Application Information Bulletin: 13 colors on CytoFLEX

Advanced analysis of human T cell subsets on the CytoFLEX flow cytometer using a 13 color tube based on DuraClone dry reagent technology.

Authors: Nicole Weit[1], Michael Kapinsky[2], Andreas Böhmler[1]
E-mail: aboehmler@beckman.com

Affiliation: [1] Beckman Coulter GmbH, Krefeld, Germany
[2] Beckman Coulter Immunotech S.A.S, Marseille, France

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PRINCIPAL OF THE TECHNIQUE

Summary

Multi-color flow cytometry is a powerful tool to analyze the highly heterogeneous human T cell compartment. Using the 10 color DuraClone IM T Cell Subset dry reagent kit (CD45RA-FITC, CD197-PE, CD28-ECD, CD279-PC5.5, CD27-PC7, CD4-APC, CD8-Alexa-Fluor* 700, CD3-APC-Alexa-Fluor* 750, CD57-Pacific Blue*, CD45-Krome Orange) plus 3 additional liquid antibodies for the violet laser (Brilliant Violet 605™ anti-human CD95, Brilliant Violet 650™ anti-human CD25, and Brilliant Violet 785™ anti-human CD127 antibodies) we defined a 13-color tube which allows for the identification of major peripheral T cell subsets according to classical and more recent characterization criteria, with a minimum of sample preparation effort.

488 Excitation					638 Excitation			405 Excitation				
FITC	PE	ECD	PC5.5	PC7	APC	APC-A700 (1)	APC-A750 (2)	Pacific Blue*	Krome Orange	BV (3) 605	BV 650	BV 785
CD45RA	CCR7 (CD197)	CD28	PD1 (CD279)	CD27	CD4	CD8	CD3	CD57	CD45	CD95	CD25	CD127

(1) APC-Alexa Fluor* 700 (2) APC-Alexa Fluor* 750 (3) Brilliant Violet*

INTRODUCTION

T lymphocytes (T cells) form an essential part of the adaptive immune system and are therefore of major interest for the research community. Two recent examples are the work of the international ONE Study (www.onestudy.org) and BIO-DrIM (www.biodrim.eu) consortia, international groups of experts in the field of immune monitoring, funded by the European Commission. Within these approaches, marker and dye selections for flow cytometry have been designed and optimized by expert flow labs to monitor the human immune response [1].

The close cooperation between these groups and Beckman Coulter resulted in the development of the DuraClone IM brand of dry reagents for the analysis of human immune cells. Using the 10 color DuraClone IM T Cell Subsets kit plus 3 additional liquid markers, a 13 color tube has been designed for the phenotypic analysis of major T cell sub-populations in the human peripheral blood.

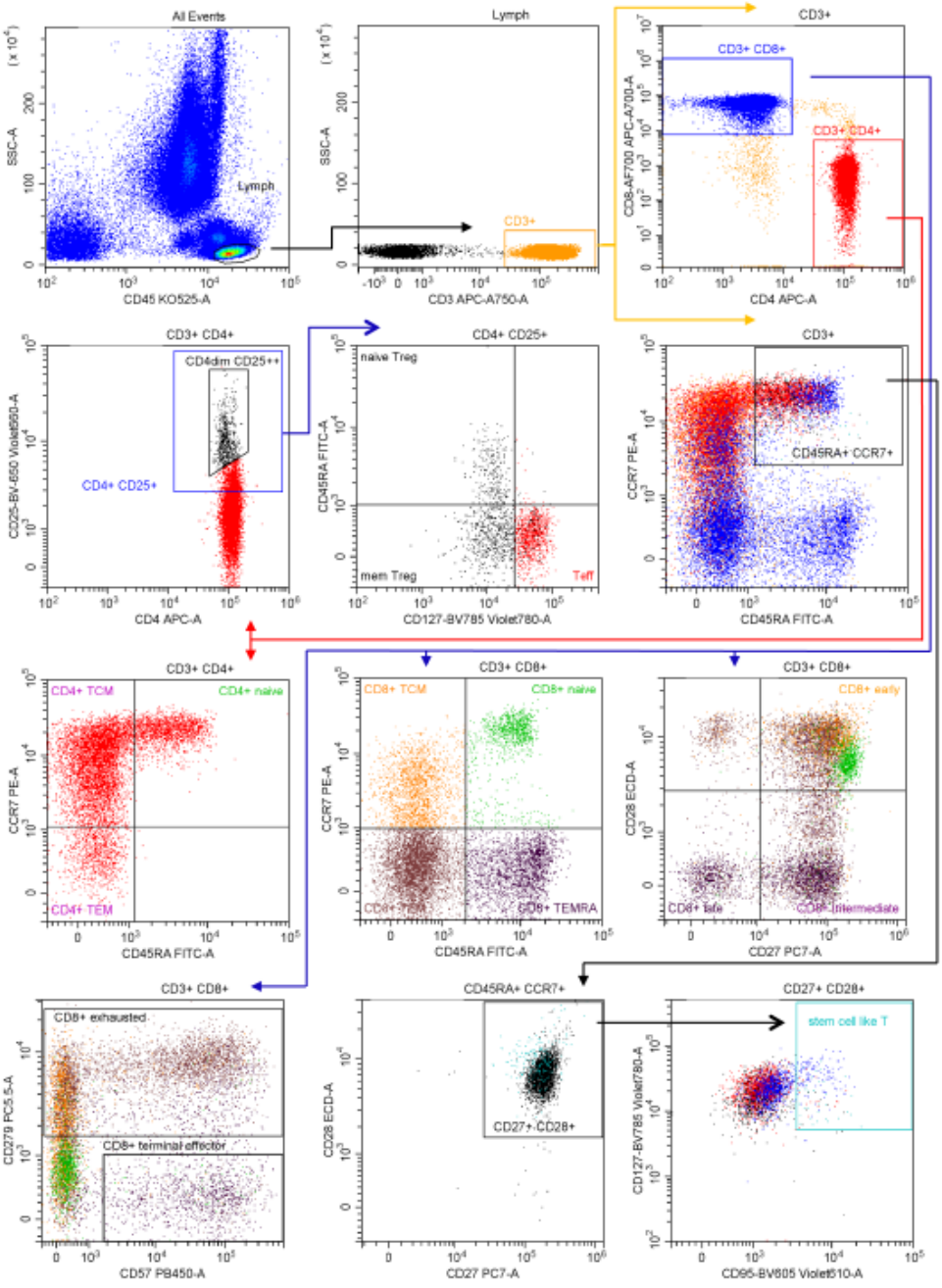
PROTOCOL

Standard Procedure:

After informed consent, 100 µL of human peripheral blood from a healthy donor was added to a DuraClone IM T Cell Subset dry reagent tube (Beckman Coulter), followed by 5 µL each of Brilliant Violet 605™ anti-human CD95, Brilliant Violet 650™ anti-human CD25, and Brilliant Violet 785™ anti-human CD127 antibodies (BioLegend). Cells were mixed for 8 seconds, incubated for 15 minutes at room temperature (RT) in the dark, and red blood cells were lysed by adding 2 mL of VersaLyse Lysing solution plus 50 µL of IOTest 3 Fixative Solution (both from Beckman Coulter). Following incubation (20 min at RT), the suspension was spun down (200 x g, 5 min), the supernatant discarded, and the pellet resuspended in 3 mL 1 x PBS. After an additional centrifugation step (see above), the cell pellet was resuspended in 500 µL 1 x PBS for subsequent analysis on a 13 color / 3 laser CytoFLEX flow cytometry system (Beckman Coulter).

CytoFLEX is the first commercial flow cytometer to utilize fiber array photo diodes (FAPD)s for fluorescence channel detection. The FAPD provides low-noise detection with high quantum efficiency and minimum light loss ensuring high signal to noise ratio and optical resolution especially with small particle measurements and dim fluorescence detection.

RESULTS



Analyses of T cell subsets based on the differential expression of surface molecules related to cell function, differentiation, or activation have evolved [2]. As a result, T cell analysis requires a multitude of markers to capture the various populations that have been described [3]. The 13 color tube presented here, allows for the identification of most T cell memory subpopulations that can be characterized by surface marker expression patterns. It is suitable for all flow cytometers with a 5-3-5 (488 & 561 nm / 638 nm / 405 nm) optical layout and reduces sample preparation to basically only 4 pipetting steps.

REFERENCES

1. Streitz M. et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. Transplantation Research 2013 2:17.
2. Appay V. et al. Phenotype and function of human T lymphocyte subsets: consensus and issues. Cytometry A 2008 73A: 975.
3. Mahnke Y.D. et al. The who's who of T-cell differentiation: Human memory T-cell subsets. Eur. J. Immunol. 2013 43: 2797.

NOTES

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer with 488 nm / 638 nm / 405 nm laser configuration. As differences exist in the performance between analyzers, the author cannot guarantee a similar appearance with the use of other Flow Cytometers.

REAGENT DETAILS

Reagent	Supplier	Order Details
DuraClone IM T Cell Subsets Kit	Beckman Coulter	B53328
VersaLyse Lysing Solution	Beckman Coulter	A09777 or§ IM3648
IOTest 3 Fixative Solution	Beckman Coulter	A07800 or§ IM3515
Brilliant Violet 605 anti-human CD95	BioLegend	305628
Brilliant Violet 650 anti-human CD25	BioLegend	302634
Brilliant Violet 785 anti-human CD127	BioLegend	351330

§ Depending on geography.

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

CytoFLEX is a trademark of Xitogen Technologies (Suzhou), Inc., a Beckman Coulter company. CytoFLEX with 561 nm laser not yet available.

*Alexa Fluor and Pacific Blue are registered trademarks of Molecular Probes, Inc.; Brilliant Violet is a trademark of Sirigen Group Ltd.