Product Information

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Fas/Fc Chimera
mouse, recombinant
expressed in *Sf* 21 cells

Catalog Number **F8799**
Storage Temperature –20 °C

Synonyms: Apo-1; CD95

Product Description

Recombinant mouse Fas (CD95, Apo-1)/Fc Chimera is a transmembrane glycoprotein receptor expressed in insect *Sf* 21 cells using a baculovirus expression system. A cDNA sequence encoding the extracellular domain (amino acids 1-169) of mouse Fas antigen[1] is fused to the carboxy-terminal 6× histidine tagged Fc region of human IgG1 by a linker peptide. The recombinant protein is a disulfide-linked homodimer with a blocked amino-terminus. The reduced monomer of mouse Fas/Fc has a calculated molecular mass of 46 kDa. However, due to glycosylation, recombinant mouse Fas/Fc migrates as a 55 kDa protein in SDS-PAGE under reducing conditions. Mouse Fas cDNA encodes a 327 amino acid type 1 membrane protein that belongs to the TNF and NGF receptor family.

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 embryo-genesis, metamorphosis, endocrine-dependent tissue atrophy, a variety of pathologic conditions, and normal tissue turnover, is called programmed cell death (PCD). Most of 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 cellular pathways that control apoptosis are critical to the maturation, selection, and survival of lymphocytes. Apoptosis is the physiological mode of lymphoid cell death in the negative selection of T cells in the thymus, ligation of CD4 and CD3 in mature T cells, down-regulation of the immune response, clonal deletion of B cells, death of killer cell targets, cytokine-mediated killing, and tumor regression.

The most studied receptors involved in apoptosis are CD95/Fas/Apo-1 (apoptosis inducing protein 1) and TNF receptor I (TNF RI). Apoptosis mediated by both signaling cascades 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 TNF RI. Engagement of Fas by its ligand (Fas ligand, FasL, CD95L), or by an appropriate antibody, results in the rapid induction of PCD in susceptible cell lines. This process bypasses the usual long sequence of signaling enzymes and immediately activates preexisting caspases.[3]

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. Primary sequence analysis of the extracellular portion of CD95/Fas/Apo-1 reveals strong homologies with the extracellular domain of receptors belonging to the TNF receptor family. This family includes TNF receptor types I and II, the low affinity nerve growth factor receptor, and lymphocyte receptors such as CD27, CD30, CD40, and OX40.[1,4] An integral membrane protein, with strong homology to TNF-α and TNF-β, has been identified as Fas ligand (CD95L/FasL).[1]

A moderate degree of homology (26% identity in a stretch of 65 amino acids) between the intracellular portion of the human CD95 (Fas) and the 55 kDa TNF RI, has been observed. Mutational analysis of this domain has revealed its involvement in the generation of the apoptotic signal from both Fas and TNF RI.[1] Thus, a common effector may transduce the apoptotic signal from both receptors.

Fas is highly expressed in epithelial cells, hepatocytes, activated mature lymphocytes,[5] virus-transformed lymphocytes, and tumor cells. It is found on a number of lymphoma cell lines. Upon contact with an anti-Fas antibody, some lymphocytes expressing Fas antigen undergo apoptosis.[6,7] Fas is also expressed in mouse thymus, liver, heart, lung, kidney, and ovary. A soluble form of Fas has been detected that plays a role in regulating certain aspects of immune system function. Elevated levels of soluble Fas have been detected in sera from patients with leukemic diseases, as well as in patients with systemic lupus erythematosus. Therefore, altered levels of secreted Fas protein are likely to be involved in the abnormal growth regulation of lymphoid cells. The production of excess soluble Fas protein prevents cells from undergoing Fas ligand induced apoptosis and, thereby, permits tumor cells to escape immunosurveillance. Antibodies reacting specifically with CD95 (Fas, Apo-1) 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, and its essential role in mammalian development especially in immune system homeostasis.

This product is lyophilized from a 0.2 μm filtered solution of phosphate buffered saline containing 50 μg bovine serum albumin per 1 μg of the cytokine.

Recombinant mouse Fas is measured by its ability to inhibit FAS-ligand induced apoptosis of Jurkat cells.[8] Resazurin dye was used in the assay as a fluorescence indicator for cell survival.

The ED_{50} for this effect is generally 0.03–0.1 μg/ml in the presence of 20 ng/ml of recombinant human Fas ligand.

The ED_{50} is defined as the effective concentration of growth factor that elicits a 50% increase in cell growth in a cell based bioassay.

Purity: >97% (SDS-PAGE, visualized by silver stain)

Endotoxin: <1.0 EU per 1 μg of the cytokine
[LAL (Limulus amebocyte lysate) method]

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reconstitute the contents of the vial using sterile phosphate buffered saline. Prepare a stock solution of ≥50 μg/mL.

Storage/Stability
Store the product at –20 °C. Upon reconstitution, the product may be stored at 2–8 °C for up to 3 months without detectable loss of activity. For extended storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended.

References

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