



Cytek® cFluor® Immunoprofiling Assay & Kits

Turnkey Solutions for Basic and In-Depth Immunophenotyping

To empower scientific discovery, Cytek® scientists designed and developed a series of Immunoprofiling Kits to jumpstart immunology research. When used with Cytek full spectrum flow cytometers, the cFluor® Immunoprofiling Kits provide turnkey solutions for basic to in depth research needs in immunophenotyping of human PBMC and whole blood. Users with different levels of expertise are now empowered to get optimal results.

These pre-optimized kits take 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

Start with our 8-color kit to obtain basic immunophenotyping data. The 14-color kit adds several markers useful for the identification of B- and T-cell subsets.

The 25-color panel provides detailed immunophenotyping across many subsets to reproducibly uncover maximum data from precious samples. These panels can be easily expanded to a larger panel. Not only have the panels been designed to provide optimal performance, but also to allow for drop-ins without impacting resolution of the markers included in each kit.

cFluor® TBMNK Kit, 8-Color

The cFluor TBMNK kit is designed to quickly perform basic immunophenotyping and analyze human B-cell, T-cell, monocyte, and NK cell populations on the Cytek Aurora and Northern Lights™ flow cytometers equipped with violet, blue, and red lasers. Compared to conventional TBMNK panels, adding CD14 and assigning CD16 and CD56 to separate channels allows delineation of classical, intermediate, and nonclassical monocytes in addition to better phenotyping of NKT-like cells.

The cFluor TBMNK Kit can be used as a backbone to further characterize populations of interest. This ready-to-use cFluor TBMNK Kit is also a good learning tool for new spectrum flow cytometer users to get trained on Cytek Aurora and Northern Lights flow cytometers.

8-Color Panel

Violet		Blue		Red	
Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome
CD3	cFluor V420	CD8	cFluor B515	CD16	cFluor R668
CD14	cFluor V450	CD19	cFluor BYG710	CD56	cFluor R720
CD45	cFluor V547			CD4	cFluor R780
				Viability	ViaDye Red

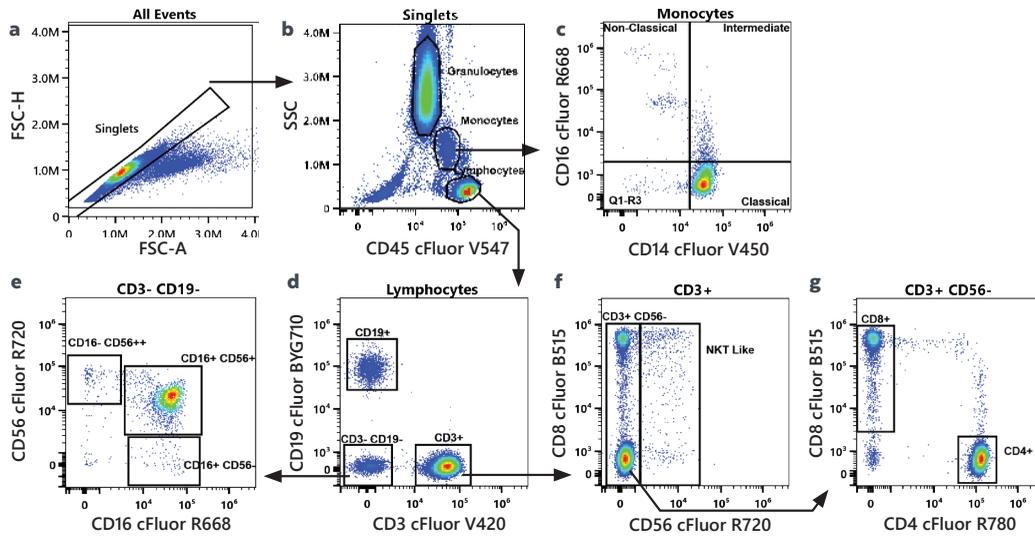


Figure 1: Results of human blood sample from a healthy donor stained with the 8-color panel. a) Single cells are identified. b) Gating of lymphocytes and monocytes using side scatter plus CD45. c) Identification of subpopulations of monocytes with CD14 and CD16. d) Lymphocytes were gated on CD19 (B-cells) and CD3 (T-cells). e) The double negative population (CD3- CD19-) was further defined with CD16 and CD56 to identify NK cells. f) CD3+ cells were gated on CD56 and CD8 to identify NKT-like cells. g) CD4 and CD8 T-cell populations were identified from the CD3+ CD56- population.

cFluor® Immunoprofiling Kit, 14 Color

The cFluor 14-color Immunoprofiling Kit allows for the analysis of human B-cell and T-cell, monocytes, and NK cell populations. Furthermore, this kit contains reagents to identify B- and T-cell subsets, such as memory cell populations. This cFluor panel, together with our Aurora and Northern Lights full spectrum flow cytometers, enables you to quickly identify the main cell subsets present in human blood sample.

The cFluor Immunoprofiling Kit, 14 Color can also be used as a backbone to expand to a panel of interest for more in depth immunophenotyping.

14-Color Panel

Violet		Blue		Red	
Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome
CD3	cFluor V420	CD8	cFluor B515	CD127	cFluor R659
CD14	cFluor V450	CCR7	cFluor BYG575	CD16	cFluor R668
CD45	cFluor V547	IgD	cFluor BYG667	CD56	cFluor R720
		CD45RA	cFluor B690	CD4	cFluor R780
		CD19	cFluor BYG710	Viability	ViaDye Red
		CD25	cFluor BYG781	CD27	cFluor R840

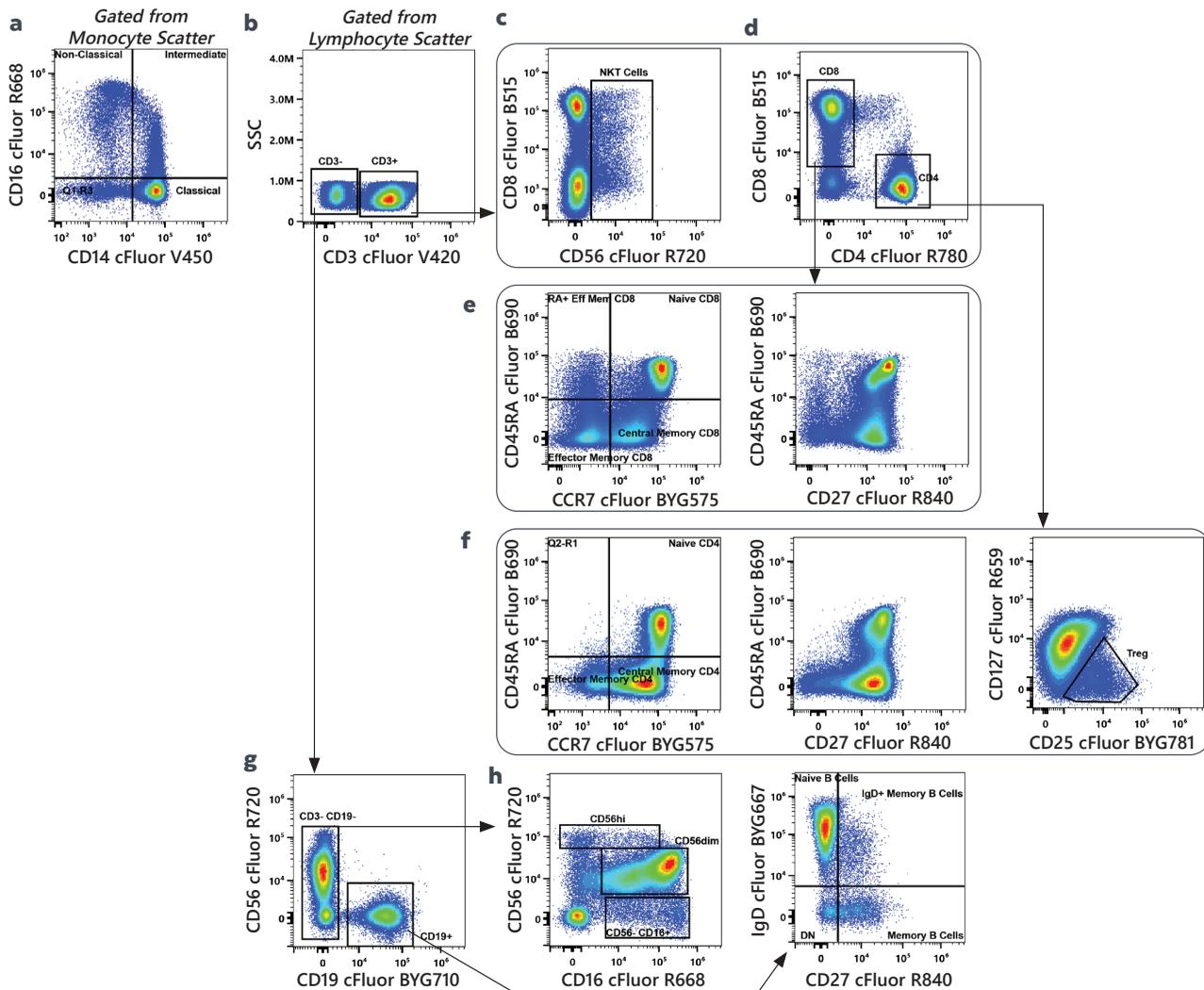


Figure 2: Results of healthy donor PBMCs stained with the 14-color panel. a) Identification of monocytes with CD14 and CD16. b) CD3 was used to gate T-cells. c) From the T-cell population, CD8 and CD56 were used to obtain the NKT-like population. d) CD4 and CD8 T-cell populations. e) Characterization of memory and effector CD8 T-cells. f) Characterization of memory and effector CD4 T-cells and identification of regulatory T-cells. g) The CD3- population further divided into CD19+ and CD56+ subsets. h) NK cell populations. i) Characterization of naive and memory B-cells.

Cytek® 25-Color Immunoprofiling Assay

The Cytek 25-Color Immunoprofiling Assay is designed based on our experience and learning from the 40-color OMIP-69 panel that was published in Cytometry Part A (97A: 1044-1051, 2020). This assay provides a turnkey solution for identification of major human immune subpopulations for T, B, NK cells, monocytes, dendritic cells, and basophils, which all 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.

25-Color Panel

Violet		Blue		Yellow-Green		Red	
Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome	Specificity	Fluorochrome
CCR7	BV421*	CD141	cFluor B515	CD25	cFluor BYG575	CD127	cFluor R659
CD45RA	cFluor V450	CD8	cFluor B532	CD4	cFluor YG584	CD1c	cFluor R668
IgM	BV510*	CD14	cFluor B548	CD16	cFluor BYG610	CD19	cFluor R685
CD20	cFluor V547	HLADR	cFluor B690	IgD	cFluor BYG667	CD123	cFluor R720
CD3	BV570*			TCRgd	cFluor BYG710	CD45	cFluor R780
CD28	BV650*			CD11c	cFluor BYG781	Viability	ViaDye Red
CD38	BV711*					CD27	cFluor R840
CD56	BV750*						
PD-1	BV785*						

*Brilliant Violet™ reagents are available from BioLegend

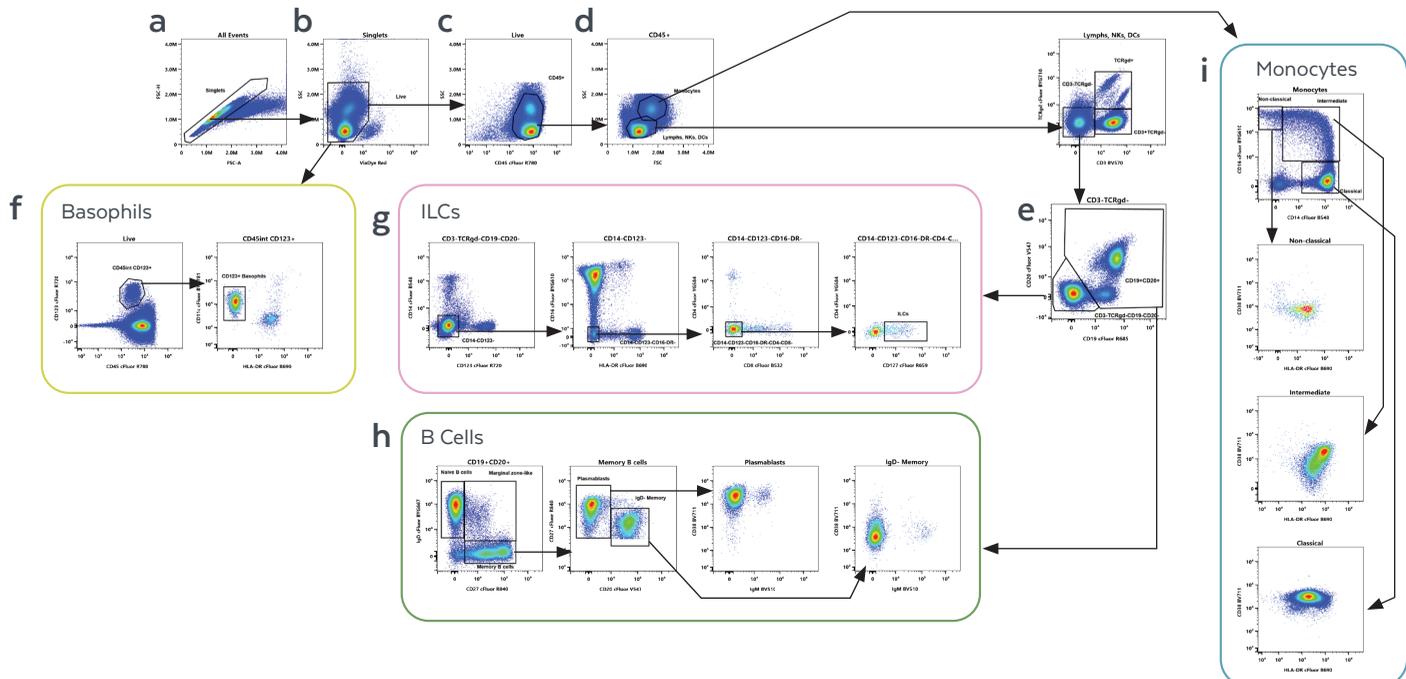


Figure 3: Upstream cell gating for population identification. Cells are first gated on a) singlets, b) viable cells, c) CD45⁺, and d) lymphocytes or monocytes. e) B cells are identified as a subset of CD3⁺TCRγδ⁻ lymphocytes. g) Innate lymphoid cells and h) B cells and their related populations. f) Basophils are classified within viable cells. i) Monocytes are further segregated into phenotypes by expression of CD14 and CD16.

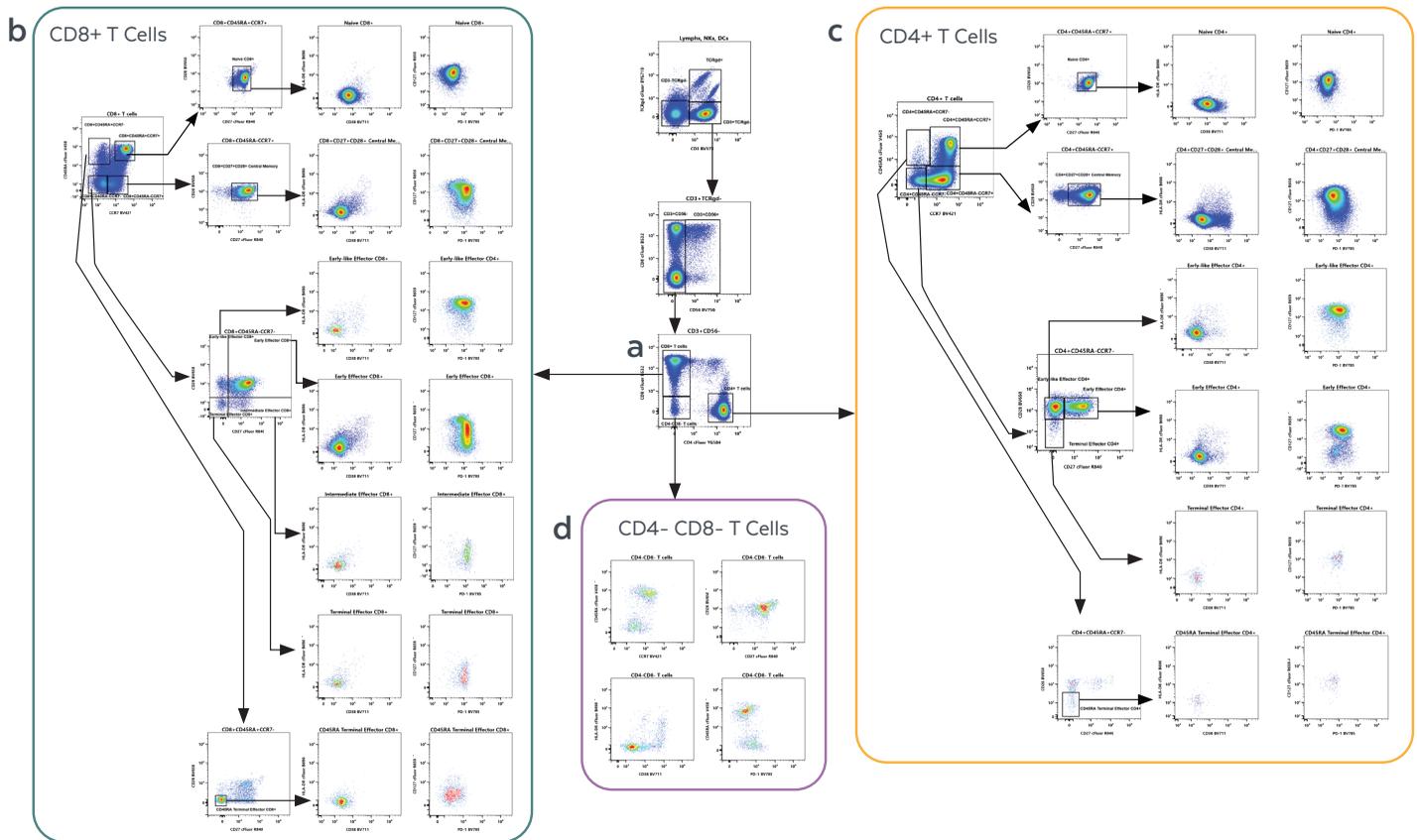


Figure 4: T_H and T_C characterization in 25-color panel. a) Within the T lymphocyte population, $CD4^+$, $CD8^+$, and $CD4-CD8^-$ T cells can be quantified. Further characterization of b) $CD8^+$, c) $CD4^+$, and d) $CD4-CD8^-$ can segregate naïve, memory, effector, and terminal effector subsets.

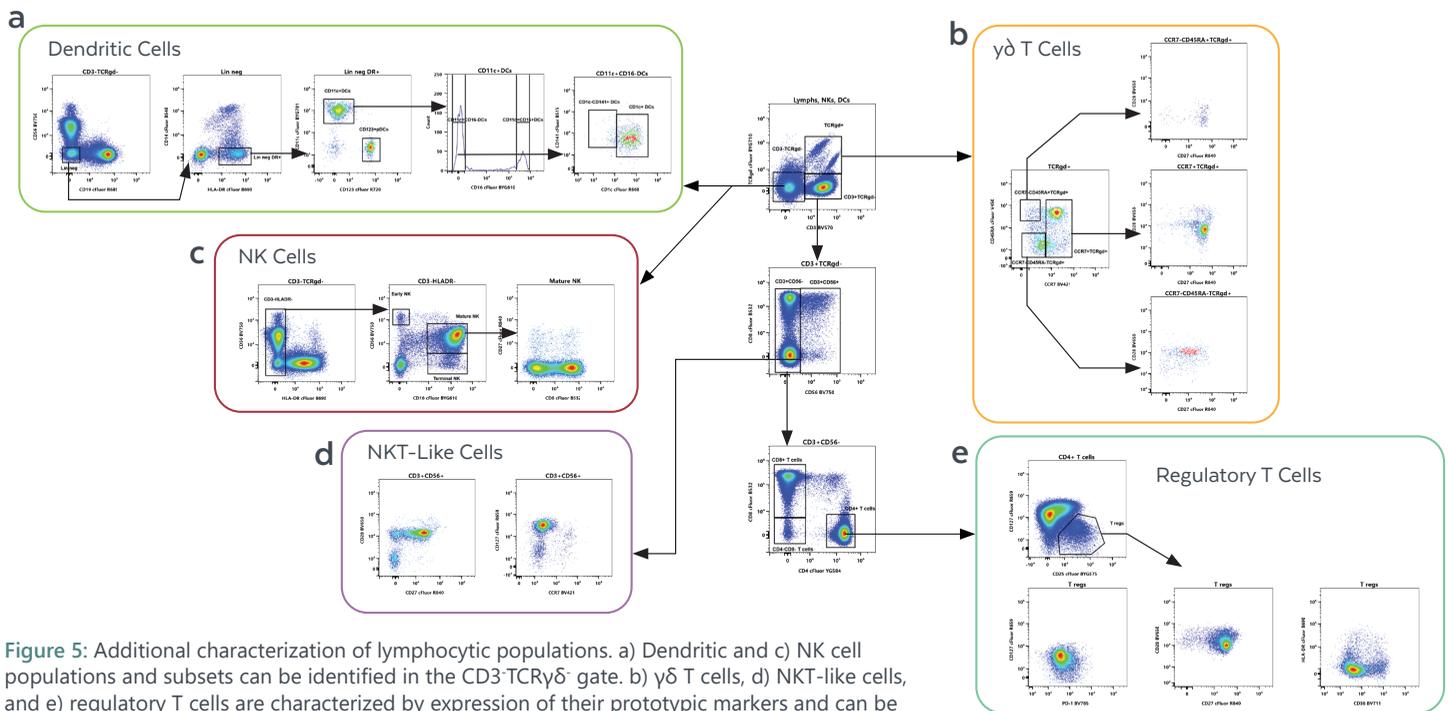


Figure 5: Additional characterization of lymphocytic populations. a) Dendritic and c) NK cell populations and subsets can be identified in the $CD3^+TCR\gamma\delta^-$ gate. b) $\gamma\delta$ T cells, d) NKT-like cells, and e) regulatory T cells are characterized by expression of their prototypic markers and can be further interrogated for different subsets and activation states.

Ordering Information:

Description	Size	P/N
cFluor® TBMNK Kit, 8 Color	25 Tests	R7-40001
cFluor® Immunoprofiling Kit, 14 Color	25 Tests	R7-40000
Cytek 25-Color Immunoprofiling Assay, cFluor® Reagent Kit (18C)	25 Tests	R7-40002
Immunoprofiling Kit, 7 Color (Brilliant Violet™), BioLegend	25 Tests	900004160
ViaDye™ Red Fixable Viability Dye Kit	100 Tests	R7-60008

cFluor® V547, cFluor® B515, cFluor® R668 and cFluor® R720 are equivalent to CF®405L, CF®488A, CF®647 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.

Cytek dye cFluor® R840 and its conjugates were developed, manufactured, and is commercialized by BioLegend, Inc. under the trademark APC/Fire810™. Cytek dye cFluor® BYG610 was developed, manufactured, and is commercialized by BioLegend, Inc. under the trademark PE-Dazzle 594™. *Fluor conjugated antibody manufactured and supplied by BioLegend Inc.

Brilliant Violet 421™, Brilliant Violet 510™, Brilliant Violet 570™, Brilliant Violet 605™, Brilliant Violet 650™, Brilliant Violet 711™, and Brilliant Violet 785™ are trademarks of Sirigen Group Ltd.

"Cytek," "Northern Lights" and "cFluor" are trademarks or registered trademarks of Cytek Biosciences, Inc. All other service marks, trademarks and tradenames appearing herein are the property of their respective owners.

Find out more at www.cytexbio.com/collection/reagents

Please contact sales@cytekbio.com with any questions

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

© 2021 Cytek Biosciences, Inc. All rights reserved. All trademarks are the properties of Cytek Biosciences, Inc. and its subsidiaries unless otherwise specified.

N9-20084 Rev. A
October 2021

