Technical Data Sheet

BV421 Mouse Anti-Human CD28

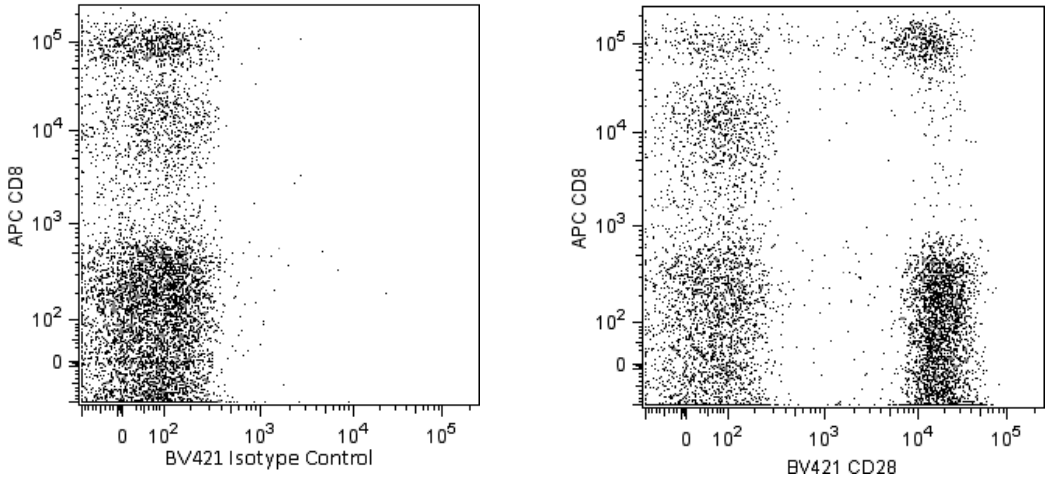
Product Information

Material Number:	**562613**
Alternate Name:	CD28 antigen; T44; Tp44; TP44
Size:	100 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.

The antibody was conjugated to BD Horizon BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue conjugates.



Multicolor flow cytometric analysis of CD28 expression on human peripheral blood lymphocytes. *Whole blood was stained with APC Mouse anti-Human CD8 antibody (Cat. No. 555369/561952/561953) and either BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438; Left Panel) or BD Horizon™ BV421 Mouse anti-Human CD28 antibody (Cat. No. 562613; Right Panel). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The two-color flow cytometric dot plots showing the correlated expression of CD28 (or Ig Isotype Control staining) versus CD8 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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

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Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
562438	BV421 Mouse IgG1, k Isotype Control	50 µg	X40
554656	Stain Buffer (FBS)	500 mL	(none)
555899	Lysing Buffer	100 mL	(none)
555369	APC Mouse Anti-Human CD8	100 Tests	RPA-T8
561952	APC Mouse Anti-Human CD8	25 Tests	RPA-T8
561953	APC Mouse Anti-Human CD8	500 Tests	RPA-T8

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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. An isotype control should be used at the same concentration as the antibody of interest.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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
8. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.

References

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. (Clone-specific: Calcium Flux, (Co)-stimulation, Functional assay)

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. (Immunogen: Calcium Flux, (Co)-stimulation, Flow cytometry, Functional assay, IC/FCM Block, Immunoprecipitation, Stimulation)

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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