Technical Data Sheet

PE-CF594 Rat Anti-Human CD294 (CRTH2)

Product Information

Material Number:	563501
Alternate Name:	CRTH2; PTGDR2 ; Prostaglandin D2 receptor 2; DL1R; DP2; GPR44
Size:	50 tests
Vol. per Test:	5 μl
Clone:	BM16
Immunogen:	Human CRTH2 Transfected Cell Line
Isotype:	Rat (WI) IgG2a, ĸ
Reactivity:	QC Testing: Human
Workshop:	VIII
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BM16 monoclonal antibody specifically binds to CD294. CD294 is encoded by PTGDR2 (Prostaglandin D2 receptor 2) and is also known as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), GPR44 (G protein-coupled receptor 44), DL1R, and DP2. CD294 is a member of the G protein-coupled leukocyte chemoattractant receptor family and it is expressed on Th2, but not Th1 type cells. CD294 is detectable on CD4+ T cells in fresh PBMC but not on B cells and NK cells. CD294 is also expressed on peripheral blood basophils and eosinophils, suggesting its involvement allergic reactions. Phenotypic analysis of CD4+ T cells expressing CRTH2 demonstrated that they were also CD45RA-negative and CD45RO+ and CD25+. These cells produce Th2- but little or no Th1-type cytokines upon stimulation with PMA and Ionomycin.

This antibody is conjugated to BD Horizon[™] PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Two-color flow cytometric analysis of CD294 expression on human peripheral blood cells. Whole blood was stained with APC Mouse Anti-Human CD45 antibody (Cat. No. 555485/560973/561864) and either BD Horizon™ PE-CF594 Rat IgG2a, κ Isotype Control (Cat. No. 562302; Left Panel) or BD Horizon™ PE-CF594 Rat Anti-Human CD294 antibody (Cat. No. 563501; Right Panel). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). Two-color dot plots showing the correlated expression of CD45 versus CD294 (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable leukocytes. Flow cytometry was performed using a BD™ LSR II Flow Cvtometer Svstem.

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594

were removed **Application Notes**

Application

Application				
Flow cytometry	ow cytometry Routinely Tested			
Suggested Compar	nion Products			
Catalog Number	Name	Size	Clone	
554656	Stain Buffer (FBS)	500 ml	(none)	
562302	PE-CF594 Rat IgG2a, κ Isotype Control	0.1 mg	R35-95	
555899	Lysing Buffer	100 ml	(none)	
555485	APC Mouse Anti-Human CD45	100 tests	HI30	

25 tests

500 tests

HI30

HI30

Product Notices

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561864

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^{6} cells in a 100-µl experimental 1. sample (a test).
- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 2.

APC Mouse Anti-Human CD45

APC Mouse Anti-Human CD45

- 3 An isotype control should be used at the same concentration as the antibody of interest.
- 4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem 5 fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 6. discarding to avoid accumulation of potentially explosive deposits in plumbing.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at 7. www.bdbiosciences.com/colors.
- 8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- 9. CFTM is a trademark of Biotium, Inc.
- 10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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- 12. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.

References

Cosmi L, Annunziato F, Galli MIG , Maggi RME , Nagata K, Romagnani S. CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. Eur J Immunol. 2000; 30(10):2972-2979. (Clone-specific: Flow cytometry)

Nagata K, Hirai H, Tanaka K, et al. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). FEBS Lett. 1999; 459(2):195-199. (Clone-specific: Flow cytometry)

Nagata K, Tanaka K, Ogawa K, et al. Selective expression of a novel surface molecule by human Th2 cells in vivo. J Immunol. 1999; 162(3):1278-1286. (Immunogen: Blocking, Cell separation, Flow cytometry, Western blot)

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