

## Technical Data Sheet

**BV605 Mouse Anti-Human CD28****Product Information**

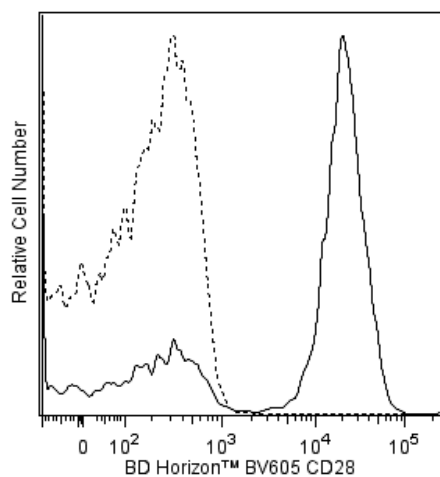
<b>Material Number:</b>	<b>562976</b>
<b>Alternate Name:</b>	CD28 antigen; T44; Tp44; TP44
<b>Size:</b>	100 Tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	CD28.2
<b>Immunogen:</b>	Human CD28 Transfected Cell Line
<b>Isotype:</b>	Mouse (C3H x BALB/c) IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Workshop:</b>	V 5T CD28.05
<b>Storage Buffer:</b>	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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup> influx in Jurkat T cells.

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794).



**Flow cytometric analysis of CD28 expression on human peripheral blood lymphocytes.** Whole blood was stained with either BD Horizon™ BV605 Mouse IgG1, κ Isotype Control (Cat. No. 562652; dashed line histogram) or BD Horizon™ BV605 Mouse anti-Human CD28 antibody (Cat. No. 562976; solid 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™ BV605 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV605 were removed.

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## Application Notes

### Application

Flow cytometry

Routinely Tested

### Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
562652	BV605 Mouse IgG1, $\kappa$ Isotype Control	50 $\mu$ g	X40
555899	Lysing Buffer	100 mL	(none)
563794	Brilliant Stain Buffer	5 mL	(none)

### Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ L experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/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. 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. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
8. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon™ BV605 conjugate.
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### 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. *Leukocyte 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. *Leukocyte 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)

Verwilghen J, Vandenberghe P, Wallays G, et al. Simultaneous ligation of CD5 and CD28 on resting T lymphocytes induces T cell activation in the absence of T cell receptor/CD3 occupancy. *J Immunol*. 1993; 150(3):835-846. (Biology)

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