

Technical Data Sheet

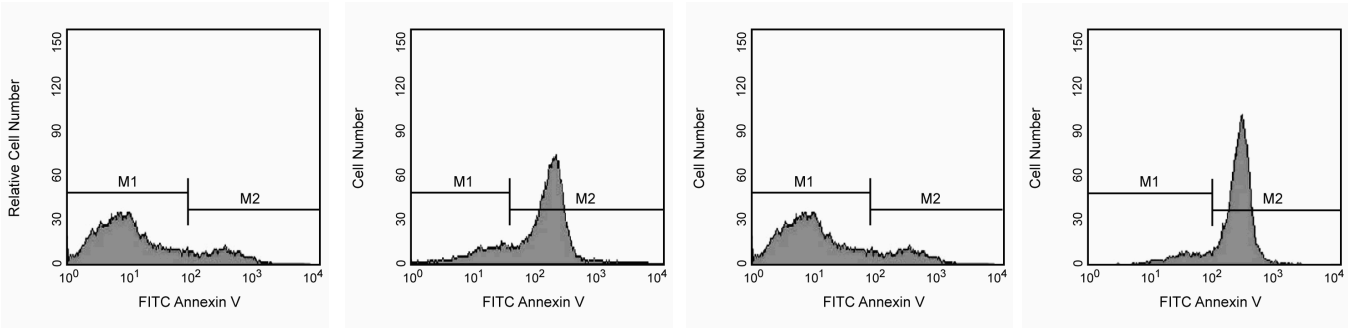
Purified NA/LE Mouse Anti-Human CD253

Product Information

Material Number:	550912
Alternate Name:	TNFSF10; TRAIL; APO-2L; TL2
Size:	0.25 mg
Concentration:	1.0 mg/ml
Clone:	RIK-2
Immunogen:	Human TRAIL Transfected Cell Line
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
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 RIK-2 monoclonal antibody specifically binds to CD253 which is also known as TRAIL (TNF-Related Apoptosis-Inducing Ligand) and Apo2 ligand (APO-2L). CD253 is a member of the Tumor Necrosis Factor Superfamily and is encoded by the *TNFSF10* gene. CD253 is a type II membrane protein that may be expressed as a full-length, cell surface associated protein as well as in a soluble form. Both surface and soluble forms of TRAIL rapidly induce apoptosis by a wide range of cell lines but not by normal cells. TRAIL is expressed by activated T cells, NK cells, dendritic cells, monocytes and by a variety of non-lymphoid cells. TRAIL can bind to and exert apoptosis through DR4 (TRAIL-R1) and DR5 (TRAIL-R2) receptors. It can also bind to decoy receptors, including DcR1/TRID/TRAIL-R3 and DcR2/TRUNDD/TRAIL-R4, and possibly OPG/TNFRSF11B, which may serve to regulate TRAIL activity. TRAIL has been shown to be involved in T cell-mediated cytotoxicity, however its mechanism of action remains to be fully elucidated. The RIK-2 clone was selected based on its ability to block TRAIL-mediated cytotoxic activity.



Flow Cytometric Analysis of TRAIL-induced Killing and RIK-2 Blocking using FITC Annexin V staining. Jurkat T cells were left untreated (far left/first panel) or treated for 16 hours under the following conditions: Cells were incubated with 20 ng/ml of recombinant human TRAIL and 10 µg/ml anti-histidine antibody (second panel); or with 20 ng/ml of recombinant human TRAIL and 10 µg/ml anti-histidine antibody preincubated with RIK-2 antibody (third panel); or with 20 ng/ml of recombinant human TRAIL and 10 µg/ml anti-histidine antibody preincubated with mlgG1 antibody (negative control) (far right/fourth panel). Following treatments, cells were evaluated for Annexin-V staining. Cells induced to undergo apoptosis by treatment with recombinant human TRAIL gave a population of cells that was Annexin V-FITC positive (second panel, M2). Annexin V-FITC staining was blocked when cells were incubated with a mixture of recombinant human TRAIL and RIK-2 antibody (third panel, M2). Cells treated with the mixture of recombinant TRAIL and mlgG1 antibody could not block the killing (far right/fourth panel). A small number of Annexin-V positive cells in the untreated population represents a basal level of apoptosis (far left/first panel). The results indicate that clone RIK-2 can block cell mediated killing induced by recombinant human TRAIL as measured by Annexin V-FITC staining of Jurkat cells.

Preparation and Storage

Store undiluted at 4°C.
This preparation contains no preservatives, thus it should be handled under aseptic conditions.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Flow cytometry	Routinely Tested
Bioassay	Tested During Development
Blocking	Tested During Development

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Suggested Companion Products

Catalog Number	Name	Size	Clone
553447	Purified NA/LE Mouse IgG1 κ Isotype Control	0.5 mg	107.3
550516	PE Mouse Anti-Human CD253	0.1 mg	RIK-2

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

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Marsters SA, Pitti RM, Donahue CJ, Ruppert S, Bauer KD, Ashkenazi A. Activation of apoptosis by Apo-2 ligand is independent of FADD but blocked by CrmA. *Curr Biol.* 1996; 6(6):750-752. (Biology)

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Sheridan JP, Marsters SA, Pitti RM, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science.* 1997; 277(5327):818-821. (Biology)

Wiley SR, Schooley K, Smolak PJ, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity.* 1995; 3(6):673-682. (Biology)

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