

# Human <sub>His6</sub> Fas Ligand/TNFSF6 (h<sub>His6</sub> FasL)

SC 10 µg  
(With Carrier)

LC 50 µg  
(Carrier Free)

SF 10 µg  
(With Carrier)

LF 50 µg  
(Carrier Free)

Multi-milligram quantities available

rev. 02/23/11



Cell Signaling  
TECHNOLOGY®

Orders 877-616-CELL (2355)

orders@cellsignaling.com

Support 877-678-TECH (8324)

info@cellsignaling.com

Web www.cellsignaling.com

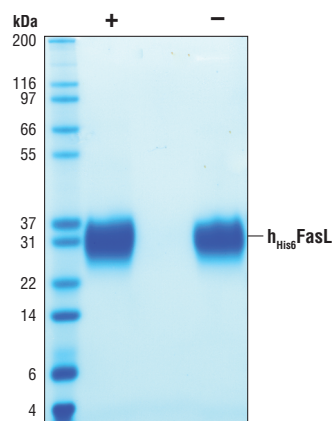
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

**Source:** Recombinant human<sub>His6</sub> FasL (h<sub>His6</sub> FasL) Pro134-Leu281 (Accession #NP\_000630) was expressed in human 293 cells at Cell Signaling Technology.

**Molecular Characterization:** Recombinant N-terminally His6-tagged hFasL has a calculated MW of 19,834. DTT-reduced and non-reduced protein migrate as 31-36 kDa polypeptides. Lower mobility in SDS-PAGE is due to glycosylation.

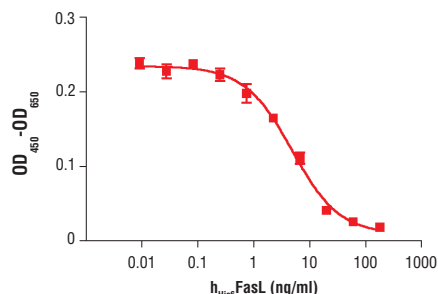
**Endotoxin:** Less than 0.01 ng endotoxin/1 µg h<sub>His6</sub> FasL.

**Purity:** >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant h<sub>His6</sub> FasL. All lots are greater than 98% pure.

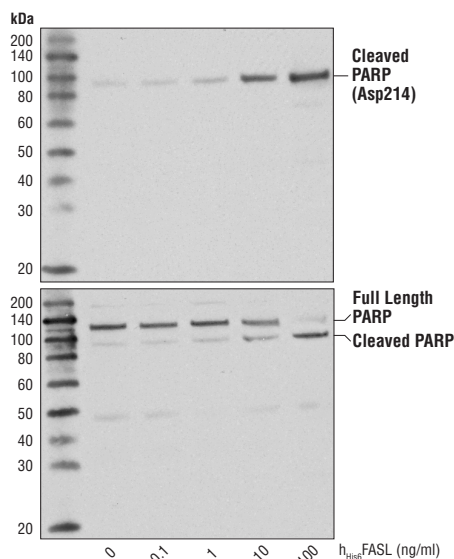


The purity of recombinant h<sub>His6</sub> FasL was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant h<sub>His6</sub> FasL and staining overnight with Coomassie Blue.

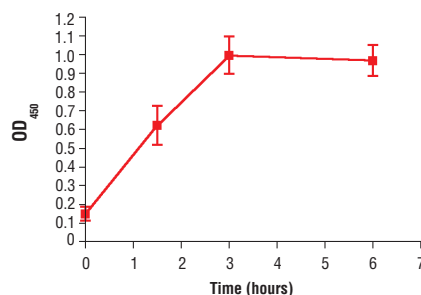
**Bioactivity:** The bioactivity of h<sub>His6</sub> FasL was determined in a Jurkat cell viability assay. The ED<sub>50</sub> of each lot is between 1-5 ng/ml.



The viability of Jurkat cells treated with increasing amounts of h<sub>His6</sub> FasL in the presence of 10 µg/ml anti-His antibody was assessed. After 24 hour treatment with h<sub>His6</sub> FasL, cells were incubated with a tetrazolium salt and the OD<sub>450</sub> - OD<sub>650</sub> was determined.



Western blot analysis of extracts from Jurkat cells, untreated or treated with h<sub>His6</sub> FasL for 3 hours, using Cleaved PARP (Asp214) Antibody (Human Specific) #9541 (upper) or PARP Antibody #9542 (lower).



Treatment of Jurkat cells with h<sub>His6</sub> FasL induces caspase-3 cleavage as detected by PathScan® Cleaved Caspase-3 (Asp175) Sandwich ELISA Kit #7190.

**Formulation:** Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg h<sub>His6</sub> FasL.

Carrier free: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2

## Reconstitution:

Add sterile PBS or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final h<sub>His6</sub> FasL concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS or PBS containing protein to minimize absorption of h<sub>His6</sub> FasL to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock h<sub>His6</sub> FasL should be greater than 50 µg/ml.

**Storage:** Stable in lyophilized state at 4°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

**Applications:** Optimal concentration for the desired application should be determined by the user.

**Background:** FasL is a member of the TNF-superfamily family of proteins and is expressed primarily on the cell surface of activated T and NK cells (1). FasL regulates the immune response through its ability to induce apoptosis. The immunoregulatory role of FasL is underscored by lymphadenopathy associated with FasL or Fas knockout mice and the fraction of autoimmune lympho-proliferative syndrome (ALPS) patients that have mutations in the FasL receptor, Fas (1). FasL is a membrane protein that can be cleaved into a soluble trimeric form by metalloproteinases (1,2). The soluble form of FasL retains the ability to bind to Fas, however, its ability to induce apoptosis is diminished (2). The ligation of Fas by FasL leads to the assembly of death-inducing signaling complex (DISC) and the recruitment and activation of caspase-8/caspase-10 (1,3). Active caspase-8/caspase-10 subsequently activates the "effector" caspases caspase-3 and caspase-7, and cleavage of BID (1,3).

## Background References:

- (1) Strasser, A. et al. (2009) *Immunity* 30, 180-92.
- (2) Schneider, P. et al. (1998) *J Exp Med* 187, 1205-13.
- (3) Guicciardi, M.E. and Gores, G.J. (2009) *FASEB J* 23, 1625-37.