Cytek® 25-Color Immunoprofiling Assay

The Cytek 25-color immunoprofiling assay is designed and optimized by Cytek scientists to provide a turnkey solution for identifying major human immune subpopulations for T, B, NK cells, monocytes, dendritic cells, and basophils – all of which play important roles in the innate and adaptive immune response in various diseases.

To support the new 25-Color Immunoprofiling Assay, Cytek released an 18-color cFluor® reagents kit that can be used with seven reagents from other suppliers. The 25-color assay has been optimized and titrated for use with 7 Brilliant Violet™ reagents from BioLegend for analyzing human PBMC and whole blood on Cytek's Aurora systems equipped with violet, blue, yellow-green, and red lasers.

Product details for Cytek 25-Color Immunoprofiling Assay, cFluor® Reagent Kit (18C)

Catalog number: R7-40002 (25 Tests)

Category: Immunophenotyping

Format: cFluor®* conjugated antibodies in individual tubes

Target	Clone	Fluorochrome
CD45RA	HI100	cFluor® V450
CD20	2H7	cFluor® V547
CD141	M80	cFluor® B515
CD8	SK1	cFluor® B532
CD14	63D3	cFluor® B548
HLA-DR	L243	cFluor® B690*
CD25	BC96	cFluor® BYG575*
CD4	SK3	cFluor® YG584
CD16	3G8	cFluor® BYG610*
IgD	IA6-2	cFluor® BYG667
TCRγδ	B1	cFluor® BYG710
CD11c	3.9	cFluor® BYG781*
CD127	A019D5	cFluor® R659*
CD1c	L161	cFluor® R668
CD19	HIB19	cFluor® R685
CD123	6H6	cFluor® R720
CD45	2D1	cFluor® R780*
CD27	QA17A18	cFluor® R840

Test Dilution: 5µL per test

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Application:	Flow cytometry
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% BSA
Storage:	2-8°C and protected from light. **Do not freeze**

PRODUCT DETAILS for BioLegend Immunoprofiling Kit, 7 Color (Brilliant Violet™)	
Catalog number:	900004160 (25 Tests)
Category:	Immunophenotyping

Format:

Target	Clone	Fluorochrome
CD197 (CCR7)	G043H7	Brilliant Violet 421™
IgM	MHM-88	Brilliant Violet 510™
CD3	UCHT1	Brilliant Violet 570™
CD28	CD28.2	Brilliant Violet 650™
CD38	HIT2	Brilliant Violet 711™
CD56	5.1H11	Brilliant Violet 750™
CD279 (PD-1)	EH12.2H7	Brilliant Violet 785™

Test Dilution:	5µL per test
Application:	Flow cytometry

PRODUCT DESCRIPTION

Cytek 25-Color Immunoprofiling Assay allows for the identification of helper T cells, cytotoxic T cells, B cells, NK cells, NKT cells, Basophils, Dendritic cells, ILCs, and monocytes in human peripheral blood mononuclear cells and in whole blood. The reagents in this kit help to distinguish different subsets of T, B, NK, NKT, DCs; including regulatory T cells, naïve T cells, activated T cells, memory T cells, effector T cells, naïve B cell, memory B cells, Early NK, Mature NK, Terminal NK, nonclassical and classical monocytes.

CD1c belongs to the major histocompatibility complex (MHC)-like CD1 family and is expressed on dendritic cells, monocytes, and B cells. CD1c interacts with self or microbial derived lipid and glycolipid antigens for presentation to T cells.

CD3 is expressed on all mature T cells, NK T cells, and some thymocytes. CD3, a part of the CD3/T cell receptor complex, plays a role in antigen recognition, signal transduction, and T cell activation.

CD4, is expressed on most thymocytes, a major subset of T cells, and on monocytes/macrophages. Functionally, CD4 is associated with thymic differentiation, in conjunction with MHC class II molecules in antigen recognition and with signal transduction.

CD8 is found on thymocytes, on a subset of T cells and on NK cells. This molecule acts as a co-receptor in MHC class I molecules in antigen recognition, has a role in T cell activation and in thymic differentiation.

CD11c is expressed in high levels on dendritic cells. The molecule is also expressed on monocytes, macrophages, neutrophils, NK cells and subsets of T and B cells. CD11c heterodimerizes with Integrin beta

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2 chain to form CD11c/CD18 complex, which binds to fibrinogen, CD54, and iC3b to play a role in leukocyte adhesive interactions.

CD14 is also known as a high affinity LPS receptor and is highly expressed on monocytes and macrophages. It is also expressed on granulocytes, but at a lower level. In addition, CD14 is found on interfollicular dendritic cells, reticular dendritic cells, and Langerhans cells.

CD16 is expressed on NK cells, monocytes and macrophages in the form of CD16a. Another form of CD16, CD16b is expressed on neutrophils. CD16 engagement of IgG leads to NK cell activation, antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis

CD19 is expressed in the B cell lineage, from pro-B to blastoid B cells. However, it is absent on plasma cells. It is also expressed on follicular dendritic cells. CD19 is involved in B cell development, activation, and differentiation.

CD20 is a membrane protein specific to mature B cells and is involved in the differentiation of B cells into plasma cells. The expression of CD20 is also found on a variety of malignant B cells.

CD25 is a low affinity IL-2 receptor that is expressed on progenitor lymphocytes, activated T & B cells, and monocytes, as well as on regulatory T cells. When CD25 is in association with CD122, IL-2 receptor beta chain and with CD132, the common gamma chain, the resulting complex is the high-affinity IL-2 receptor. IL-2 receptor signaling is key for lymphocyte proliferation and survival.

CD27 is a lymphocyte specific member of the TNF receptor superfamily. CD27 is expressed on a subset of thymocytes, mature T cells. The expression is further upregulated during T cell activation. Subpopulations of B and NK cells also express CD27. In T cell-B cell interaction, CD27 binds to CD70 to provide co-stimulation to result in T cell activation and B cell differentiation and proliferation.

CD28 is a costimulatory molecule on T cells that promotes signaling involved in actin remodeling, cytokine production, and T cell survival and differentiation. The main ligands for CD28 are B7-1 (CD80) and B7-2 (CD86) on antigen presenting cells. Plasma cells, neutrophils, and eosinophils also express CD28, however, the role CD28 plays in these cells is not clear.

CD38 is expressed on most leukocytes and has a dual role as a receptor and enzyme. As a receptor, CD38 is involved in cell adhesion and signal transduction. As an enzyme, CD38 metabolizes extracellular NAD+ to regulate extracellular nucleotide homeostasis. CD38 is highly expressed on plasma cells and multiple myeloma cells.

CD45 is expressed on all hematopoietic cells, except erythrocytes and platelets. CD45 is a signaling molecule that is involved in cellular proliferation, differentiation and in regulation of immune cell functions.

CD45RA is an isoform of CD45 due to alternate splicing. CD45RA is expressed on naïve or resting CD4+ and CD8+ T cells, as well as on B cells and monocytes.

CD56, within the hematopoietic system, is expressed on NK cells and NKT cells, a subset of T cells. In the nervous system, CD56 is expressed by neurons and plays a role in the homotypic adhesion of neural cells.

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The CD123 molecule is expressed predominantly on hematopoietic progenitor cells, macrophages, dendritic cells, basophils, eosinophils, monocytes, megakaryocytes, and some B cells. It is also present in myeloid, lymphoid, and vascular endothelial cells

CD127, when it heterodimerizes with the common gamma chain (CD132), forms the IL-7 receptor. This receptor is involved in the development of B cells in the bone marrow, and the control of T cell proliferation in the periphery. CD127 expression is downregulated in regulatory T cells.

CD141 binds to its ligand, thrombin, to form a thrombin-thrombomodulin complex that activates protein C and initiates the Protein C anticoagulant pathway. It is expressed on macrophages, monocytes, dendritic cells, and vascular endothelial cells.

CD197 (CCR7) is a chemokine receptor for chemokines CCL19 and CCL21. CCR7 and its ligands are involved in trafficking and migration of immune cells into immunological milieus. For example, B cell migration and organization into secondary lymphoid tissues, and migration of memory T cells into inflamed tissue. CCR7 is expressed on T cells and the detection of CCR7, in combination with CD45RA, can be used to distinguish naive T cells from central and effector memory T cells.

CD279 (PD-1) is an inhibitory receptor on activated T and B cells, although most research has been focused on T cells. The binding of PD-1 to its ligands (PD-L1 and PD-L2) induces T cell tolerance, inhibiting T cell effector functions including proliferation, differentiation, and cytokine production.

IgD is a membrane B cell receptor (BCR) co-expressed with IgM on naive B cells. IgD is expressed in B cells upon exiting the bone marrow to the periphery. IgD initiates BCR signaling when bound to antigen. Following B cell activation and differentiation into memory and plasma cells, IgD expression is downregulated and stopped.

IgM is a BCR expressed on immature and naïve B cells. IgM is the first BCR isotype expressed on B cells. On naïve B cells, IgM is co-expressed with IgD. The binding of antigen to IgM initiates signaling and activation of the B cell. Following B cell activation, IgM expression is eventually stopped as B cells differentiate into memory and plasma cells that express IgG, IgA, or IgE isotypes.

HLA-DR is present on the surface of antigen-presenting cells, including B cells, dendritic cells, macrophages, monocytes and activated T cells. MHC class II regulates the immune system by playing a critical role in binding and presenting antigen-derived peptides to peptide-MHC II-specific CD4 T cells.

TCRγδ is a T cell receptor consisting of two glycoprotein chains, a 45-60 kDa gamma chain and a 40-60 kDa delta chain. Unlike the conventional αβ T cell receptor, which recognizes peptide bound to MHC, γδ TCR recognizes antigen in a non-MHC restricted way. TCR γδ associates with CD3 to play a role in signal transduction and is found on thymocytes and subsets of T cells.

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RECOMMENDED USAGE

Human peripheral mononuclear cells (PBMC) or whole blood have been tested to validate the performance of this kit. Please refer to the product web page for the staining protocols, fluorochrome list, experiment template and data acquisition protocol.

For Research Use Only. Not intended for use in diagnostic procedures.

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