Technical Data Sheet

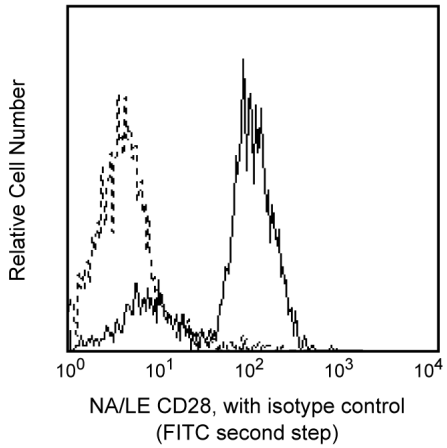
Purified NA/LE Mouse Anti-Human CD28

Product Information

Material Number:	**555725**
Alternate Name:	CD28 antigen; T44; Tp44; TP44
Size:	0.5 mg
Concentration:	1.0 mg/ml
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:	No azide/low endotoxin: Aqueous buffered solution containing no preservative, 0.2μm sterile filtered. Endotoxin level is ≤0.01 EU/μg (≤0.001 ng/μg) of protein as determined by the LAL assay.

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.



Profile of peripheral blood lymphocytes analyzed by flow cytometry. *Second step staining using FITC Goat Anti-Mouse IgG/IgM (Cat. No. 555988).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

Application Notes

Application

Flow cytometry	Routinely Tested
Bioassay	Tested During Development

Recommended Assay Procedure:

Suggested protocol for functional studies using CD28.2 mAb:

1. Isolate human PBMC with Ficoll-Paque™ Plus
2. Suspend PBMC in medium supplemented with 1% glutamine, 1% penicillin/streptomycin and 10% FBS at 10^6 cells/ml
3. Incubate 1 x 10^6 PBMC with soluble NA/LE™ format of CD3 mAb (Cat. No. 555336) at 1μg/ml final concentration in 7% CO2, 37°C for 3

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days
4. Wash cells twice and resuspend in the above medium at 10^6 cells/ml
5. Distribute cells in 96-well round bottom plate at 10^5 cells/well
6. Add soluble NA/LE™ format of CD28.2 mAb at 5 µg/ml final concentration
7. Incubate the plate for an additional three days
8. Pulse the plate with [3H]TdR (1 µCi/well). Harvest the plate 20 hours after pulse and count

Comment: Addition of Protein G (Sigma) 5 µg/ml together with CD28.2 will significantly enhance the costimulation, presumably through cross-linking.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
555336	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	HIT3a

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
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References

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