Cytek® cFluor® Human B Cell Monitoring Kit

The Cytek® cFluor® Human B Cell Monitoring Kit detects B cell subsets that are useful for monitoring the responses in anti-CD20 treated autoimmune patients or for measuring the efficacy of B cell depleting treatments in clinical trials. The kit consists of a 13-color panel designed for identifying antibody secreting cells (plasmablasts/plasma cells), naïve and memory B cell subsets, as well as T cell subsets. The panel also includes granulocyte and monocyte markers allowing for cleaner lymphocyte gating.

The 13-color cFluor® B Cell Monitoring assay can be analyzed on Cytek® Full Spectrum cytometers equipped with blue and red lasers.

R8-50006 Rev. A, Effective Date: 10/28/2022
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Product details for Cytek® cFluor® Human B Cell Monitoring Reagent Kit (13C)	
Catalog number:	R7-40008 (25 Tests)
Category:	Immunophenotyping
Format:	cFluor® conjugated antibodies in individual vials

Target	Clone	Fluorochrome
IgM	CH2	cFluor® B515[1]
CD4	SK3	cFluor® B532[1]
CD15	HI98	cFluor® B548[1]
CD38	HB7	cFluor® BYG575
CD27	O323	cFluor® BYG610[2]
CD14	MEM-15	cFluor® BYG667[2]
IgD	IgD26	cFluor® BYG710[2]
CD19	SJ25C1	cFluor® BYG781[2]
CD3	SK7	cFluor® R659
IgG	4A11	cFluor® R668[1]
CD8	SK1	cFluor® R685[1]
CD20	2H7	cFluor® R720[1]
CD45	2D1	cFluor® R780[2]

Test Dilution:	5 µL per test
Application:	Flow cytometry
Formulation:	Phosphate-buffered saline, pH 7.2, containing 0.09% sodium azide and 0.2% BSA (BSA Country of Origin USA)
Storage:	2-8°C and protected from light. **Do not freeze**

PRODUCT DESCRIPTION

The Cytek® cFluor® Human B Cell Monitoring Reagent Kit allows for the identification of B cell subsets in human peripheral blood mononuclear cells and in whole blood collected in EDTA, heparin, or CytoChex® BCT tubes. The reagents in this kit distinguish plasmablasts/plasma cells (CD19+CD20-CD27+CD38++), naïve B cells (CD27-IgD+), IgG class switched memory B cells (CD27+IgG+), and class unswitched memory B cells (CD27+IgD+ and/or IgM+). Additionally, the kit identifies the T cell (CD3+) subsets of CD4+ and CD8+ T cells. Granulocyte and monocyte markers, CD15 and CD14, are included in the panel to allow for a cleaner lymphocyte gate.

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

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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.

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.

CD15 is commonly used as a marker for human myeloid cells and is highly expressed on granulocytes, including neutrophils and eosinophils. Monocytes have lower expression of CD15 while lymphocytes and basophils have no expression. CD15 is involved in various cell functions including phagocytosis, neutrophil adhesion to dendritic cells, and chemotaxis.

CD19 is expressed in the B cell lineage. 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. However, it is absent on plasmablasts and plasma cells. The expression of CD20 is found on a variety of malignant B cells.

CD27 is a lymphocyte specific member of the TNF receptor superfamily. Subpopulations of B and NK cells express CD27. For B cells, it is commonly used to identify memory cells. CD27 is expressed on a subset of thymocytes, mature T cells. The expression is further upregulated during T cell activation. 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.

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 regulation of immune cell functions.

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.

IgG is a membrane BCR that is produced when activated naïve B cells undergo class switch recombination of the Ig heavy chain constant region. This class-switching results in IgM and IgD being replaced by IgG as the activated B cell develops into memory and antibody secreting cells. On memory B cells, when IgG binds to its target antigen, it initiates signaling and activation that rapidly leads to proliferation and differentiation into antibody secreting cells.

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IgM is a membrane 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.

RECOMMENDED USAGE

Human peripheral mononuclear cells (PBMC) or whole blood collected in EDTA, heparin, or CytoChex® BCT tubes 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.

Please briefly centrifuge the reagent vial before use.

Use appropriate personal protective equipment per the product safety data sheet when using this product.

REFERENCES

Clevers H et al. Ann Rev Immunol. **6,** 629-62 (1988)
Evans RL et al. Immunol. **78,** 544-8 (1981)
Cole DK et al. Cell Mol Immunol. **1,** 81-8 (2004)
Ferrero E et al. J. Immunol. **145,** 331-6 (1990)
Otero D et al. J Immunol. **170,** 73-83 (2003)
Vossen M et al. J Immunol. **180,** 3739-45 (2008)
Fritsch R et al. J Immunol. 175, 6489-97 (2005)
Loken MR et al. Cytometry. **11,** 453-9 (1990)
Kumar, Connors & Farber. Immunity. **48,** 202-213 (2018)
Goodwin R et al. Cell. **73,** 447-56 (1993)
Macardle PJ, et al. Cell Immunol. **168**, 59-68 (1996)
Naeim, F, et al. Atlas of Hematopathology 2nd Edition (2018)
Morandi, F, et al. Cells. **8**, 1527 (2019)
Pieper, K, et al. J Allergy Clin Immunol **131**, 959-71 (2013)
Stavnerzer J, et al. J Immunol. **193**, 5370-8 (2014)
Lund-Johansen F, et al. J Immunol. **148** 3221-9 (1992)

[1]cFluor® B515, cFluor® B532, cFluor® B548, cFluor® R668, cFluor® R685, and cFluor® R720 are equivalent to CF®488A, CF®503R, CF®514, CF®647, CF®660C and CF®700 respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.
[2]cFluor® BYG610, cFluor® BYG667, cFluor® BYG710 and cFluor® BYG781 are tandem dyes made with R-PE. cFluor® R780 is a tandem dye made with APC. Caution – Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.

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

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