Development of an anti-human TRBC2 antibody for flow cytometry

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Abstract 5029

Abstract

Each chain of the αβ T-cell receptor (TCR) contains a constant (C) region and an antigen-binding variable (V) region. There are two human isoforms of the TCR β chain, T-cell receptor constant β chain (TRBC)1 and TRBC2, differing slightly in the constant region, and individual αβ T-cells express one isoform or the other. The ratio of TRBC1$^+$ to TRBC2$^+$ αβ T-cells falls within a characteristic range of healthy donors; monoclonal T-cell malignancies skew this ratio dramatically. This effect makes TRBC1 and TRBC2 more specific targets for diagnosis and treatment of T-cell malignancies than the typical T-cell markers. A monoclonal antibody specific for TRBC1 (clone JOVI.1) has proven useful in this regard but is unable to directly identify both TRBC1$^+$ and TRBC2$^+$ T cell malignancies as is done for B cell cancers using kappa and lambda-specific antibodies.

Here we characterize clone SAM.2.rMAb, to our knowledge the first monoclonal antibody that selectively binds human TRBC2 and demonstrate its use by flow cytometry in combination with JOVI.1 to confirm the mutually exclusive expression of TRBC1 and TRBC2 in CD4$^+$ and CD8$^+$ T-cells, NKT cells, MAIT cells and Treg. This new clone permits direct identification of TRBC2$^+$ cells, is compatible with BD Pharm Lyse™ Lysing Buffer, BD FACS™ Lysing Solution, BD Pharmingen™ Transcription Factor Buffer Set and can be used in phenotyping panels that include TCR and CD3 antibodies. SAM.2.rMAb is thus a valuable flow cytometry reagent to potentially combine with JOVI.1 in building rapid and low-cost research use only immunophenotyping assays for basic and applied research on human T-cell malignancies.

Methods

Materials:

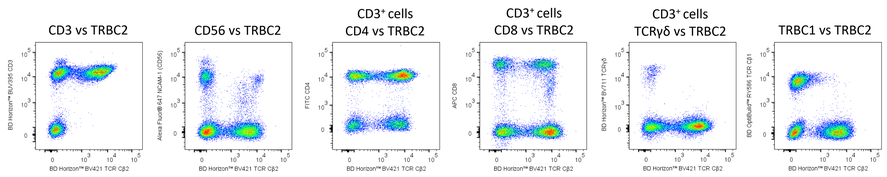
1. Human TCR Cβ2 (TRBC2) clone SAM.2.rMAb BD Horizon™ BV421 (BD Cat. No. 571543/571585)
2. Human TCR Cβ2 (TRBC2) clone SAM.2.rMAb PE (BD Cat. No. 571190/571191)
3. Human TCR Cβ1 (TRBC1) clone JOVI.1 BD OptiBuild™ RY586 (BD Cat. No. 753522)
4. Human CD3 clone SP34-2 BD Horizon™ BUV395 (BD Cat. No. 564117)
5. Human CD3 clone SP34-2 Alexa Fluor™ 488 (BD Cat. No. 557705)
6. Human CD3 clone SP34-2 APC (BD Cat. No. 557597)
7. Human CD3 clone UCHT1 FITC (BD Cat. No. 555332)
8. Human CD3 clone UCHT1 BD Horizon™ BUV395 (BD Cat. No. 563546)
9. Human CD3 clone OKT3 FITC (BD Cat. No. 566783)
10. Human CD3 clone HIT3a FITC (BD Cat. No. 555339)
11. Human CD3 clone SK7 BD OptiBuild™ RB780 (BD Cat. No. 755579)
12. Human CD4 clone SK7 FITC (BD Cat. No. 570821)
13. Human CD4 clone SK7 BD Horizon™ BV711 (BD Cat. No. 563028)
14. Human CD8 clone HIT8a APC (BD Cat. No. 566852)
15. Human CD8 clone HIT8a BD Horizon™ BUV395 (BD Cat. No. 569178)
16. Human NCAM-1 (CD56) clone R19-760 Alexa Fluor™ 647 (BD Cat. No. 563443)
17. Human CCR7 clone 2-L1-A BD Horizon™ RB780 (BD Cat. No. 568748)
18. Human CD45RA clone HI100 APC (BD Cat. No. 550855)
19. Human CD161 clone HP-3G10 Alexa Fluor™ 647 (BD Cat. No. 566708)
20. Human TCR Vα7.2 clone OF-5A12 BD OptiBuild™ RB780 (BD Cat. No. 755441)
21. Human FoxP3 clone 259D/C7 BD Horizon™ RB780 (BD Cat. No. 568682)
22. Human TCRγδ clone 11F2 BD Horizon™ BV711 (BD Cat. No. 568490)
23. Human TCRαβ clone IP26 BD Horizon™ R718 (BD Cat. No. 567676)
24. Human TCRαβ clone T10B9 BD Horizon™ BV510 (BD Cat. No. 563625)
25. Human TCRαβ clone WT31 FITC (BD Cat. No. 347773)
26. Mouse IgG, κ clone X40 BD Horizon™ BV421 (BD Cat. No. 562438)
27. BD Pharmingen™ Human BD Fc Block™ Reagent (BD Cat. No. 564220)
28. BD Horizon™ Brilliant Stain Buffer (BD Cat. No. 566349)
29. BD Pharmingen™ Stain Buffer (FBS) (BD Cat. No. 554656)
30. BD Pharmingen™ DAPI Solution (BD Cat. No. 564907)
31. BD Pharmingen™ 7-AAD (BD Cat. No. 559925)
32. BD Pharm Lyse™ Lysing Buffer (BD Cat. No. 555899)
33. BD FACS™ Lysing Solution 10X Concentrate (BD Cat. No. 349202)
34. BD Pharmingen™ Transcription Factor Buffer Set (BD Cat. No. 562574)

Methods:

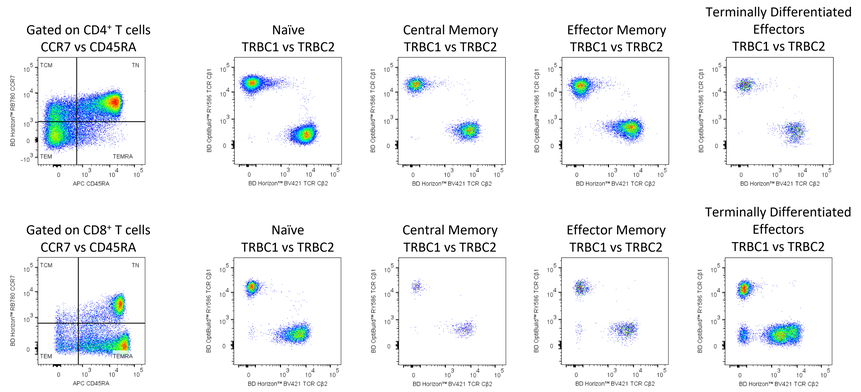
1. Human whole blood from healthy donors was stained with a cocktail of antibodies against surface markers (TRBC1 and TRBC2 were stained simultaneously), then erythrocytes were removed by treating the blood with either BD Pharm Lyse™ Lysing Buffer or BD FACS™ Lysing Solution.
2. Fresh human peripheral blood mononuclear cells (PBMC) from healthy donors were isolated using Ficoll-Paque™ PLUS (GE Healthcare) gradient centrifugation, then cells were stained with a cocktail of antibodies against surface markers in BD Pharmingen™ Stain Buffer (FBS) (TRBC1 and TRBC2 were stained simultaneously).
3. For intracellular staining, freshly isolated PBMCs were either stained first with a cocktail of antibodies against surface markers in BD Pharmingen™ Stain Buffer (FBS), then fixed and permeabilized with the BD Pharmingen™ Transcription Factor Buffer Set and stained with antibodies against intracellular markers, or the PBMCs were directly fixed and permeabilized with the BD Pharmingen™ Transcription Factor Buffer Set, then the cells were stained with a cocktail of antibodies against surface and intracellular markers. In both conditions, TRBC1 and TRBC2 were stained simultaneously.
4. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ Software.

SAM.2.rMAb selectively binds human TRBC2 and can be used in combination with JOVI.1 to confirm the mutually exclusive expression of TRBC1 and TRBC2 in human T cells

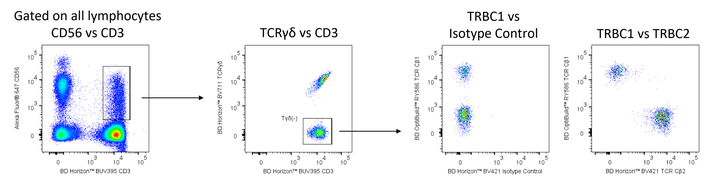
1A Phenotyping Panel – All lymphocytes



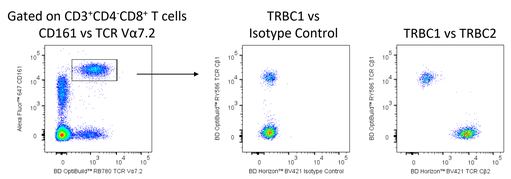
1B Effector/Memory T Cells



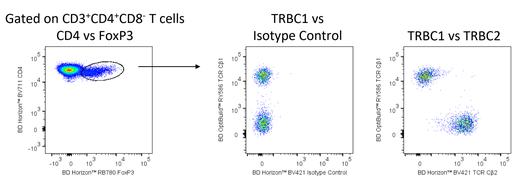
1C Natural Killer T (NKT)-like Cells

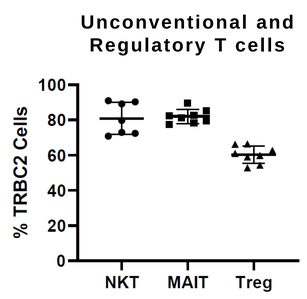


1D Mucosal-Associated Invariant T (MAIT) Cells



1E Regulatory T Cells (Treg)





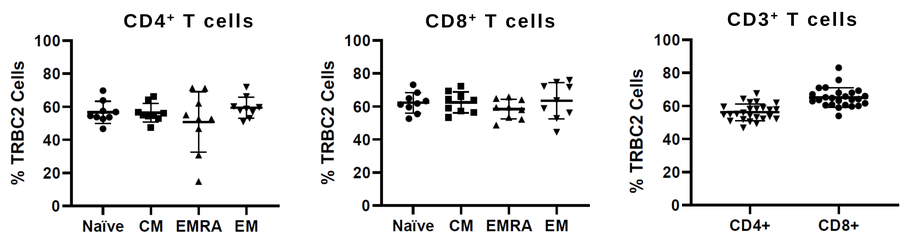


Figure 1: Analysis of TRBC2 expression in immune cell populations. (A) Human blood was stained with CD3, CD56, CD4, CD8, TCRγδ, TRBC1 and TRBC2. Erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer. Cells in the lymphocyte gate are shown here. TRBC2 is expressed on CD3$^+$ cells, CD56$^+$ cells, CD4$^+$ cells and CD8$^+$ cells, and is not expressed on TCRγδ$^+$ cells. TRBC1 and TRBC2 expression is mutually exclusive, as shown by the absence of double positive cells in the last panel on the right. (B) Human PBMCs were stained with specific antibodies to identify subpopulations of T cells. TRBC1 and TRBC2 expression is shown on CD4$^+$ (top row) and CD8$^+$ (bottom row) naïve cells (CCR7$^+$CD45RA$^+$), central memory cells (CCR7$^+$CD45RA$^-$), effector memory cells (CCR7$^-$CD45RA$^-$) and terminally differentiated effector cells (CCR7$^-$CD45RA$^+$). (C) Human PBMCs were stained with specific antibodies to identify NKT cells. TRBC1 and TRBC2 (or isotype control) expression is shown on CD3$^+$CD56$^+$TCRγδ$^-$ NKT cells. (D) Human PBMCs were stained with specific antibodies to identify MAIT cells. TRBC1 and TRBC2 (or isotype control) expression is shown on CD3$^+$CD4$^-$CD8$^-$CD161$^+$TCR Vα7.2$^+$ MAIT cells. (E) Human PBMCs were fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set and the cells were stained with specific antibodies to identify regulatory T cells. TRBC1 and TRBC2 (or isotype control) expression is shown on CD3$^+$CD4$^+$CD8$^-$FoxP3$^+$ Treg cells. % of TRBC2 positive cells is shown for different T cell populations.

SAM.2.rMAb is compatible with specific CD3 and TCR clones, and can be used with BD buffers

2A Costaining of TRBC2 with CD3 and TCR clones

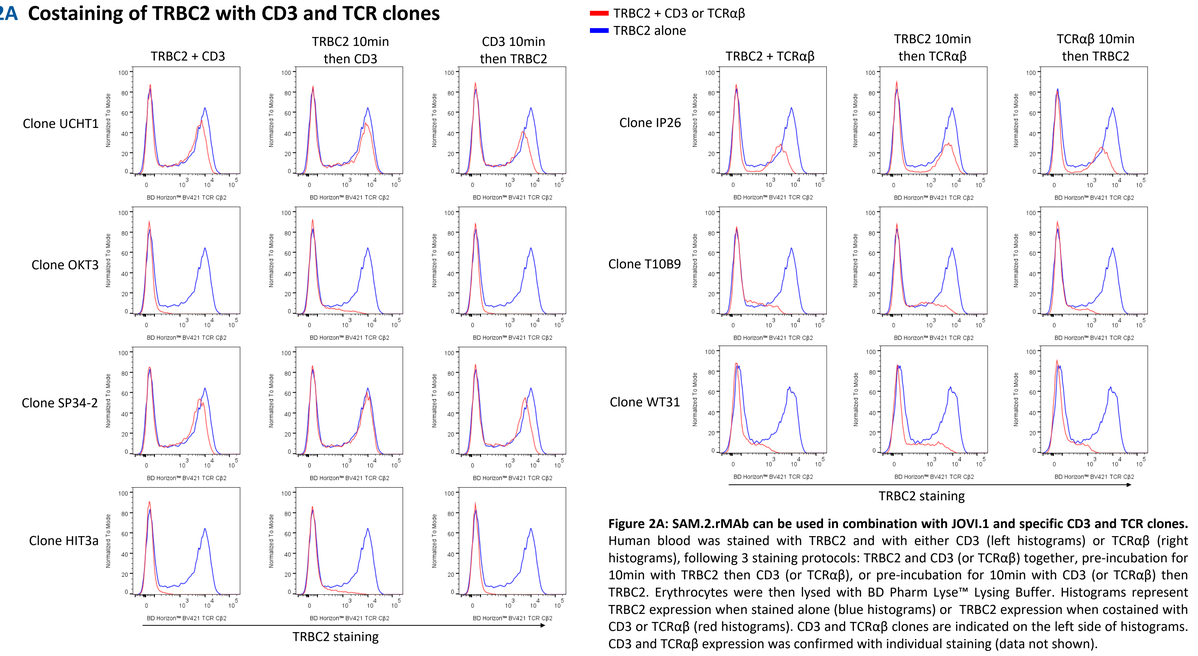


Figure 2A: SAM.2.rMAb can be used in combination with JOVI.1 and specific CD3 and TCR clones. Human blood was stained with TRBC2 and with either CD3 (left histograms) or TCRαβ (right histograms), following 3 staining protocols: TRBC2 and CD3 (or TCRαβ) together, pre-incubation for 10min with TRBC2 then CD3 (or TCRαβ), or pre-incubation for 10min with CD3 (or TCRαβ) then TRBC2. Erythrocytes were then lysed with BD Pharm Lyse™ Lysing Buffer. Histograms represent TRBC2 expression when stained alone (blue histograms) or TRBC2 expression when costained with CD3 or TCRαβ (red histograms). CD3 and TCRαβ clones are indicated on the left side of histograms. CD3 and TCRαβ expression was confirmed with individual staining (data not shown).

2B BD lysing buffers and permeabilization buffers

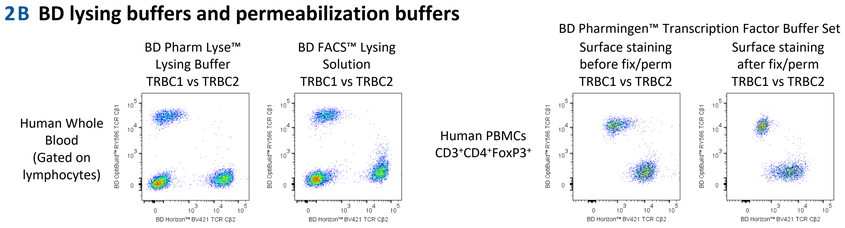


Figure 2B: SAM.2.rMAb is compatible with BD buffers. Human blood was stained with TRBC1 and TRBC2 followed by treatment with BD Pharm Lyse™ Lysing Buffer or BD FACS™ Lysing Solution to remove erythrocytes. TRBC1 and TRBC2 expression is shown on total lymphocytes (left plots). Human PBMCs were stained with TRBC1 and TRBC2 before or after fixation and permeabilization using the BD Pharmingen™ Transcription Factor Buffer Set. TRBC1 and TRBC2 expression is shown on total lymphocytes (right plots).

Conclusions

- SAM.2.rMAb **selectively binds TRBC2** in human lymphocytes, and it **can be used with JOVI.1** to confirm that TRBC1 and TRBC2 expression is mutually exclusive in T cells.
- CD4$^+$ and CD8$^+$ differentiation subpopulations, as well as Treg, all contain TRBC1$^+$ and TRBC2$^+$ cells, in a ratio that is comparable to the ratio observed for the total CD3$^+$ T cell population.
- NKT cells and MAIT cells, two populations of unconventional αβ T cells expressing an invariant or semi-invariant restricted TCR, also contain TRBC1$^+$ and TRBC2$^+$ cells but both populations showed a higher proportion of TRBC2$^+$ cells, when compared to the total T cell population.
- SAM.2.rMAb is **compatible with BD Pharm Lyse™ Lysing Buffer**, BD **FACS™ Lysing** Solution and BD Pharmingen™ T**ranscription Factor Buffer Set** for easy use in phenotyping panels.
- SAM.2.rMAb **can be used in combination with specific CD3 and TCR clones**, using our optimized staining protocol; per the table below:

Specificity	Clone	TRBC2 + TCR	TRBC2 10min then TCR	TCR 10min then TRBC2
CD3	UCHT1	✓	✓	✓
CD3	OKT3	X	X	X
CD3	SP34-2	✓	✓	✓
CD3	HIT3a	X	X	X
CD3	SK7	X	✓	X
TCRαβ	IP26	X	X	X
TCRαβ	T10B9	X	X	X
TCRαβ	WT31	X	X	X
TCRγδ	11F2	✓	✓	Not tested

✓ = compatible (% positive cells and MFI show <20% difference vs staining alone)
✓ = partially compatible (% positive cells and MFI show ~20% difference vs staining alone)
X = not compatible (no SAM.2.rMAb staining detected, or % positive cells and/or MFI show >50% difference vs staining alone)