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Product Information

Monoclonal Anti-Fas Ligand
Clone 101624, produced in rat
purified immunoglobulin

Catalog Number **F2928**

Product Description

Monoclonal Anti-Fas Ligand (CD95L) (rat IgG1 isotype) is produced from a murine hybridoma elicited from a rat immunized with purified recombinant mouse Fas ligand, amino acids 132–279.[1] The antibody is expressed in mouse NSO cells. It is purified from the IgG fraction of tissue culture supernatant using Protein G affinity chromatography.

Monoclonal Anti-Fas Ligand specifically recognizes recombinant mouse Fas ligand. The antibody may be used for neutralization and immunoblotting. By immunoblotting, the antibody shows ~10% cross-reactivity with human TRAIL and mouse TRANCE. 5% cross-reactivity may be seen with human Fas ligand, rat Fas ligand, mouse TNF-α, human April, and human VEGI. There is no cross-reactivity with human GITR ligand and human Light.

Fas Ligand (Fas ligand, FasL, CD95L), a 40 kDa type II membrane protein, belongs to the tumor necrosis factor (TNF) family,[2] which includes TNFα, α- and β-chains of lymphotoxin (LT), CD40 ligand, and CD30 ligand.[3,4] In the new TNF superfamily nomenclature, FasL is referred to as TNFSF6. The specific receptor for FasL is Fas (CD95, Apo-1), a 45 kDa type I membrane protein that is a member of the TNF receptor family.[5,6] FasL has four potential N-glycosylation sites which appear to be variably used.[3] Consequently, the apparent molecular mass of FasL may vary, per glycosylation and breakdown patterns in a certain preparation.[7] The amino acid sequences of human and murine FasL are 76.9% identical, and they are not species-specific.[8]

Membrane bound FasL (mFasL) is a 40 kDa protein, while the active soluble form of FasL (sFasL) was identified as a 26 kDa protein from the supernatant of activated peripheral T cells and cultured cells transfected with the full-length FasL DNA. Like other members of the TNF family membrane-bound FasL can be cleaved to generate the soluble Fas ligand, a non-covalently linked homotrimer. Membrane-bound FasL and TNFα are primary activators of their receptors. Soluble FasL may inhibit the killing effect of membrane FasL.

The Fas/FasL system plays an important role in modulating immune response by inducing cell apoptosis to maintain homeostasis, self-tolerance of lymphocytes, and immune privilege. Engagement of Fas by its ligand, results in the rapid induction of programmed cell death (PCD) in susceptible cells. This process bypasses the usual long sequence of signaling enzymes and immediately activates preexisting caspases.[9]

Fas Ligand is a potent chemoattractant for neutrophils, suggesting that it has a proinflammatory function. FasL is predominantly expressed on activated T cells and NK cells, whereas Fas is expressed on various cell types.[10]

The activation of mature T cells with phorbol myristic acetate (PMA) and ionomycin, concanavalin A (Con A) or anti-CD3, induces FasL gene expression. Herpes Simplex virus type 2 (HSV-2) but not HSV-1, potentially inhibits FasL surface expresssion in infected cells and thereby suppresses FasL-mediated cell death.[11]

Reagent

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
To one vial of lyophilized powder, add 1 mL of 0.2 μm filtered phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody.

Storage/Stability
Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2–8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing. Do not store in a "frost-free" freezer.

Procedure
Monoclonal Anti-Mouse Fas Ligand has the ability to neutralize the bioactivity of mouse Fas Ligand on Jurkat cells. Crosslinking antibody (mouse anti-6× histidine) and recombinant mouse Fas ligand are incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this pre-incubation, Jurkat cells are added. The assay mixture in a total volume of 100 μL, containing anti-Fas ligand at concentrations of 0.01–100 μg/mL, recombinant mouse Fas ligand at 5 μg/ml, crosslinking antibody (mouse anti-6× hisitidine) at 10 μg/mL, and cells at 5×10^4 cells/mL is incubated at 37 °C for 2 days in a humidified CO_2 incubator. The mixture is pulsed with ^{3}H-thymidine during the final 4 hours. The cells are detached and harvested onto glass fiber filters, and the ^{3}H-thymidine incorporated into the DNA is measured.

The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize recombinant mouse Fas Ligand bioactivity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity.

Product Profile
Monoclonal Anti-Mouse Fas Ligand has the ability to neutralize the biological activity of recombinant mouse Fas Ligand using Jurkat cells.

Immunoblotting: a working concentration of 1–2 μg/mL is recommended. The detection limit for recombinant mouse Fas ligand is ~5 ng/lane under non-reducing and reducing conditions.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

References

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TT,KAA,PHC,TMS,MAM 07/19-1