

Product Information

MONOCLONAL ANTI-FAS LIGAND (CD95L), Human CLONE Alf1.2

Purified Mouse Immunoglobulin

Product Number **F 1926**

Product Description

Monoclonal Anti-Human Fas Ligand (CD95L) (mouse IgG1 isotype) is derived from the Alf1.2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant soluble active extracellular domain of human Fas ligand.^{1,2} The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-Human Fas Ligand (CD95L) specifically recognizes the extracellular domain of human Fas ligand.² The antibody may be used for ELISA, immunoprecipitation (may precipitate an additional protein of 55 kDa) and flow cytometry.²

Homeostasis of multicellular organisms is controlled not only by the proliferation and differentiation of cells but also by cell death. The death of cells during embryogenesis, endocrine-dependent tissue atrophy, metamorphosis, a variety of pathologic conditions, and normal tissue turnover, is called programmed cell death (PCD). Most PCD proceeds by apoptosis, a process that includes condensation and segmentation of nuclei, condensation and fragmentation of the cytoplasm, and often extensive fragmentation of chromosomal DNA into nucleosome units.²

Many cells can be activated to undergo apoptosis following the interaction of selected ligands with cell surface receptors. The most well-studied receptors are CD95/Fas/Apo-1 (apoptosis inducing protein 1) and tumor necrosis factor receptor 1 (TNFR1). Apoptosis mediated by either of these receptors results in activation of a family of cysteine proteases known as caspases. However, Fas-mediated death occurs much more rapidly than that triggered by the TNFR1.

The action of Fas is mediated via FADD (Fas-associated death domain)/MORT1, an adapter protein that has a death domain at its C-terminus and binds to the cytoplasmic death domain of Fas. Human

CD95/Fas/Apo-1 antigen is a single transmembrane glycoprotein receptor (325 amino acids, 45-48 kDa).^{3,4} An integral membrane protein, has been identified as the Fas ligand (Fas ligand, FasL, CD95L).⁵

FasL is a type II transmembrane glycoprotein and is a member of the TNF family, which includes TNF α , α - and β -chains of lymphotoxin (LT), CD40 ligand and CD30 ligand.^{6,7} 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 in the supernatant of activated peripheral T cells and cultured cells transfected with the full-length FasL DNA. FasL has four potential N-glycosylation sites which appear to be variably used.⁶ Consequently, the apparent MW of FasL may vary, per glycosylation and breakdown patterns in a certain preparation.⁷

Engagement of Fas by its ligand, results in the rapid induction of PCD in susceptible cells. This process bypasses the usual long sequence of signaling enzymes and immediately activates preexisting caspases.⁹ FasL is expressed on activated T cells, whereas Fas is expressed on various types of cells.¹⁰ The cellular pathways that control apoptosis are critical to the maturation, selection, and survival of lymphocytes.

Apoptosis or cell suicide is the physiological mode of lymphoid cell death in circumstances like negative selection of T cells in the thymus, ligation of CD4 and CD3 in mature T cells, downregulation of the immune response, clonal deletion of B cells by antigen, death of killer cell targets, cytokine-mediated killing, and tumor regression. Fas is also expressed on a number of lymphoma cell lines, on Epstein-Barr virus-transformed B lymphoblasts, and on a proportion of activated B and T cells. The production of excess soluble Fas protein would prevent cells from undergoing Fas ligand induced apoptosis and thereby permit tumor cells to escape immunosurveillance. 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 expression in infected cells and thereby suppresses FasL-mediated cell death.¹¹ Antibodies reacting specifically with Fas ligand are useful tools in the study of the intracellular pathways leading from membrane receptor engagement to apoptotic cell death, the tissue distribution and developmental expression pattern of Fas ligand, and its essential role during mammalian development especially in immune system homeostasis.

Reagent

Monoclonal Anti-Human Fas Ligand (CD95L) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1 % bovine serum albumin and 15 mM sodium azide.

Antibody Concentration: Approx. 1 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A minimum working concentration of 1-2 µg/test is determined by flow cytometry, using cultured KFL9 cells (human chronic myelogenous leukemia cell line K562, stably-transfected with human Fas ligand).

When assayed by flow cytometric analysis, using the antibody at working concentration to stain 1×10^6 to 2×10^6 cells/0.1ml/test, fluorescence intensity is observed similar to that obtained with saturating antibody levels. The percentage population positive is also at the maximum percentage positive, using saturating antibody levels.

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

References

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