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Application Note

Multi-Color Flow Cytometry Panels for Immunophenotyping of Expanded T Cells

Introduction

Bio-Techne offers many immune cell therapy solutions, including fluorescent-conjugated antibodies for flow cytometry to assess T cell memory phenotype, T cell activation, and T cell exhaustion. Together with our Fluorokines™ fluorescent-labeled proteins, these panels will streamline the path to immune cell analysis during CAR-T cell development.

Immune cell therapy encompasses several approaches to harness the host immune response for treating immune disorders and cancer, including chimeric antigen receptor (CAR) T cell therapy, regulatory T cell (Treg) therapy and tumor infiltrating lymphocytes (TILs) therapy. Production of any therapeutic involving biological materials has a high level of inherent variability. Personalized cell therapies like CAR-T cell therapy add additional layers of complexity.

CAR-T cell therapy manufacturing is a multi-stage process that involves isolating T cells from donors and engineering them to express a chimeric antigen receptor (CAR) to target specific antigens on a patient's tumor cells. After expansion, these CAR-T cells are transfused back into the patient, resulting in T cell activation and destruction of the tumor cells. Throughout the process, it is critical to monitor the memory phenotype, activation status, and exhaustion markers on the T cells. The most common way to monitor T cell phenotype is by flow cytometry to ensure successful activation, expansion, CAR-T expression and functionality of the CAR-T cells post-infusion.

Our experts have selected antibody-fluorochrome combinations that work together for these flow cytometry panels to save you time and money developing a flow cytometry panel from scratch. In this application note, we'll show how Bio-Techne fluorescent-conjugated antibodies and Fluorokines™ can be used to monitor the frequency and phenotype of peripheral blood mononuclear cells (PBMCs) and expanded T cells in immune cell therapy workflows.

When to Use Multi-Color T Cell Panels in Immune Cell Therapy Workflow

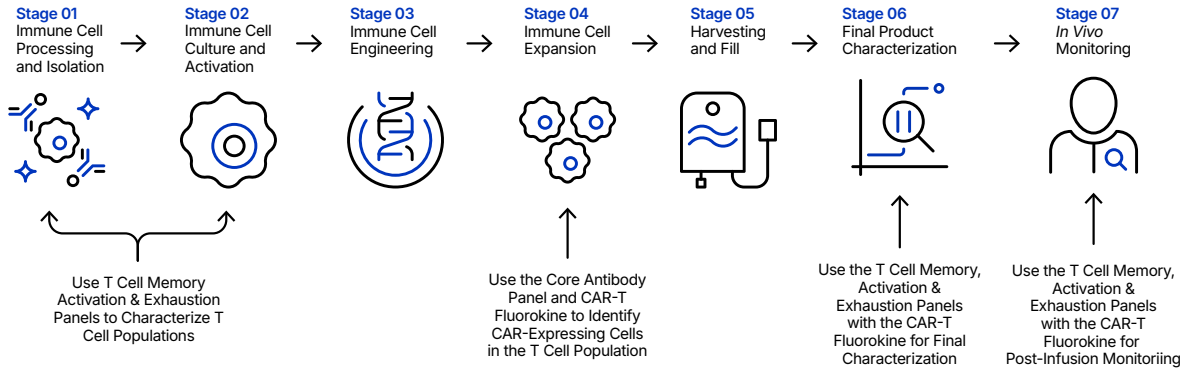

Figure 1: Overview of Patient-Specific Immunotherapy Workflow. Donor PMBC sample is processed for T cell selection **(Stage 1)** and enrichment **(Stage 2)**. T cells are modified for chimeric antigen receptor (CAR) expression (Stage 3) to recognize tumor cell receptors and expanded in cell culture **(Stage 4)** before harvesting **(Stage 5)**. The final CAR-T cell product is characterized **(Stage 6)** prior to re-introduction back into the patient **(Stage 7)**.

Benefits of Using Multi-Color T Cell Panels

- Eliminate the guesswork from flow cytometry panel design by entrusting it to our team of antibody specialists.
- Enhance reproducibility and standardize immunophenotyping in your lab.
- Achieve reliable results with Bio-Techne's flow-validated antibody panels, featuring our rigorously tested R&D Systems™ antibodies.
- Compatible with Alexa Fluor® 488-conjugated Fluorokines and Near IR viability dyes.

Flow Cytometry Panel for Immunophenotyping T Cell Memory Markers

The frequency of various memory T cell subsets in a sample can significantly influence the expansion potential and functionality of the host immune response. Utilize this validated multi-color flow cytometry panel to phenotype human PBMCs for memory T cell markers, such as CD45RO and CCR7.

TABLE // 01

Marker	Fluorochrome	Catalog #
CD3	mFluor™ Violet 450	FAB100MFV450
CD4	mFluor™ Violet 500	FAB3791MFV500
CD8	Alexa Fluor® 700	FAB1509N
CD45RA	mFluor™ Violet 610	FAB11444MFV610
CD45RO	Alexa Fluor® 647	FAB10642R
CCR7	PE	FAB197P

Flow Cytometry Panel for Immunophenotyping T Cell Activation Markers

T cells are activated when exposed to their specific antigen or external stimuli *in vitro*. Analyzing the expression patterns of early and late activation markers can enhance the understanding of the host immune response. Use this validated multi-color flow cytometry panel to identify activation markers, such as CD25, CD69, and CD38 on your T cells.

TABLE // 02

Marker	Fluorochrome	Catalog #
CD3	mFluor™ Violet 450	FAB100MFV450
CD4	mFluor™ Violet 500	FAB3791MFV500
CD8	Alexa Fluor® 700	FAB1509N
CD25	mFluor™ Violet 610	FAB1020MFV610
CD38	Alexa Fluor® 647	FAB2404R
CD69	PE	FAB23591P

Flow Cytometry Panel for Immunophenotyping T Cell Exhaustion Markers

The expression of inhibitory receptors is essential for regulating immune responses. However, abnormal and prolonged expression of these receptors can cause immune dysfunction. Utilize this validated multi-color flow cytometry panel to analyze your T cells for exhaustion markers, such as PD-1, TIM-3, and LAG-3.

TABLE // 03

Marker	Fluorochrome	Catalog #
CD3	mFluor™ Violet 450	FAB100MFV450
CD4	mFluor™ Violet 500	FAB3791MFV500
CD8	Alexa Fluor® 700	FAB1509N
LAG-3	mFluor™ Violet 610	FAB23193MFV610
PD-1	Alexa Fluor® 647	FAB10863R
TIM-3	PE	FAB2365P

TABLE // 04

Selection of **Fluorokines for CAR-Detection**

BCMA	CD19	CD30/TNFRSF8	CD40/TNFRSF5	CD300e/LMIR6
DLL3	EGFR	EMMPRIN/CD147	EpCAM/TROP1	ErbB2/Her2
Fc gamma RIIA/CD32a (R167)	Fc gamma RIIIA/CD16a	Glypican 3	IL-13 R alpha2	Mesothelin
MUC-1	OX40	PD-1	Siglec-2/CD22	Siglec-3/CD33
TRAIL R2	TSLPR	VEGFR2/KDR		

Flow Cytometry Panels Can Be Used to Detect T Cell Memory, Exhaustion, and Activation Markers in Human PBMCs

Initially, PBMCs are isolated from patient donors. Since the final output of CAR-T cells can be affected by the starting raw materials, the frequency of memory T cells, activated T cells and exhausted T cells by flow cytometry sets a baseline for the heterogeneity of the immune cell populations and, if isolating T cells before engineering, the overall purity of the sample (Figure 1: Stage 1).

Figure 2 shows the subpopulations of T cells expressing markers of memory (CD45RO/CD45RA), activation (CD69/CD38), and exhaustion (TIM-3/LAG-3) from a donor's PBMCs at Day 0, or Stage 1, of the immune cell therapy process.

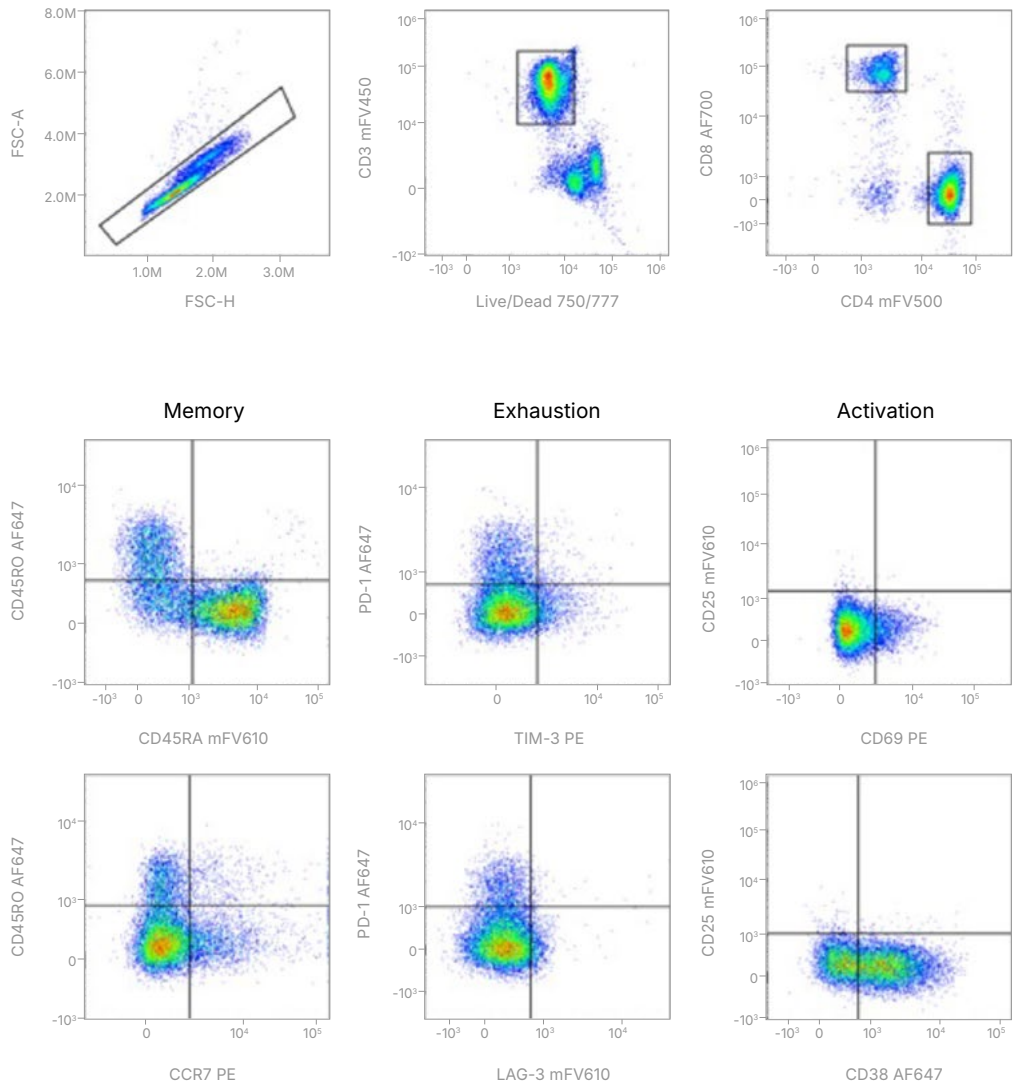

Figure 2: Characterization of PBMC Cells for T Cell Memory, Activation and Exhaustion with Multi-Color T Cell Panels using Flow Cytometry. Cryopreserved human (Day 0) PBMCs were thawed and stained with three flow cytometry panels to assess their naïve, memory, exhaustion, and activation phenotypes prior to T cell activation. PBMCs were stained with anti-human CD3 mFluor™ Violet 450, CD4 mFluor™ Violet 500, CD8 Alexa Fluor® 700, and Live/Dead 750/777 viability dye, followed by staining with three different panels to asses T cell 1) Memory (CD45RO Alexa Fluor® 647, CD45RA mFluor™ Violet 610, and CCR7 PE), 2) Exhaustion (PD-1 Alexa Fluor® 647, TIM-3 PE, and LAG-3 mFluor™ Violet 610), and 3) Activation (CD25 mFluor™ Violet 610, CD69 PE, and CD38 Alexa Fluor® 647).

Flow Cytometry Panels Identify Changes in Human T Cell Phenotypes After Activation

During T cell activation and cell culture expansion (Figure 1: Stage 2), immunophenotyping with flow panels is used to evaluate changes in T cell activation and overall changes in the distribution of T cell subtypes. These can be critical for understanding the success of T cell engineering and output of the final product. Figure 3 shows representative staining using Memory T Cell, T Cell Exhaustion, and T Cell Activation flow cytometry panels from Bio-Techne.

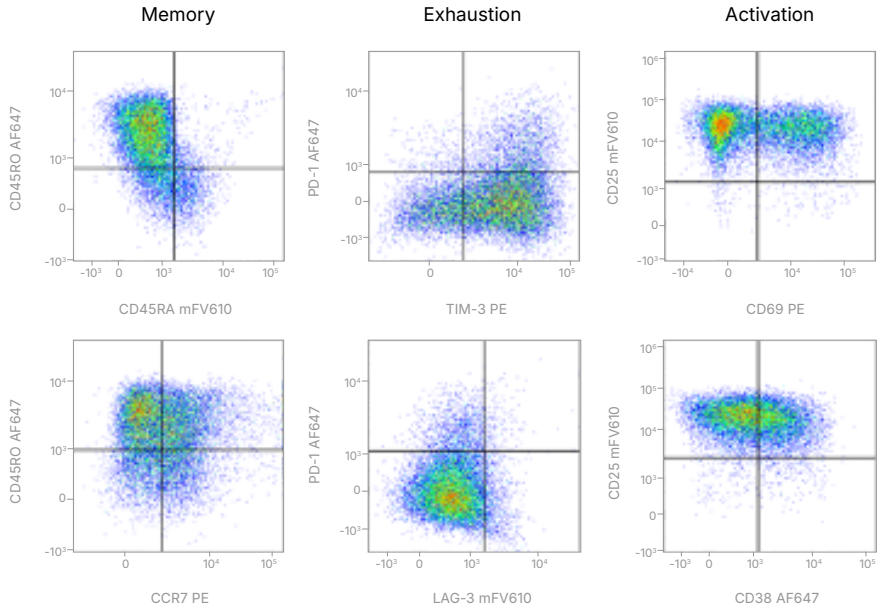

Figure 3: Characterization of Activated T Cells for T Cell Memory, Activation and Exhaustion with Multi-Color T-Cell Panels using Flow Cytometry. Day 2 activation and Day 9 memory and exhaustion phenotypes of expanded T cells. Human PBMCs stimulated with biotinylated versions of GMP Anti-Human CD3 (Cat# MAB11411-GMP) and GMP Anti-Human CD28 (Cat# MAB11412-GMP) coupled to M-270 Dynabeads + 200 IU/mL GMP Recombinant Human IL-2 (Cat# BT-202-GMP) for 9 days. Day 9 expanded T cells were stained with Anti-Human CD3 mFluor™ Violet 450, CD4 mFluor™ Violet 500, CD8 Alexa Fluor® 700, and Live/Dead 750/777 viability dye, followed by staining with three different panels to asses T cell 1) Memory (CD45RO Alexa Fluor® 647, CD45RA mFluor™ Violet 610, and CCR7 PE), 2) Exhaustion (PD-1 Alexa Fluor® 647, TIM-3 PE, and LAG-3 mFluor™ Violet 610), and 3) Activation (CD25 mFluor™ Violet 610, CD69 PE, and CD38 Alexa Fluor® 647).
Learn more about GMP antibodies for immune cell therapy.

Flow Cytometry Panels Can Detect Donor-to-Donor Variability in Memory Phenotype, Activation, and Exhaustion Markers

Figure 4 demonstrates how using flow panels from Day 0 to Day 2 (for activation) or Day 9 (for memory and exhaustion) can provide quantitative data on how T cells from donors can change over time.

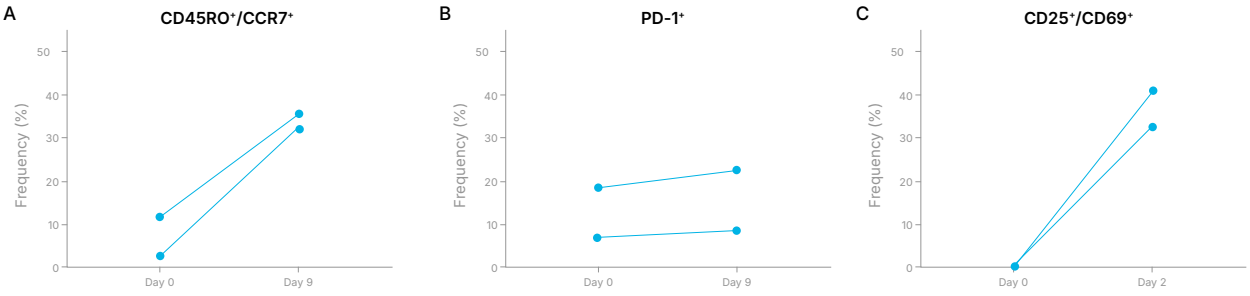

Figure 4: T Cell Memory, Activation and Exhaustion Panels Track Changes in T Cell Populations Over Time. Human PBMCs were stimulated with biotinylated versions of GMP Anti-Human CD3 (Cat# MAB11411-GMP) and GMP Anti-Human CD28 (Cat# MAB11412-GMP) coupled to M-270 Dynabeads + 200 IU/mL GMP Recombinant Human IL-2 (Cat# BT-002-GMP) for 9 days, as described in Figure 2. Differences in expression of CD45RO+/CCR7+ central memory (A), PD-1+ (B), and CD25+/CD69+ (C) cells were analyzed at Day 0 and Day 9 (A, B), and Day 2 (C) after activation.

Multi-Color Antibody Panels are Compatible with Fluorokines for Flow Cytometry

Upon vector delivery and genetic engineering of the CAR-T cell, using flow panels to identify CAR-expressing T cells is necessary to ensure successful engineering of the cells and final product characterization before infusion back into the patient (Figure 1: Stage 4, Stage 6). Post-infusion of CAR-T cells, flow panels can be used to monitor disease progression, immune response, and the efficacy of CAR-T cell therapy (Figure 1: Stage 7).

Bio-Techne offers a wide selection of fluorescent-labeled recombinant proteins called Fluorokines™. Fluorokines are conjugated to Alexa Fluor® 488, Alexa Fluor® 647, Atto 488, or Atto 647N, offering intense fluorescence and excellent photostability. As demonstrated in Figure 5 below, Fluorokines can be combined with T cell antibody panels for comprehensive characterization of CAR-T cells using multi-color flow cytometry.

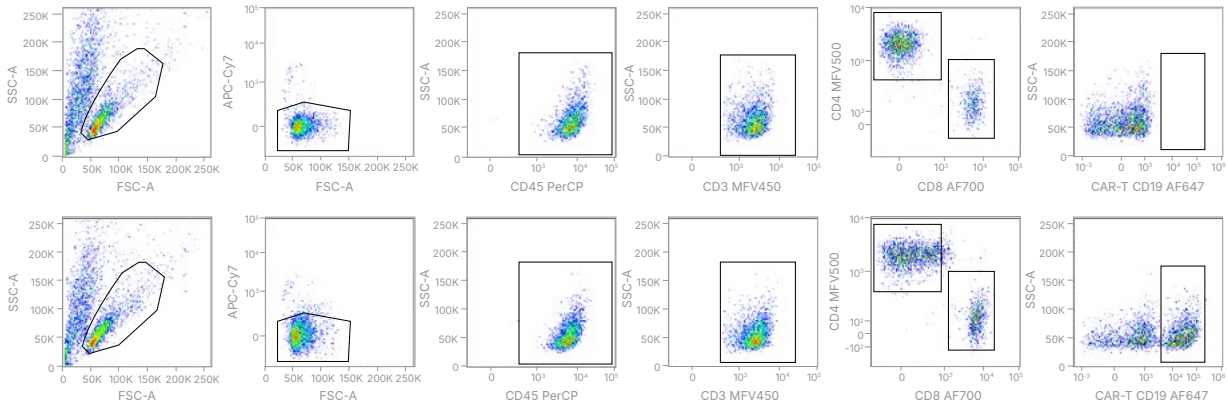

Figure 5. Characterization of CD4⁺/CD8⁺ CD19-CAR-T Cells by Multi-Color Flow Cytometry with a Recombinant Human CD19 CAR (AF647) Fluorokine and a Panel of Fluorochrome-Conjugated Antibodies. Two samples of CD4⁺/CD8⁺ T cells transduced with a hCD19-CAR and cultured were stained with the following panel of monoclonal antibodies: PerCP-conjugated Mouse Anti-Human CD45 (R&D Systems, Cat# FAB1430C), mFluor™ Violet 450-conjugated Mouse Anti-Human CD3 epsilon (R&D Systems, Cat# FAB100MFV450), Alexa Fluor® 700-conjugated Mouse Anti-Human CD8 alpha (R&D Systems, Cat# FAB1509N), mFluor™ Violet 500-conjugated Mouse Anti-Human CD4 (R&D Systems, Cat# FAB3791MFV500), along with the Fluorokine, Recombinant Human CD19 Fc Chimera Alexa Fluor® 647 Protein (R&D Systems, Cat# AFR9269). Live/dead cells were identified using APC-Cy®7. Cells were initially gated on singlets and live cells.

Conclusions

Bio-Techne's portfolio of immune cell marker antibodies enables multi-color T cell panels for comprehensive T Cell Memory, Activation, and Exhaustion immunophenotyping. These multi-color flow cytometry panels are compatible with our Fluorokines fluorescent-labeled proteins products, enabling detailed characterization of CAR-T cells. The flow cytometry panels used in this application note show that Bio-Techne's R&D Systems antibodies can be used to immunophenotype naive PBMCs, activated PBMCs, and engineered CAR-T cells. All Bio-Techne reagents, including flow cytometry antibodies and Fluorokines, are backed by our 100% Guarantee. Bio-Techne ensures confidence in your results and the reliability of your data.