T Cell Receptor Characterization of PBMCs Using the CytoFlex

APPLICATION NOTE

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IN THIS PAPER YOU WILL

- Learn how to prepare PBMC and lyse red blood cells for flow cytometric staining
- Learn how to stain PBMC for T cell receptor analysis by flow cytometry utilizing DuraClone
- Identify TCR subpopultaions using the CytoFLEX flow cytometer and DuraClone

Introduction

T cells play a pivotal role in the function and regulation of the immune system. Mature T cells express both CD3 and a T Cell Receptor (TCR). They can further be divided into two subpopulations based on their type of TCR: αβ T cells (95% of the T cell repertoire) and γδ T cells (5% of the T cell repertoire). TCR αβ T cells are then differentiated into CD4 T cells or CD8 T cells based on their affinity recognition of antigens bound to MHC Class II or MHC Class I, respectively. The largest subset of γδ T cells express the Vδ2 TCR complex. A smaller subset of γδ T cells express the Vδ1 TCR complex. The study of TCR γδ Vδ2 T cells and TCR γδ Vδ1 T cells is important in feto-maternal[1] and allograft tolerance[2]. The understanding of the many T cell subpopulations is important for a variety of normal and pathological studies.

DuraClone IM panels are unitized, dry format reagent cocktails that are room temperature stable. Tube formulations were based on the ONE Study as well as design input from expert flow Cytometry labs for use in clinical research studies. Here, we look at the DuraClone IM TCRs tube with normal PBMCs on the CytoFLEX.

Methods

Peripheral Blood Mononuclear Cell (PBMC) isolation

Ten milliliters of whole blood was diluted to 21 mL with 1X PBS + 3% FCS. Six mL of Ficoll (Sigma:Histopaque 1077 cat # 10771) was added to three, 15 mL conical vials. Seven mLs of diluted blood were added to the Ficoll by addition with a 10 mL pipet at a very steep angle. Diluted blood was then slowly added to over-lay on to the Ficoll. Cells were spun at 1500 RPM for 30 minutes in a swinging bucket centrifuge with the brake turned off. Supernatant was aspirate and samples were pooled and washed in PBS + 5% FCS. Cell pellets were resuspended and counted using the ViCell and diluted to a final concentration of 1 x 10^6 cells/100 µL per tube for staining.

DuraClone Staining Procedure

One million PBMCs were added to a DuraClone IM TCRs tube and then vortexed for 5 seconds. After 20 minutes incubation at room temperature in the dark, the PBMCs were washed with 3 mL of PBS and centrifuged at 400g for 5 minutes. The PBMCs were resuspended in 250 µL of PBS and acquired on the CytoFLEX.

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Laser	405 nm					488 nm					638 nm		
Fluor	Krome Orange	Pacific Blue	V610	V660	V780	FITC	PE	ECD	PC5	PC7	APC	AF700	APC AF750
Marker	CD45	TCR Vδ2				TCR γδ	TCR αβ	HLA-DR		TCR Vδ1	CD4	CD8	CD3
Clone	J.33	IMMU 389				IMMU510	IP26A	Immu-357		R9.12	13B8.2	B9.11	UCHT-1

Results

Rapid TCR Subsetting with the DuraClone IM TCRs Tube

Staining of normal PBMCs with the DuraClone IM TCRs tube depicted here suggests that T cell subsets can be rapidly determined with the CytoFLEX. After approximately 30 minutes, PBMCs were accurately characterized into their appropriate compartments. Using the DuraClone IM TCRs tube is a viable alternative to laborious staining procedures.

References

1- Pregnancy and gamma/delta T cells: taking on the hard questions. Mincheva-Nilsson L. Reprod Biol Endocrinol. 2003 Dec 2; 1:120. Review.

2- Characteristics of Vδ1 (+) and Vδ2 (+) γδ T cell subsets in acute liver allograft rejection. Yu X, Liu Z, Wang Y, Wang H, Zhang M, Sun Y, Su H, Jin L, Wang F, Shi M. Transpl Immunol. 2013 Dec; 29(1- 4):118-22.

Notes

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer. 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 TCRs Tube	Beckman Coulter	B53340
Lymphocyte Separation Medium	Corning	25-072-CI
DPBS, 1X (Dulbecco's Phosphate-Buffered Saline	Corning	21-031-CV

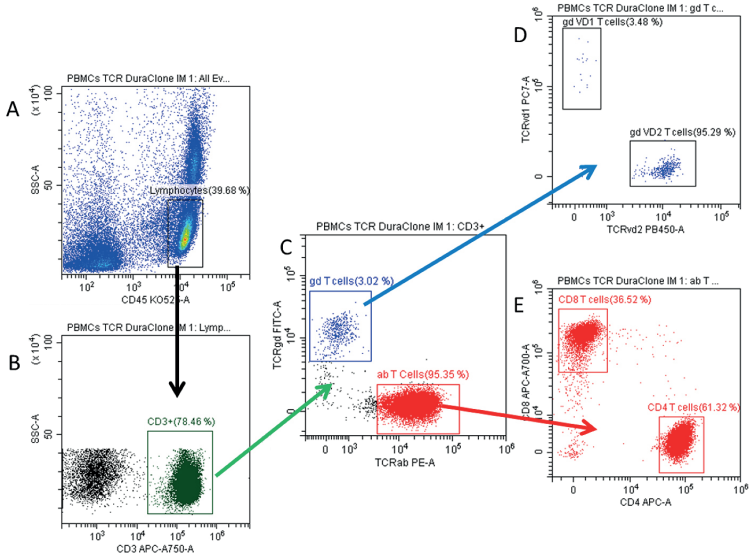

Figure 1. Rapid T Cell Receptor Subsetting with the DuraClone IM TCRs Tube

PBMCs were gated on Lymphocytes by CD45 staining (A). CD3+ lymphocytes (B) were then gated based on expression of TCR γδ or TCR αβ (C). Of the γδ T cells, expression of Vδ1 and Vδ2 was characterized (D). Of the αβ T cells, CD8 and CD4 expression is characterized (E).

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