

Technical Data Sheet

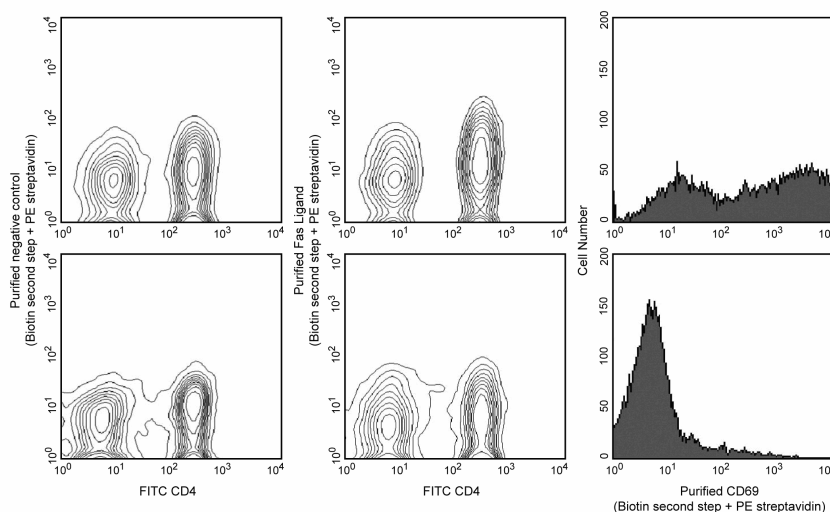
Purified Hamster Anti-Mouse CD178

Product Information

Material Number:	555291
Alternate Name:	Fas Ligand, CD95 Ligand
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	MFL3
Immunogen:	Mouse FasL-transfected cells
Isotype:	Armenian Hamster IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The MFL3 antibody reacts with CD178 (Fas Ligand, CD95 Ligand) on all strains tested. In the mouse, Fas Ligand is expressed on activated T cell lines and in spleen, testis, and eye. FasL mRNA has been demonstrated at various levels in bone marrow, thymus, spleen, lymph node, lung, small intestine, testis, and uterus. Moreover, T-cell activators, but not B-cell activators, enhanced the expression of FasL mRNA in splenocytes; and FasL mRNA was restricted to the T-cell lineage among a panel of cell lines from lymphoid tissues. Fas Ligand is not functional in mice homozygous for the gld (generalized lympho-proliferative disease) mutation; these mice cannot limit the expansion of activated lymphocytes and develop autoimmune disease. Fas Ligand is a member of the TNF/NGF family, which binds to CD95 (Fas), inducing apoptotic cell death. This Fas/Fas Ligand interaction is believed to participate in T-cell development, the regulation of immune responses, and cell-mediated cytotoxic mechanisms. There is mounting evidence that Fas Ligand is also proinflammatory, mediating neutrophil extravasation and chemotaxis. Fas Ligand is released from the surface of transfectant cells by metalloproteinases, and the soluble Fas Ligand may block the activities of the membrane-bound molecule. The MFL3 mAb has been reported to efficiently inhibit the cytotoxicity of mouse Fas Ligand-transfected cells against human Fas-transfected cells. This hamster mAb to a mouse leukocyte antigen does not cross-react with rat leukocytes.



Expression of Fas Ligand on T lymphocytes. T lymphocytes from BALB/c spleen (mouse T Cell Enrichment Column, R&D Systems, Minneapolis, MN), cultured for 7 hours on plate-bound anti-mouse CD3 mAb 17A2 (Cat. No. 555273, top panels) or on uncoated plates (bottom panels), were simultaneously stained with FITC-conjugated anti-mouse CD4 mAb RM4-5 (Cat. No. 553046/553047, top and bottom, left and center panels) and purified mAb MFL3 (center panels), or stained with purified anti-mouse CD69 mAb H1.2F3 (Cat. No. 553234, right panels). The purified antibodies were detected with biotinylated goat anti-Armenian hamster IgG polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) followed by Streptavidin-PE (Cat. No. 554061, all panels). Staining with anti-mouse CD69 mAb demonstrates the activation state of the T cells (right panels). 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.

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Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

We have found that enriched splenic T cells are induced to express Fas Ligand by 6-8-hour culture with plate-bound anti-mouse CD3 mAb 17A2 (Cat. No. 555273), mAb 145-2C11 (Cat. No. 557306/553058), or mAb 500A2 (Cat. No. 553238). Because Fas Ligand is expressed at low density on activated cells, it may be desirable to amplify staining by using a biotinylated second-step antibody with a "bright" third-step reagent, such as Streptavidin-PE (Cat. No. 554061). The BD Biosciences anti-hamster IgG mAb cocktails, either biotin- or PE-conjugated (Cat. No. 554010 or 554056, respectively) are not effective for staining with mAb MFL3 (see Figure).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	PE Streptavidin	0.5 mg	(none)
555273	Purified Rat Anti-Mouse CD3 Molecular Complex	0.5 mg	17A2
557306	Purified Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
553238	Purified Hamster Anti-Mouse CD3e	0.5 mg	500A2
553969	Purified Hamster IgG1, κ Isotype Control	0.5 mg	A19-3

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.
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. 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 affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
5. 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/documents/hamster_chart_11x17.pdf.

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