3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

# ProductInformation

**Fas LIGAND/TNFSF6**
Human, Recombinant
Expressed in CHO cells

Product Number **F 0427**

Synonyms: FasL, CD95 ligand, Apo-1 ligand, TNFSF6

## Product Description

Recombinant human Fas ligand (FasL) consists of amino acid residues 134-281 of human FasL[1] fused at the amino terminus to a 6X histidine tag which is then linked at the carboxy-terminus to a CD33 (Met1 – Met17) signal peptide. The N-terminal 6X his-tagged recombinant protein is expressed in a CHO cells. Recombinant human Fas ligand, after removal of the CD33 signal peptide, is a non-covalently linked trimer. The monomer has a calculated molecular weight of approximately 18 kDa. As a result of glycosylation, recombinant Fas Ligand migrates as an approximately 26 to 28 kDa protein on SDS-PAGE under reducing and non-reducing conditions.

Native Fas ligand is a 40 kDa, type II membrane protein belonging to the TNF super family. Its specific receptor, Fas (CD95/Apo-1), is a 45 kDa protein that transduces the apoptotic signal into cells. Fas Ligand is expressed primarily by T cells and NK cells and to a lesser degree in the testes, cornea, and several malignant tumor cell types. Human Fas ligand shares 85% sequence identity with mouse Fas ligand and 78% sequence identity with rat Fas ligand.

The Fas/FasL system modulates the immune response by inducing cell apoptosis to maintain homeostasis and in the regulation of immune responses and privilege.[2] Cysteine-rich repeats of the Fas receptor are required for binding by the Fas ligand.[3] This binding induces trimerization of Fas in the target cell membrane and activation. Activation of Fas causes the recruitment of Fas-associated protein with death domain (FADD) via interactions between the death domains of Fas and FADD.[4] Pro-caspase 8 binds to Fas-bound FADD via interactions between the death effector domains (DED) of FADD and pro-caspase 8 leading to the activation of caspase 8. Activated caspase 8 then cleaves (activates) nine other procaspases, a process that ultimately leads to apoptosis.

B cell antigen receptor signaling inhibits Fas-mediated apoptosis via up-regulation of cellular FLICE-inhibitory protein (c-FLIP).[5] The expression of the Fas ligand is regulated by protein phosphatases(s) sensitive to okadaic acid.[6] Serum withdrawal-induced apoptosis is mediated partially by the Fas/FasL interactions.[7] Studies suggest the Fas ligand is also a potent chemotactic factor in polymorphonuclear neutrophils which may be independent of the death-domain mediated apoptosis previously described.[8]

## Reagent

Recombinant human Fas ligand is supplied as approximately 10 μg of protein lyophilized from a sterile filtered phosphate-buffered saline (PBS) solution containing 50 μg bovine serum albumin per μg of cytokine.

## Preparation Instructions

Reconstitute the vial contents with sterile PBS containing a minimum of 1% human or bovine serum albumin. Stock solution concentration should be no less than 10 μg/ml.

## Storage/Stability

Lyophilized samples are stable for at least six months at –20 °C. Upon reconstitution, store at 2-4 °C for up to one month. For extended storage, store in working aliquots at –20 °C. Repeated freeze-thaw cycles should be avoided. Do not store in frost-free freezer.

## Product Profile

Recombinant Fas Ligand activity is measured by its ability to induce apoptosis in Jurkat cells. Recombinant human Fas ligand effective range is 0.2 to 0.5 μg/ml in the presence of a cross-linking anti-6X histidine antibody. Optimal dilutions should be determined by each laboratory for each application.

Purity: >95% by SDS-PAGE, visualized by silver stain.

Endotoxin level: < 0.1 ng/μg of protein as determined by the LAL (Limulus amebocyte lysate) method.

**References**


1. Mita, E., et al., Role of Fas ligand in apoptosis induced by hepatitis C virus infection. Biochem. Biophys. Res. Commun., **204,** 468-474 (1994).
2. Starling, G. c., et al., Identification of amino acid residues important for ligand binding to Fas. J. Exp. Med., **185**, 1487-1492 (1997).
3. Orlinick, J. R., et al., Requirement of cysteine-rich repeats of the Fas receptor for binding by the Fas ligand. J. Biol. Chem., **272**, 28889-28894 (1997).
4. Juo, P., et al., FADD is required for multiple signaling events downstream of the receptor Fas. J. Cell Growth Differ., **10**, 797-804 (1999).
5. Wang, J., et al., Inhibition of Fas-mediated apoptosis by the B cell antigen receptor through c-FLIP. Eur. J. Immunol., **30**, 155-163 (2000).
6. Morimoto, Y., et al., Upregulation of the expression of Fas antigen and Fas ligand in a human submandibular gland ductal cell line by okadaic acid. Arch. Oral Biol., **45**, 657-666 (2000).
7. Hu, C. L., Apoptosis of bovine granulosa cells after serum withdrawal is mediated by Fas antigen (CD95) and Fas ligand. Biol. Reprod., **64**, 518-526 (2001).
8. Seino, K., et al., Cutting Edge: Chemotactic Activity of Soluble Fas Ligand Against Phagocytes. J. Immunol., **161**, 4484-4488 (1998).


lcm 2/03