ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Fas Ligand (FASL) Antibodies and Positive Control

Cat. FASL11-S	Rabbit Anti-Rat FASL Antiserum # 1	**SIZE**: 100 ul
Cat. FASL11-A	Rabbit Anti-Rat FASL IgG # 1, Aff. pure	**SIZE**: 100 ug
Cat. FASL11-P	Rat FASL Control/blocking peptide	**SIZE**: 100 ug
Cat. FASL11-C	Recombinant Rat FASL protein for WB	**SIZE**: 100 ul

Apoptosis occurs not only during programmed cell death, but also during the death process induced by some cytotoxic T cells. A protein ligand, FASL, was identified that triggers cell death by binding to the cell surface receptor variously known as FAS or APT1 family of receptors that includes the 2 tumor necrosis factor (TNF) receptors. The FAS antigen is expressed not only in the cells of the immune system but also in the liver, lung, ovary, and heart, where its function is unclear. FAS ligand (soluble Fas Ligand (sFasL), TNFSF6, CD95L, Apo I Ligand, APTL; human 281-aa, ~ 32 kDa, chromosome 1q23) is a type II transmembrane protein that belongs to the tumor necrosis factor family. FASL is expressed in activated splenocytes and thymocytes, consistent with its involvement in T-cell-mediated. Like other members of the TNF family, the membrane-bound FasL can be cleaved by metalloproteinase to generate the soluble Fas ligand (sFasL), which is mainly a non-covalently linked homotrimer. It has been shown that the membrane-bound TNF-á and FasL are primary activators of their receptors. In contrast to soluble TNF-á, sFasL is much less cytotoxic. FasL may competitively inhibit the killing effect of FasL, indicating that the cleaving of FasL might be a mechanism to down-regulate FASL activities. Rat FasL shares 93.3% and 78% amino acid identity with that of mouse and human, respectively. FASL is alternative spliced into two forms: Defects in TNFSF6 are a cause of autoimmune lymphoproliferative syndrome (ALPS), also known as Canale-Smith syndrome (CSS), a childhood syndrome involving hemolytic anemia and thrombocytopenia.

Source of Antigen and Antibodies

Antigen	18-aa Synthetic peptide, 130-146aa of **rat FASL** (1); **Designation (FASL11-P, or control peptide**)
Location	~Mid-region, Extracellular domain
Ab Host/type	Rabbit, Polyclonal
Ab Format	Unpurified antiserum (**cat # FASL11-S**) and Aff pure IgG purified using antigen-agarose column (**cat # FASL11-A**) supplied in PBS+0.1% BSA+
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Rat FASL (Accession #P36940, 104-278 aa, extracellular domain) was expressed as fusion protein containing 1-16 aa of human CD33 and 6XHis tag at the N-terminus. The protein was purified to >95% (mol wt 35-37 kda).

For Western blot +ve protein control (**Cat # FASL11-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **FASL11-C** for good visibility with antibody Cat # **FASL11-**S. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer (reducing) per 10 ul of the **FASL11-C** solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. This preparation is intended for qualitative purpose and not to serve as standard of known concentration. Do not freeze and thaw.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.

Recommended Usage

Western Blotting (1:1K-5K) for neat serum using Chemiluminescence technique).

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested.

Specificity & Cross-reactivity

The rat FASL11-P is 94% conserved in mouse but no significant sequence homology exists with the human FASL. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at: www.4adi.com\data\abblock.html).

General References: Suda T et al (1993) Cell 75, 1169-1178; Hakuno N et al (1996) Endocrinology 137, 1938-48; Hahne M (1995) Intl. Immunol. 7, 1381-1386

*This product is for In vitro research use only.

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