



CYTEK[®] cFLUOR[®] HUMAN B CELL MONITORING KIT

Accelerate Autoimmune Therapy Development

Cytek® cFluor® Human B Cell Monitoring Kit

Anti-CD20 monoclonal antibody (mAb) therapies are employed for a range of autoimmune diseases. The target cells for these therapies are the pathogenic memory B cells that are the source of autoantibody-secreting cells. However, anti-CD20 antibodies also deplete normal memory and naïve B cells. A successful therapy would lead to repopulation of naïve B cells over time with long-term elimination of memory B cells. The reappearance of memory B cells and antibody-secreting cells correlates with relapse.^{1,2,3}

Flow cytometry can be a valuable tool for monitoring B cell subsets and distinguishing different subsets of memory B cells from naïve B cells. This will help translational researchers evaluate whether a patient is likely in remission or at risk of resistance to therapy or relapse. Cytek® designed a 2-laser, 13-color cFluor® B Cell Monitoring assay to identify and enumerate B cell subsets in human whole blood and peripheral blood mononuclear cells using Cytek full spectrum cytometers equipped with blue and red lasers. The panel identifies plasma cells and B cell subsets including naïve, IgD⁺ and/or IgM⁺ memory, and IgG⁺ memory B cells. The assay also includes markers for CD3⁺, CD4⁺ and CD8⁺ T cells to monitor the immune status of patients with potential immunodeficiency caused by B cell depletion. In addition, this kit includes the monocyte marker CD14 and the granulocyte marker CD15 for clean and reproducible lymphoid gating.

The cFluor Human B Cell Monitoring Kit is a useful tool for monitoring B cell populations in anti-CD20 mAb treated autoimmune patients, such as Rheumatoid Arthritis (RA) patients treated with Rituximab, and as an assay to monitor the efficacy of anti-CD20 therapies in clinical trials.

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

Table 1: cFluor Human B Cell Monitoring Kit composition

This pre-optimized cFluor Human B Cell Monitoring Kit takes away the need for time consuming, labor intensive and costly panel design and optimization. Cytek also provides the following tools to streamline the laboratory workflow:

- Complete protocols for sample preparation, staining, and acquisition
- Experiment and analysis templates to streamline workflow and improve laboratory efficiency

The cFluor Human B Cell Monitoring Kit has been tested and shown to be compatible with blood samples collected in EDTA, Heparin, and Cyto-Chex blood collection tubes. This provides more flexibility for your clinical research projects.

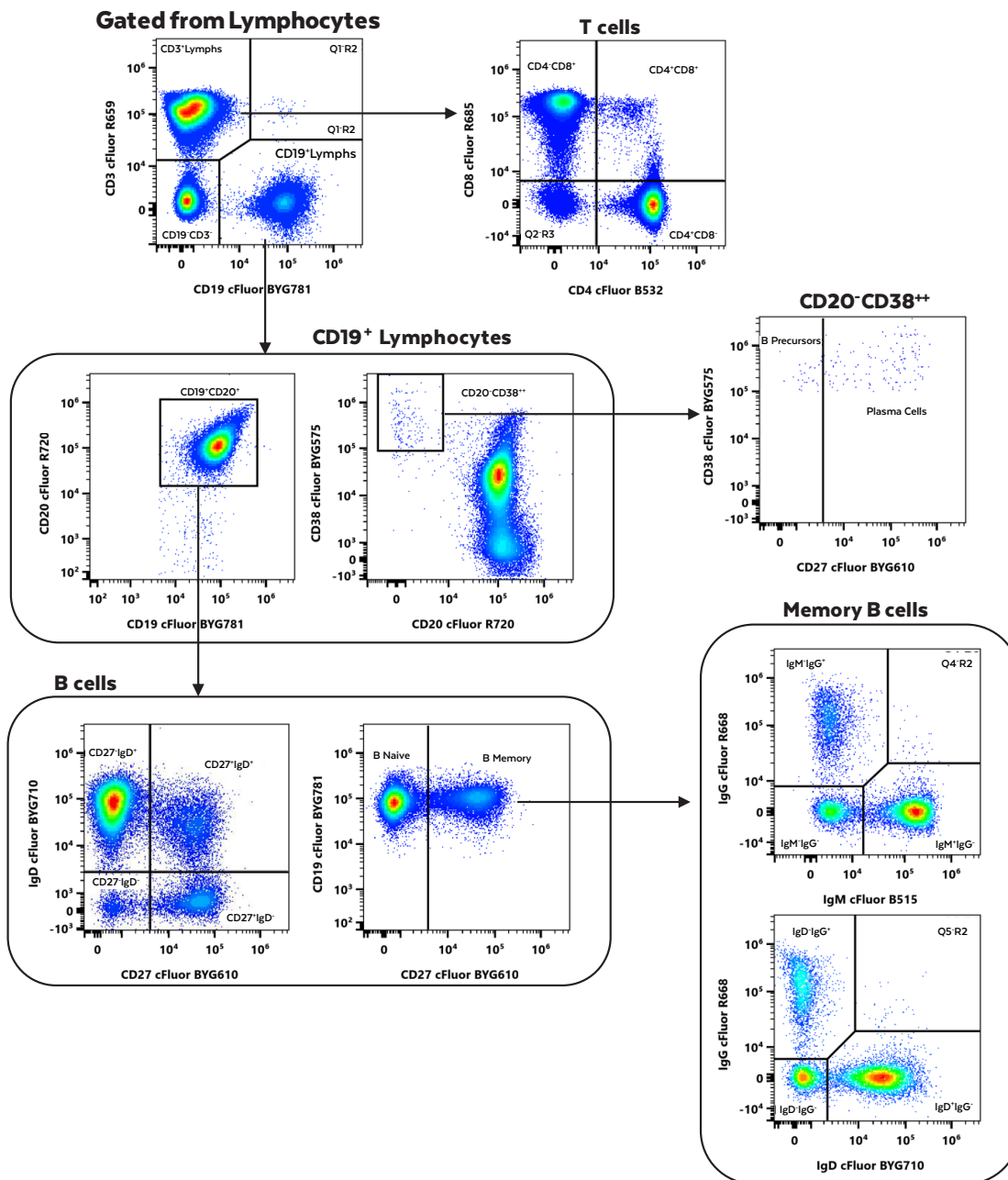


Figure 1: Cell gating strategy for identifying B cell subsets using the Cytek cFluor Human B Cell Monitoring Kit. Data were generated using whole blood collected in a heparin tube from a healthy donor. This gating strategy is based on the one published by Gatti et al.¹ Sequential gates of time, total cell, singlet, not CD15⁺ granulocyte and not CD14⁺ monocyte gates are used to exclude populations to allow for easy and cleaner lymphocyte gating (plots not shown). Lymphocytes are subsetted into CD3⁺ T cells and CD19⁺ Lymphocytes. T cells are further divided into the CD4⁺ and CD8⁺ T cells. From the CD19⁺ Lymphocytes, CD19⁺CD20⁺ B cells are selected for identifying naïve and memory subsets, while CD20⁺CD38⁺ cells are selected for identifying B cell precursors (CD27⁺) and plasma cells (CD27⁺). CD19⁺CD20⁺ B cells are classified into naïve (CD27⁺) or memory cells (CD27⁺). Plotting IgD and CD27 identifies naïve (CD27⁺IgD⁺), class switched memory (CD27⁺IgD⁺), class unswitched memory (CD27⁺IgD⁺), and double negative B cells (CD27⁺IgD⁺). From the CD19⁺CD20⁺CD27⁺ memory B cell population, plotting IgG and IgM or IgD identifies memory IgG class switched (IgM⁺IgG⁺, IgD⁺IgG⁺) and memory class unswitched (IgM⁺IgG⁺, IgD⁺IgG⁺).

References

1. Gatti A, Buccisano F, Scupoli MT, Brando B. The ISCCA flow protocol for the monitoring of anti-CD20 therapies in autoimmune disorders. *Cytometry B Clin Cytom.* 2021 Mar;100(2):194-205. doi: 10.1002/cyto.b.21930. Epub 2020 Jun 29. PMID: 32598578.
2. Van Beers JJBC, Damoiseaux JGMC. Immune Monitoring upon Treatment with Biologics in Sjögren's Syndrome: The What, Where, When, and How. *Biomolecules.* 2021 Jan 16;11(1):116. Doi: 10.3390/biom11010116. PMID: 33467204; PMCID: PMC7830440.
3. van Dam LS, Oskam JM, Kamerling SWA, Arends EJ, Bredewold OW, Berkowska MA, van Dongen JJM, Rabelink TJ, van Kooten C, Teng YKO. Highly Sensitive Flow Cytometric Detection of Residual B-Cells After Rituximab in Anti-Neutrophil Cytoplasmic Antibodies-Associated Vasculitis Patients. *Front Immunol.* 2020 Dec 15;11:566732. doi: 10.3389/fimmu.2020.566732. PMID: 33384685; PMCID: PMC7770159.

cFluor® B515, cFluor® B532, cFluor® 548, cFluor® R668, cFluor® R685 and cFluor® R720 are equivalent to CF®488A, CF®503R, CF®514, CF®647, CF®669C 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.

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

Find out more at www.cytekbio.com

Please contact sales@cytekbio.com with any questions
© 2021-2022 Cytek Biosciences, Inc. All rights reserved. All trademarks are the properties of Cytek Biosciences, Inc. and its subsidiaries unless otherwise specified.

