APA031Mu61 10µg Active Factor Related Apoptosis Ligand (FASL) Organism Species: *Mus musculus (Mouse) Instruction manual* 

#### FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

#### [PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Pro132~Leu279 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 5% Trehalose . Original Concentration: 150µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.8

Predicted Molecular Mass: 12.7kDa

**Accurate Molecular Mass:** 30kDa as determined by SDS-PAGE reducing conditions. Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.

2. Relative charge: The composition of amino acids may affects the charge of the protein.

3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.

4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

# Cloud-Clone Corp.

## [ <u>USAGE</u> ]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

#### [ STORAGE AND STABILITY ]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

#### [<u>SEQUENCE</u>]

PSTPSEKKE PRSVAHLTGN PHSRSIPLEW EDTYGTALIS GVKYKKGGLV INETGLYFVY SKVYFRGQSC NNQPLNHKVY MRNSKYPEDL VLMEEKRLNY CTTGQIWAHS SYLGAVFNLT SADHLYVNIS QLSLINFEES KTFFGLYKL

## [ACTIVITY]

Fas ligand (FASL) is a 40 kDa type II membrane protein belonging to the TNF family. In the new TNF super family nomenclature, FASL is referred to as TNFSF6. The specific receptor for FASL is FAS (CD95, Apo-1), a 45 kDa type I transmembrane protein that is a member of the TNF receptor family. FASL is predominantly expressed on activated T cells and NK cells, while FAS is expressed on various types of cells. The FAS/FASL system plays a crucial role in modulating immune response by inducing cell apoptosis to maintain homeostasis, self-tolerance of lymphocytes, and immune privilege. FASL was reported to be a potent chemoattractant for neutrophils, suggesting a novel proinflammatory function of this molecule. A functional binding ELISA assay was conducted to detect the interaction of recombinant mouse FASL and recombinant human FAS. Briefly, FASL was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate

# Cloud-Clone Corp.

samples of 100  $\,\mu$  I were then transferred to FAS-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-FASL pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50  $\mu$ L stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant mouse FASL and recombinant human FAS was shown in Figure 1, and this effect was in a dose dependent manner.

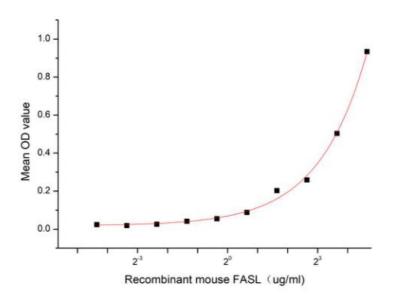


Figure 1. The binding activity of recombinant mouse FASL and recombinant human FAS

## Cloud-Clone Corp.

#### [IDENTIFICATION]

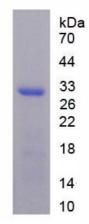


Figure 2. SDS-PAGE

Sample: Active recombinant FASL, Mouse

## [<u>IMPORTANT NOTE</u>]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.