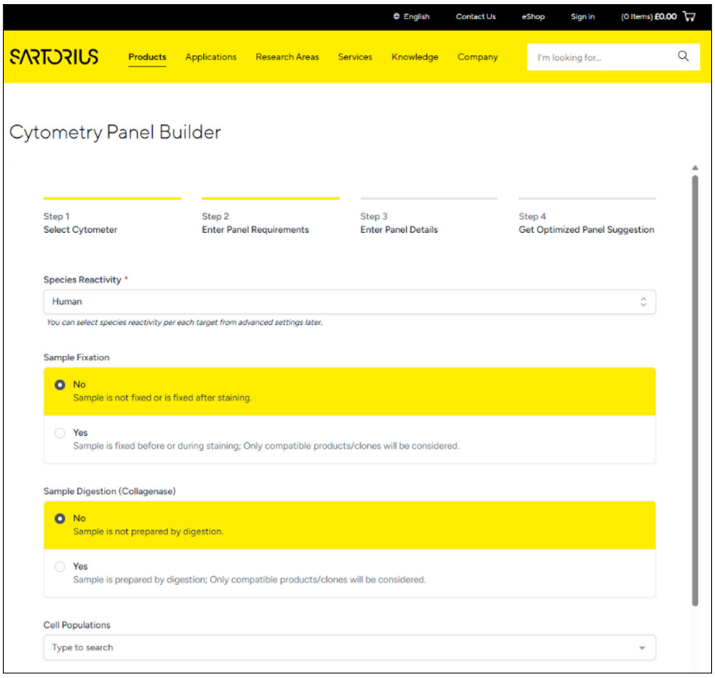
Step 1

Select instrument by clicking on the dropdown box and then click next.



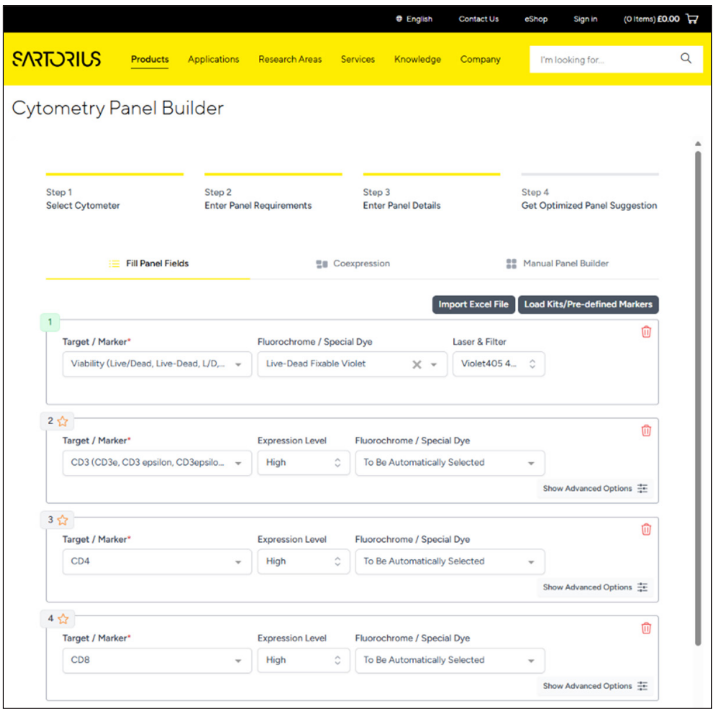
Step 2

Enter panel requirements using the menu, including species reactivity, cell type, antibody vendor, or excluded fluorochromes/channels.



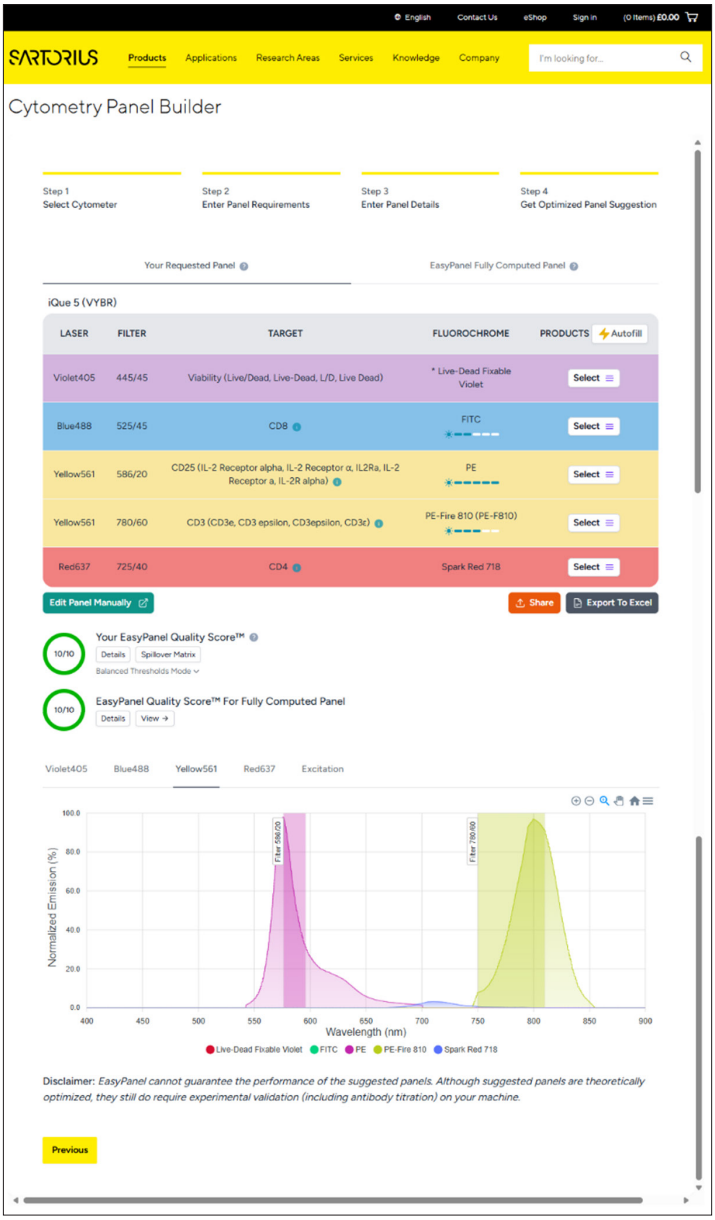
Step 3

Select your markers and optionally assign fluorochromes. This is easily editable to fit your specific requirements.



Step 4

Your panel will be generated and presented on this screen, here you will find a score of the suitability of the panel for your application, shown the spectral layout, and given more details about each antibody, including catalogue numbers for easy links to ordering. The tool can also suggest an expert gating strategy for comparison.



Example Panel Design for T-Cell Phenotyping

T-cell characterization experiments can be designed using the iQue® Cytometry Panel Builder, generating optimized panels for the iQue® platform of choice. Here we showcase the use of the panel builder in conjunction with the iQue® 5 HTS Platform.

In this example, 11 markers were selected including a viability dye (Figure 1) to characterize CD3+,CD4+ and CD8+ populations of T cells in combination with markers for activation, exhaustion and memory phenotypes.

Figure 1. Example iQue® Cytometry Panel Builder Antibody Panel for T-Cell Characterization.

Once the panel was created, it was important to perform compensation experiments using each individual antibody to generate a compensation spillover matrix in iQue Forecyt® Software. Although the panel builder provides a spillover matrix during the design process, this had to be confirmed on the instrument using the antibodies and fluorochromes selected with cells or compensation beads prior to running experiments using the panel.

To evaluate the T-cell panel, freshly isolated Human PBMCs were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and IL-2 (10 ng/mL), with or without CD3/CD28 T-cell activation beads (1 bead per cell) to activate the T-cell population. Over the following 72 hours, the cells were assessed using the recommended design from the panel builder.

Cells were assessed using the following strategy:

- All events to detect cells (FSC Vs SSC) and singlets (not shown)
- Viability marker for live cells — Activation – CD69, CD25
- CD3+ cells — Exhaustion – PD-1, LAG-3, TIM-3
- CD4+ and CD8+ populations — Memory – CCR7, CD62L

Activated Human T cells were isolated from a population of PBMCs using marker expression analysis on the iQue® 5 Platform. The gating strategy is shown in Figure 2, with membrane integrity staining to determine the live cells in the PBMC population (Figure 2A), followed by CD3 (Figure 2B), and combined CD4 and CD8 (Figure 2C) marker staining for characterization of T cells.

Evaluation of T-cell activation was performed by characterizing the marker expression of CD69 and CD25 in control and CD3/CD28 T-cell activation bead treated PBMCs. The data in Figure 3 shows that increased expression of both markers aligned with the activation status of the cells.

Further evaluation of the cell populations using the antibody panel highlighted increased expression of some key markers of exhaustion post activation (Figure 4). Comparing to non-activated controls, the activated cells all showed increased expression of PD-1, TIM-3, and LAG-3, markers of exhaustion. However, these markers are known to transiently increase in expression after activation, so at 48 hours post activation this is unlikely to be genuine exhaustion.

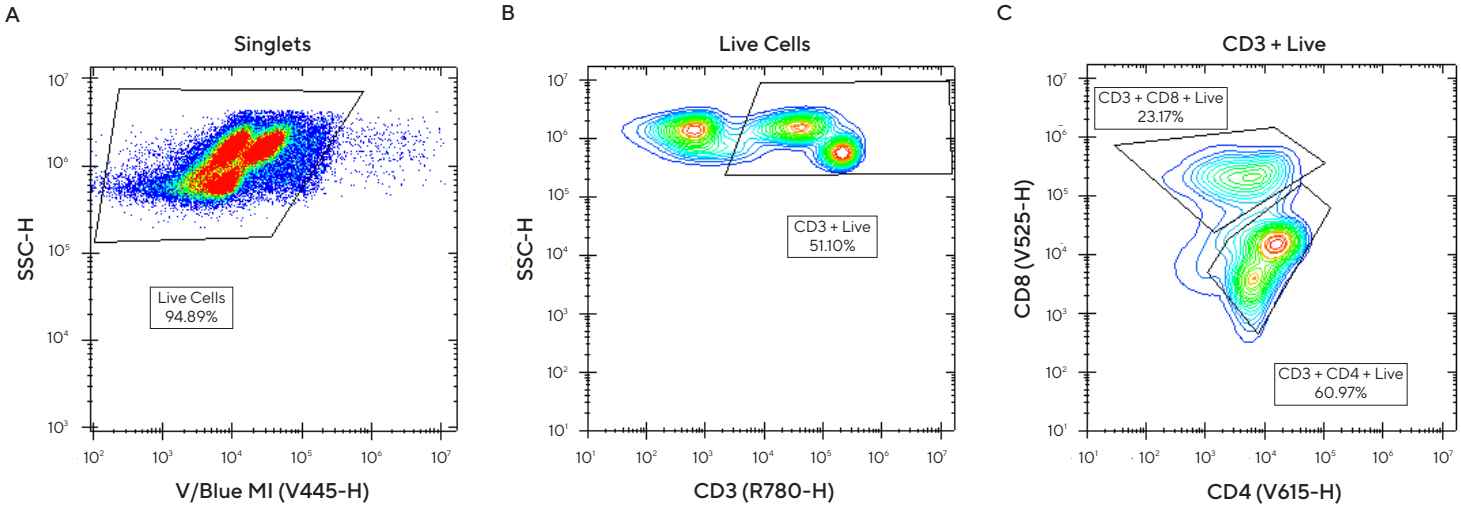


Figure 2. iQue Forecyt® Gating Strategy for Live T-Cell Populations at 48 Hours. A) Dot plot of membrane integrity dye staining for live cell gating. B) Contour plot of CD3 positive marker staining to identify T-cell populations in PBMCs. C) Contour plot of CD4/CD8 expression in CD3 positive T cells to isolate CD4 positive helper T cell and CD8 positive cytotoxic T-cell subsets.

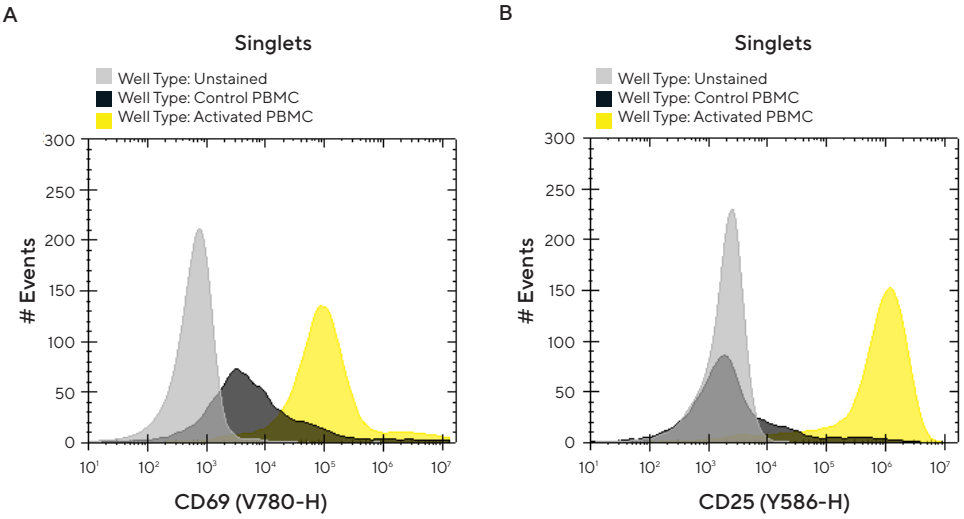


Figure 3. Activation Marker Analysis. Histograms generated in iQue Forecyt® show treatment groups for all cell events to highlight activated population. A) CD69 activation marker expression in PBMCs. B) CD25 activation marker expression in PBMCs.

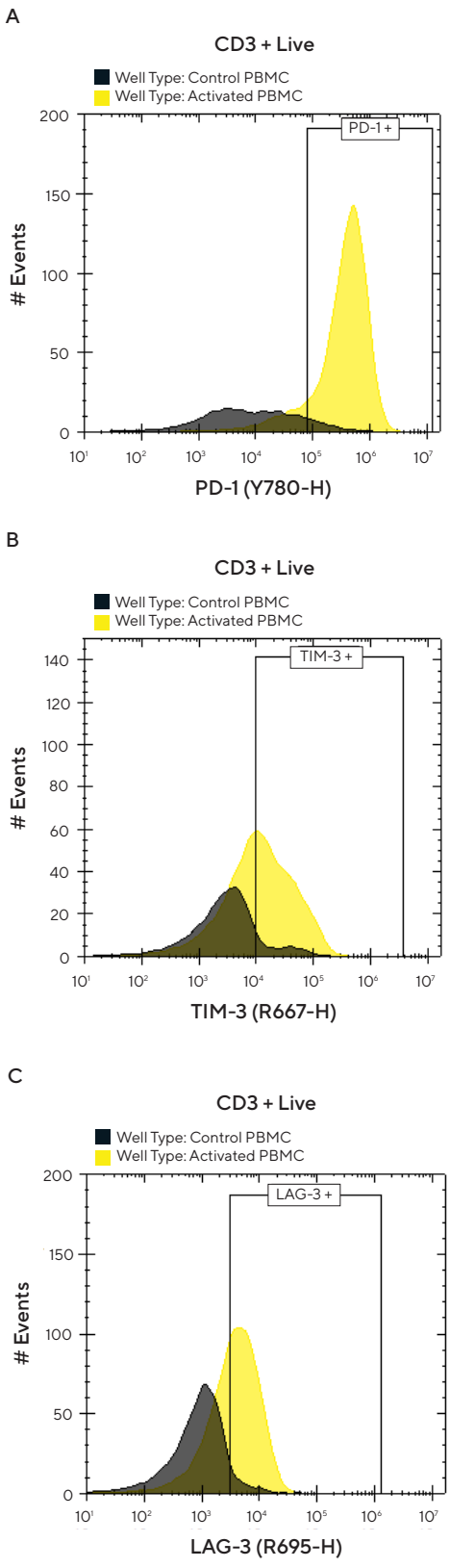


Figure 4. Exhaustion Marker Characterization. iQue Forecyt® histograms show data for the live CD3+ T-cell population gated from PBMCs with PD-1 expression (A), TIM-3 expression (B), and LAG-3 expression (C).

Combination of T-cell memory markers CD62L and CCR7 enabled the separation of populations within the CD3 positive T-cell population. CD62L and CCR7 double positive T cells fall into the T memory stem cell (Tscm) and central memory T cell category of less differentiated T cells. While CD62L and CCR7 double negative T cells are characteristic of the more differentiated phenotype of the effector memory (Tem) and effector (Teff) T cells. Data in figure 5 shows that once activated, T cells became more differentiated and lost expression of CD62L and CCR7 (76%) compared to the non-activated control (16%).

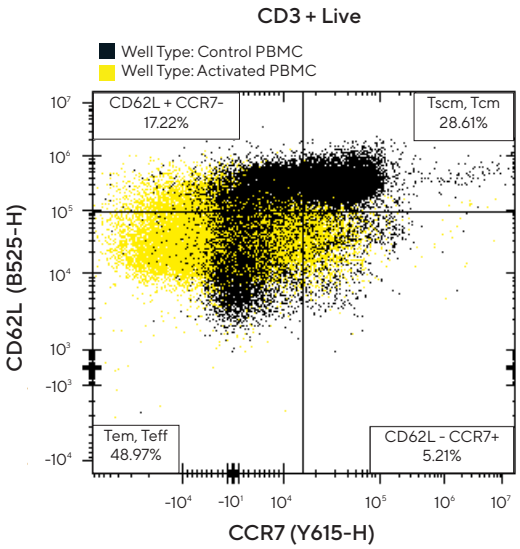


Figure 5. Potential Memory Phenotypes. iQue Forecyt® dot plot shows CCR7 and CD62L expression in control and activated PBMCs. Upper right quadrant shows double positive population Tscm, Tcm and lower left quadrant shows double negative population Tem, Teff.

Conclusion

The integration of the iQue® Cytometry Panel Builder and the iQue® HTS Platform offers a streamlined approach for creating complex panels to profile numerous cell types, in this example, immune cell populations.

The panels generated using the Panel Builder can be expanded and further enhanced by incorporating cytokine profiling using the iQue Qbead® portfolio, leveraging the iQue® Platform's capacity to independently track both cell and bead populations within the same sample.

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