Sterile

#5452

Human$_{His6}$ Fas Ligand/TNFSF6 (h_{His6}FasL)

Cell Signaling TECHNOLOGY®

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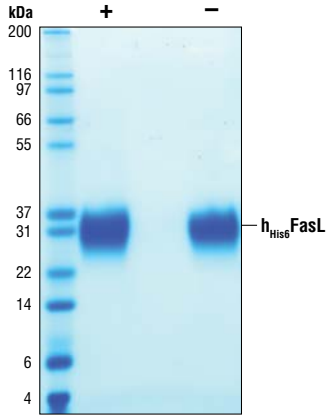
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human$_{His6}$ FasL (h_{His6}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.

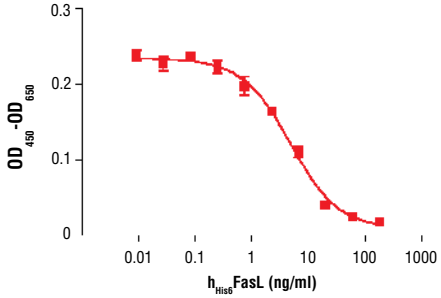
Endotoxin: Less than 0.01 ng endotoxin/1 μg h_{His6}FasL.

Purity: >98% as determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant h_{His6}FasL. All lots are greater than 98% pure.

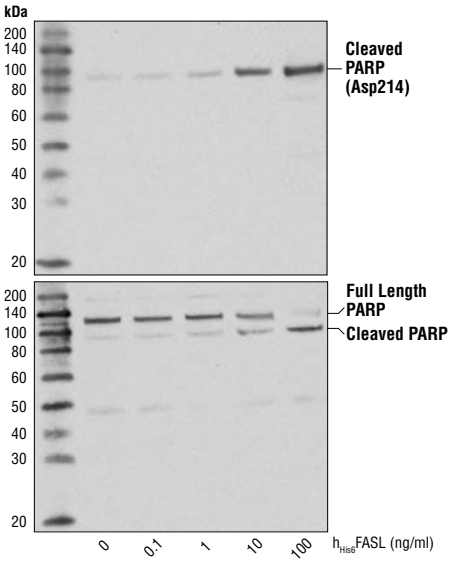


The purity of recombinant h_{His6}FasL was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant h_{His6}FasL and staining overnight with Coomassie Blue.

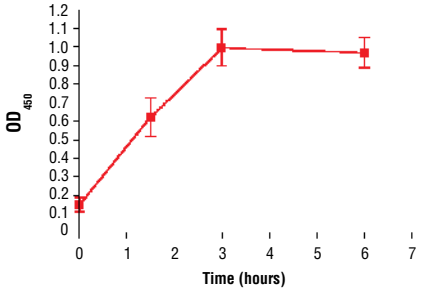
Bioactivity: The bioactivity of h_{His6}FasL was determined in a Jurkat cell viability assay. The ED_{50} of each lot is between 1-5 ng/ml.



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



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



Treatment of Jurkat cells with h_{His6}FasL induces casapase-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_{His6}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_{His6}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_{His6}FasL to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock h_{His6}FasL should be greater than 50 μg/ml.

Storage: Stable in lyophilized state at -20°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 lymphoadenopathy 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.