Technical Data Sheet

PE-CF594 Mouse Anti-Human CD28

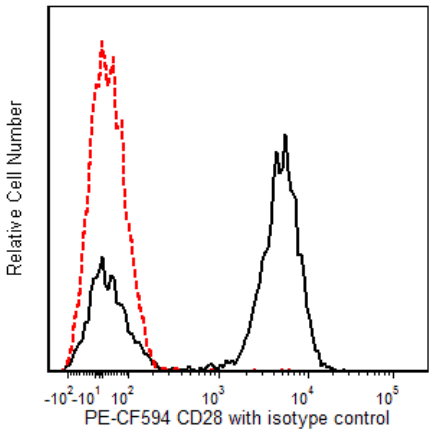
Product Information

Material Number:	**562323**
Alternate Name:	CD28 antigen; T44; Tp44; TP44
Entrez Gene ID:	940
Size:	25 Tests
Vol. per Test:	5 µl
Clone:	CD28.2
Immunogen:	Human CD28 Transfected Cell Line
Isotype:	Mouse (C3H x BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	V 5T CD28.05
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The CD28.2 monoclonal antibody specifically binds to CD28, a 44 kDa homodimeric transmembrane glycoprotein present on most mature T cells, thymocytes and plasma cells. CD28 is a costimulatory receptor that binds CD80 and CD86 as ligands and plays a very important role in T cell-B cell interactions. It has been suggested that CD28 initiates and regulates a separate and distinct signal transduction pathway from those stimulated by the TCR complex. Additionally, it has been reported that CD28 antibody clones vary in their ability to stimulate T cells to produce IL-2 and increase intracellular Ca2+ concentration. This finding suggests the existence of functionally distinct subregions on the CD28 molecule. CD28.2 has been demonstrated to bind to the same molecule as clone L293, another CD28 mAb, and has been reported to induce Ca2+ influx in Jurkat T cells.

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).



Flow cytometric analysis of CD28 expression on human peripheral blood lymphocytes. *Human whole blood was stained with the BD Horizon™ PE-CF594 Mouse Anti-Human CD28 antibody (Cat. No. 562296/562323; solid line histogram) or with a BD Horizon™ PE-CF594 Mouse IgG1, κ Isotype Control (Cat. No. 562292; dashed line histogram). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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

Flow cytometry	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40
554656	Stain Buffer (FBS)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)

Product Notices

1. 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 sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
4. 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.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. CF™ is a trademark of Biotium, Inc.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer's own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
11. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
12. This product is sold under license. Purchase of this product does not include rights to (i) incorporate this product into the purchaser's own products for resale to end-users, or (ii) use this product to conduct for-profit research for or on behalf of another party. For information on obtaining a license to this product for such prohibited uses, contact INSERM, 7 rue Watt, 75013 Paris. Telephone: +33 1 55 03 01 60. Facsimile: +33 1 55 03 01 18. Email: techtransfert@inserm-transfert.fr
13. An isotype control should be used at the same concentration as the antibody of interest.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997. (Biology)

June CH, Bluestone JA, Nadler LM, Thompson CB. The B7 and CD28 receptor families. *Immunol Today.* 1994; 15(7):321-331. (Biology)

Kuiper H, Brouwer M, Vermeire S, van Lier R. Analysis of the Workshop CD28 Panel mAb: distinct signalling pathways coupled to CD28. In: Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leucocyte Typing V: White Cell Differentiation Antigens.* Oxford: Oxford University Press; 1995:373-374. (Clone-specific: Activation, Calcium Flux, (Co)-stimulation)

Nunes J, Klasen S, Franco MD, et al. Signalling through CD28 T-cell activation pathway involves an inositol phospholipid-specific phospholipase C activity. *Biochem J.* 1993; 293(3):835-842. (Biology)

Nunes J, Klasen S, Ragueneau M, et al. CD28 mAbs with distinct binding properties differ in their ability to induce T cell activation: analysis of early and late activation events. *Int Immunol.* 1993; 5(3):311-315. (Biology)

Olive D, Cerdan C, Costello R, Sielleur I, Ragueneau M, Pages F, Klasen S, Nunes J, Imbert J. CD28 and CTLA-4 cluster report. In: Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leucocyte Typing V: White Cell Differentiation Antigens.* Oxford: Oxford University Press; 1995:360-370. (Clone-specific: (Co)-stimulation, Flow cytometry, Functional assay, Inhibition, Stimulation)

Schlossman SF, Boumsell L, Gilks W, et al, ed. *Leukocyte Typing V: White Cell Differentiation Antigens.* Oxford: Oxford University Press; 1995. (Clone-specific)

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