Technical Data Sheet

Purified Mouse Anti-Human CD178

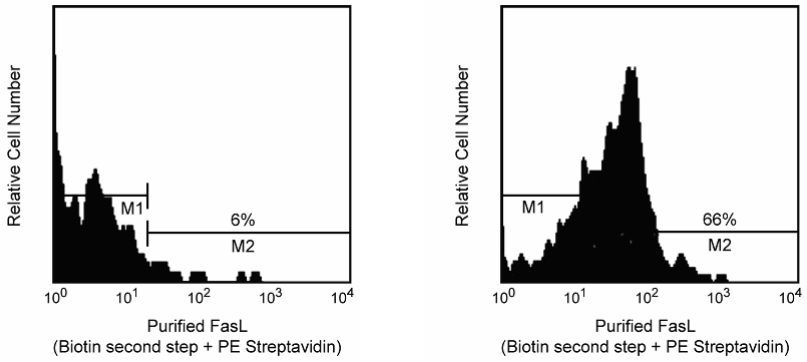
Product Information

Material Number:	**556372**
Alternate Name:	Fas Ligand; CD95 Ligand
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	NOK-1
Immunogen:	Mouse T lymphoma cells (L5178Y) expressing human FasL
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

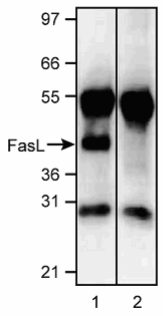
Description

Fas (CD95; APO-1) is a 45 kDa cell surface protein that mediates apoptosis when cross-linked with agonistic anti-Fas antibodies or by Fas ligand (FasL; CD178). Fas belongs to the TNF (Tumor Necrosis Factor)/NGF (Nerve Growth Factor) receptor family, and is expressed in various tissues and cells including the thymus, liver, ovary and lung. CD178 (FasL), a member of the TNF cytokine family, induces apoptosis by binding to Fas, its cell-surface receptor. FasL may exist as either membrane bound or soluble forms and is expressed by activated T and NK cells. FasL may also be constitutively expressed in some immunologically privileged sites, e.g., eye and testis. Fas and FasL play an important role in the induction of apoptosis, and thus regulate a variety of immunological responses.

The NOK-1 antibody clone has been reported to recognize human FasL, recognizing both the membrane bound (FasL) and soluble (sFasL) forms. It is reported that the epitope for NOK-1 has been mapped to the COOH-terminus of FasL, at the region implicated in Fas binding. FasL and sFasL have been reported to migrate at reduced molecular weights of 40 and 26 kDa, respectively. However, the molecular weights observed in a particular sample may vary according to FasL and sFasL glycosylation and breakdown patterns as described in the literature. The NOK-1 antibody clone is not recommended for the western blot application.



Flow cytometric analysis of human Fas Ligand (FasL) on natural killer cells (CD16+) analyzed on a FACScan™ (BDIS, San Jose, CA). *Peripheral blood mononuclear cells were cultured for 3 hours in media alone (left panel) or with ionomycin plus the metalloprotease inhibitor, KB8301 (right panel). KB8301 blocks FasL cleavage resulting in high levels of cell surface FasL. The cells were stained with purified anti-human FasL (clone NOK-1, Cat. No. 556372), followed by biotinylated goat anti-mouse Igs. The cells were then incubated with normal mouse serum before adding FITC-conjugated anti-human CD16 (Cat. No. 555406) and Streptavidin-PE (Cat. No. 554061).*



Immunoprecipitation/western blot analysis of human FasL. *Lane 1, FasL was precipitated from human peripheral blood mononuclear cells (PBMC's) with clone NOK-1 (Cat. No. 556372) and detected by western blot analysis with clone G247-4 (Cat. No. 556387). Lane 2, PBMC were immunoprecipitated with a mouse IgG1 isotype control, followed by western blot analysis with clone G247-4. The bands above and below the 40 kDa FasL band in lane 1 and lane 2 represent the heavy and light chain of IgG used for immunoprecipitation.*

Preparation and Storage

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

BD Biosciences

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Application Notes

Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Routinely Tested
Western blot	Not Recommended

Recommended Assay Procedure:

Applications include immunoprecipitation (1-2 µg/1x10^6 cells) and flow cytometry. NOK-1 has also been shown to neutralize the cytotoxic activity of FasL. Neutralization of FasL activity inhibits Fas-mediated killing. The BD NA/Le format of NOK-1 (Cat. No. 556371) should be used for all functional assays. NOK-1 and a related human FasL clone, NOK-2 [Cat. No. 556376 (purified) and No. 556375 (NA/LE)] may give different profiles in neutralization assays. It is thought that NOK-1 and NOK-2 likely recognize different FasL epitopes. Neither NOK-1 nor NOK-2 are suggested for western blot analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
556387	Purified Mouse Anti-Human CD178	0.1 mg	G247-4

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. 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.
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

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