

Technical Data Sheet

Purified Hamster Anti-Mouse CD28

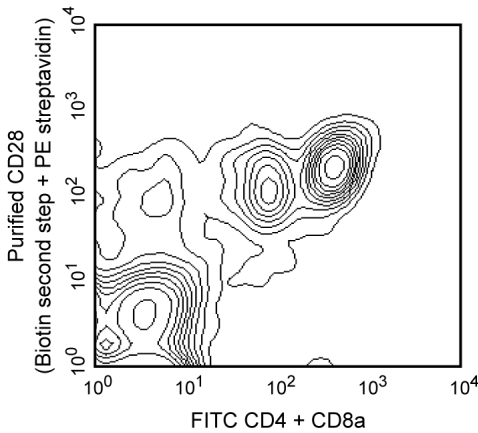
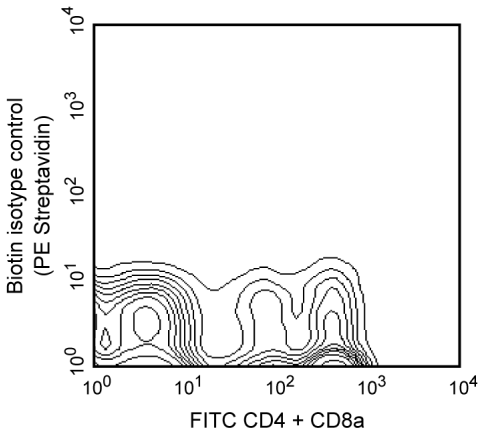
Product Information

Material Number:	557393
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	37.51
Immunogen:	Mouse EL-4 (T-cell lymphoma) Cells
Isotype:	Syrian Hamster IgG2, λ1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 37.51 antibody reacts with CD28, which is expressed on most thymocytes, at low density on nearly all CD4+ and CD8+ peripheral T cells, and at even lower density on NK cells. The expression of CD28, in splenocytes and thymocytes, has been reported to increase after activation. CD28 transcripts are found in mast cells, and cell-surface expression of CD28 is induced upon maturation or activation of mast cells. It has been reported that CD28 is not expressed on some populations of intraepithelial T lymphocytes. CD28 is a costimulatory receptor; its ligands include CD80 (B7-1) and CD86 (B7-2). The 37.51 mAb augments proliferation and cytokine production by activated T and NK cells and can provide a costimulatory signal for CTL induction. There is considerable evidence that CD28 is a costimulatory receptor involved in many, but not all, T cell-dependent immune responses.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Two-color analysis of CD28 expression on splenic T lymphocytes. BALB/c splenocytes were preincubated with purified anti-mouse CD16/CD32 mAb [Mouse BD Fc Block™] (Cat. No. 553141), then stained simultaneously with FITC-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553046) and FITC-conjugated anti-mouse CD8a mAb 53-6.7 (Cat. No. 553030) in addition to staining with purified anti-mouse CD28 clone 37.51 (right panel). Staining of CD28-positive cells was detected with biotinylated anti-Syrian hamster IgG2 mAb G192-3 (Cat. No. 554029), followed by streptavidin-PE (Cat. No. 554061). Flow cytometry was performed on a BD FACScan™ flow cytometry system.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4° C.

Application Notes

Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunoprecipitation	Reported
(Co)-stimulation	Reported
Blocking	Reported
Cytotoxicity	Reported

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2006 BD



Recommended Assay Procedure:

Precautions for flow cytometry: For flow cytometry of cell suspensions from peripheral lymphoid tissues, it is recommended that multicolor staining be performed to identify T lymphocytes and/or NK cells and that purified anti-mouse CD16/CD32 mAb 2.4G2 [Mouse BD Fc Block™] (Cat. No. 553141) be used. Since this antigen is expressed at low density on resting peripheral T lymphocytes, it may be desirable to amplify staining by using a biotinylated second-step antibody followed by a "bright" third step reagent, such as streptavidin-PE (Cat. No. 554061). If Mouse BD Fc Block™ is used, it is important that the second-step anti-hamster IgG antibody does not cross react with the Mouse BD Fc Block™. Biotinylated anti-Syrian hamster IgG2 mAb G192-3 (Cat. No. 554029) would be a suggested second-step antibody to consider.

Caution: Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also effect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format for in vitro and in vivo use.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	Streptavidin PE	0.5 mg	(none)
553962	Purified Hamster IgG2, λ 1 Isotype Control	0.5 mg	Ha4/8
553030	FITC Rat Anti-Mouse CD8a	0.1 mg	53-6.7
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553046	FITC Rat Anti-Mouse CD4	0.1 mg	RM4-5
554029	Biotin Mouse Anti-Syrian Hamster IgG2	0.5 mg	G192-3

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
3. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/pharming/hamster_chart_11x17.pdf.
4. 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.

References

Bluestone JA. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity*. 1995; 2(6):555-559.(Biology)

Cibotti R, Punt JA, Dash KS, Sharrow SO, Singer A. Surface molecules that drive T cell development in vitro in the absence of thymic epithelium and in the absence of lineage-specific signals. *Immunity*. 1997; 6(3):245-255.(Biology)

Gelfanov V, Lai YG, Gelfanova V, Dong JY, Su JP, Liao NS. Differential requirement of CD28 costimulation for activation of murine CD8+ intestinal intraepithelial lymphocyte subsets and lymph node cells. *J Immunol*. 1995; 155(1):76-82.(Biology)

Gross JA, Callas E, Allison JP. Identification and distribution of the costimulatory receptor CD28 in the mouse. *J Immunol*. 1992; 149(2):380-388.(Immunogen: (Co)-stimulation, Immunoprecipitation)

Harding FA, Allison JP. CD28-B7 interactions allow the induction of CD8+ cytotoxic T lymphocytes in the absence of exogenous help. *J Exp Med*. 1993; 177(6):1791-1796.(Biology: (Co)-stimulation)

Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature*. 1992; 356(6370):607-609.(Biology: (Co)-stimulation)

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

Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*. 1995; 182(2):459-465.(Biology: (Co)-stimulation)

Marietta EV, Weis JJ, Weis JH. CD28 expression by mouse mast cells is modulated by lipopolysaccharide and outer surface protein A lipoprotein from *Borrelia burgdorferi*. *J Immunol*. 1997; 159(6):2840-2848.(Biology)

Masten BJ, Yates JL, Pollard Koga AM, Lipscomb MF. Characterization of accessory molecules in murine lung dendritic cell function: roles for CD80, CD86, CD54, and CD40L. *Am J Respir Cell Mol Biol*. 1997; 16(3):335-342.(Biology: Blocking)

Nandi D, Gross JA, Allison JP. CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. *J Immunol*. 1994; 152(7):3361-3369.(Biology: (Co)-stimulation)

Nishio M, Spielman J, Lee RK, Nelson DL, Podack ER. CD80 (B7.1) and CD54 (intracellular adhesion molecule-1) induce target cell susceptibility to promiscuous cytotoxic T cell lysis. *J Immunol*. 1996; 157(10):4347-4353.(Biology: (Co)-stimulation, Cytotoxicity)

Rakasz E, Hagen M, Sandor M, Lynch RG. Gamma delta T cells of the murine vagina: T cell response in vivo in the absence of the expression of CD2 and CD28 molecules. *Int Immunol*. 1997; 9(1):161-167.(Biology)

Shahinian A, Pfeffer K, Lee KP, et al. Differential T cell costimulatory requirements in CD28-deficient mice. *Science*. 1993; 261(5121):609-612.(Biology)