Technical Data Sheet

BUV737 Mouse Anti-Human CD28

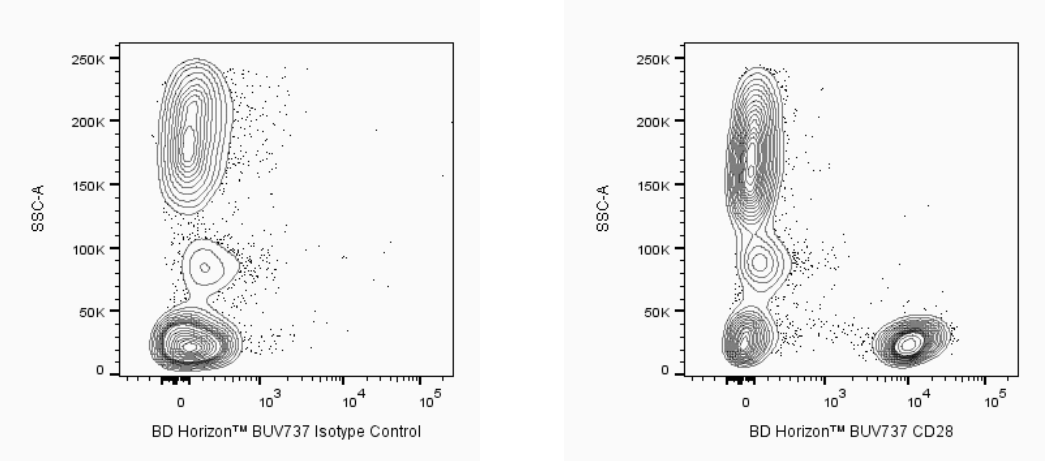
Product Information

Material Number:	**612815**
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 Tested in Development: Rhesus, Cynomolgus, Baboon
Workshop:	V 5T CD28.05
Storage Buffer:	Aqueous buffered solution containing ≤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 BUV737 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is a tandem fluorochrome with an Ex Max near 350 nm and an Em Max near 737 nm. BD Horizon Brilliant BUV737 can be excited by the ultraviolet laser (355 nm) and detected with a 740/35 nm filter. Due to the excitation of the acceptor dye by the red laser line, there may be significant spillover into red laser detectors with filters in the 700-720 nm range.



Multiparameter flow cytometric analysis of CD28 expression on human peripheral blood leucocyte populations. *Whole blood was stained with either BD Horizon™ BUV737 Mouse IgG1, κ Isotype Control (Cat. No. 612758; Left Plot) or BD Horizon BUV737 Mouse Anti-Human CD28 antibody (Cat. No. 612815; Right Plot). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). Two-parameter contour plots showing the correlated expression of CD28 (or Ig Isotype control staining) versus side-light scatter (SSC-A) signals were derived from gated events with the forward and side-light scatter characteristics of intact leucocyte populations. Flow cytometry and data analysis were performed using a BD LSRFortessa™ Cell Analyzer System and FlowJo™ software.*

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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 BUV737 under optimum conditions, and unconjugated antibody and free BD Horizon BUV737 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.

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. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

Suggested Companion Products

Catalog Number	Name	Size	Clone
612758	BUV737 Mouse IgG1, κ Isotype Control	50 µg	X40
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
555899	Lysing Buffer	100 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 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. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
7. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995:373-374. (Clone-specific: Activation, Calcium Flux, (Co)-stimulation)

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

Olive D, Cerdan C, Costello R, et al. CD28 and CTLA-4 cluster report. In: Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995:360-370. (Clone-specific: (Co)-stimulation, Flow cytometry, Functional assay, Inhibition, Stimulation)

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

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)