Technical Data Sheet

Purified NA/LE Mouse Anti-Human CD178

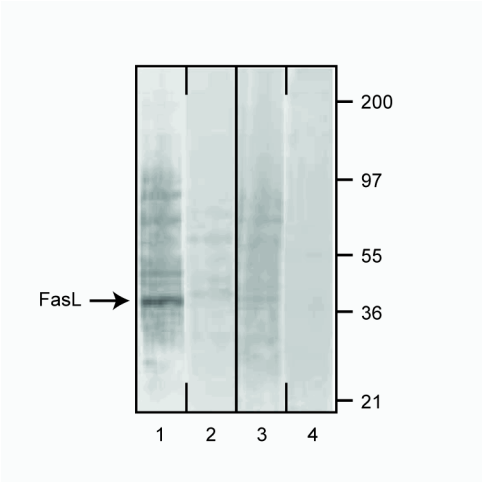
Product Information

Material Number:	**556375**
Alternate Name:	Fas Ligand, CD95 Ligand
Size:	0.25 mg
Concentration:	1.0 mg/ml
Clone:	NOK-2
Immunogen:	L51788Y Mouse T Lymphoma Cells Expressing Recombinant Human FasL
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human
Target MW:	40, 26 kDa
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

Fas (APO-1, CD95) is a 45 kDa cell surface protein that mediates apoptosis when cross-linked with agonistic anti-Fas antibodies or Fas ligand (FasL). Fas belongs to the TNF (tumor necrosis factor)/NGF (nerve growth factor) receptor family, and is expressed in various tissue and cells including the thymus, liver, ovary, and lung. FasL is a 40kDa TNF family membrane protein that induces apoptosis by binding to Fas, its cell-surface receptor. FasL is expressed on activated T and NK cells. Both Fas and FasL are thought to play an important role in the apoptotic processes that take place during T cell development.

NOK-2 recognizes human FasL. It recognizes both the membrane bound (FasL) and soluble (sFasL) forms. L5178Y mouse T lymphoma cells expressing recombinant human FasL were used as immunogen. FasL and sFasL 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 Tanaka et al.



Immunoprecipitation of Fas Ligand (FasL). *T lymphoma L5187Y cells were transfected with human FasL cDNA and either treated with metalloprotease inhibitor KB8301 or left untreated. KB8301 blocks FasL cleavage resulting in high levels of cell surface FasL. FasL is detected in [35S]-labeled cells treated with inhibitor KB8301 (lane 1), but not in untreated cells (lane 3). Isotype (negative) controls are shown in lanes 2 and 4. Purified NOK-2 (Cat. No. 556376) or Mouse IgG2a (Cat. No. 553454) was used for immunoprecipitations.*

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

Immunoprecipitation	Routinely Tested
Flow cytometry	Tested During Development
ELISA	Reported
Neutralization	Reported
Western blot	Not Recommended

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Recommended Assay Procedure:

IP: Applications include immunoprecipitation (1-2 µg/one million cells).

Functional Assay: NOK-2 has also been shown to neutralize the cytotoxic activity of FasL. Neutralization of FasL activity inhibits Fas-mediating killing. The NA/LE format of NOK-2 (Cat. No. 556375) should be used for all functional assays. NOK-2 and a related human FasL clone, NOK-1 [Cat. No. 556371 (NA/LE)] may give different profiles in neutralization assays. It is thought that NOK-1 and NOK-2 likely recognize different FasL epitopes.

Flow Cytometry: Another clone, NOK-1, (556372, 556373) are recommended for flow cytometry. NOK-2 appears weaker for flow cytometry than NOK-1.

Western Blot: Neither NOK-1 nor NOK-2 are suggested for western blot analysis. Another human FasL clone, G247-4 (Cat. No. 556387) is suggested for the western blot analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553454	Purified Mouse IgG2a κ Isotype Control	0.5 mg	G155-178
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. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Kayagaki N, Kawasaki A, Ebata T, et al. Metalloproteinase-mediated release of human Fas ligand. *J Exp Med.* 1995; 182(6):1777-1783. (Clone-specific: ELISA, Immunoprecipitation, Neutralization)

Takahashi T, Tanaka M, Brannan CI, Jenkins NA, Copeland NG, Suda T, and Nagata S. Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell.* 1994; 76(6):969-976. (Biology)

Tanaka M, Suda T, Takahashi T, Nagata S. Expression of the functional soluble form of human Fas ligand in activated lymphocytes. *EMBO J.* 1995; 14(6):1129-1135. (Biology)

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