

## Product Data Sheet

## CD40L/CD154/TRAP Protein, Human (HEK293, hFc-Flag)

Cat. No.:	HY-P72028
Synonyms:	CD40-L; T-cell antigen Gp39; NF-related activation protein; TRAP; Tumor necrosis factor ligand superfamily member 5; CD154; sCD40L;
Species:	Human
Source:	HEK293
Accession:	P29965 (M113-L261)
Gene ID:	959
Molecular Weight:	44-47 kDa

PROPERTIES	
AA Sequence	MQKGDQNPQI AAHVISEASS KTTSVLQWAE KGYYTMSNNL VTLENGKQLT VKRQGLYYIY AQVTFCSNRE ASSQAPFIAS LCLKSPGRFE RILLRAANTH SSAKPCGQQS IHLGGVFELQ PGASVFVNVT DPSQVSHGTG FTSFGLLKL
Biological Activity	<ol> <li>Measured by its binding ability in a functional ELISA. Immobilized CD40L at 2 µg/mL can bind Anti- CD40L Rabbit Monoclonal Antibody, the EC<sub>50</sub> is ≤3.3 ng/mL.</li> <li>Measured by its binding ability in a functional ELISA. Immobilized CD40 at 2 µg/mL can bind CD40L, the EC<sub>50</sub> is 3.112-3.858 ng/mL.</li> <li>Human CD40 protein hFc tag captured on COOH chip can bind Human CD40L protein hFc and Flag tag with an affinity constant &lt; 2.06 nM as detected by LSPR Assay.</li> <li>Immobilized Human CD40 Ligand Trimer, His Tag at 1 µg/mL (100 µl/Well) on the plate. Dose response curve for Human CD40, hFc Tag with the EC<sub>50</sub> of 1.29 µg/mL determined by ELISA.</li> </ol>
Appearance	Lyophilized powder
Formulation	Lyophilized from a 0.2 μm solution of PBS, 6% Trehalose, pH 7.4.
Endotoxin Level	<1 EU/µg, determined by LAL method.
Reconsititution	It is not recommended to reconstitute to a concentration less than 100 $\mu\text{g}/\text{mL}$ in ddH_2O.
Storage & Stability	Stored at -20°C for 2 years. After reconstitution, it is stable at 4°C for 1 week or -20°C for longer (with carrier protein). It is recommended to freeze aliquots at -20°C or -80°C for extended storage.
Shipping	Room temperature in continental US; may vary elsewhere.

## DESCRIPTION

Background	CD40 Ligand (CD40L; CD154; TRAP) belongs to the tumor necrosis factor (TNF) family, is the ligand for CD40/TNFRSF5, specifically expressed on activated CD4+ T-lymphocytes <sup>[1]</sup> . CD40L is a type II transmembrane protein on B cells triggers important signals for B cell differentiation, maturation, and apoptosis <sup>[4]</sup> .
	CD40L acts function by cross-linking on T-cells to generate a costimulatory signal and thus enhances the production of IL4 and IL10 in conjunction with the TCR/CD3 ligation and CD28 costimulation, as well as promoting the production of interferon-γ, and TNF-α <sup>[1][4]</sup> .
	CD40L, binding with CD40 on antigen-presenting cells (APC), activates TNFR-associated factor 2- and IKK2-dependent pathways with stimulating I-κB kinase (IKK), increasing NF-κB DNA binding, and p65 nuclear translocation. The activation of I-κB kinase leads to strongly c-Jun N-terminal kinase activation as well as GST-I-κB and GST-p65 phosphorylation <sup>[2]</sup> . CD40L involves in MAPK pathways that strongly repress Bcl-6 with inducing the phosphorylation of Erk1/2, p38 and Jnk1/2 and activating IRF4 mediated by NF-κB <sup>[3]</sup> .
	<ul> <li>CD40L also binds to and signals through several integrins, including αvβ3 and α5β1, which bind to the trimeric interface of CD40L. CD40L plays a major role in immune response and is a major target for inflammation<sup>[5]</sup>.</li> <li>CD40L is widely found in different animals, while the sequence in Human is highly similar to Rhesus macaque (98.08%), but very different from Rat and Mouse with similarities of 77.31% and 77.69%, respectively. CD40L in Human is cleaved into 2 chains of membrane form (1-261 a.a.) and soluble form (113-261 a.a.), while the soluble form derives from the membrane form by proteolytic processing. Release of soluble CD40L from platelets is partially regulated by GP IIb/IIIa, actin polymerization, and a matrix metalloproteinases (MMP) inhibitor-sensitive pathway<sup>[6]</sup>.</li> </ul>

## REFERENCES

[1]. Blotta MH, et al. Cross-linking of the CD40 ligand on human CD4+ T lymphocytes generates a costimulatory signal that up-regulates IL-4 synthesis. J Immunol. 1996 May 1;156(9):3133-40.

[2]. Schwabe RF, et al. CD40 activates NF-kappa B and c-Jun N-terminal kinase and enhances chemokine secretion on activated human hepatic stellate cells. J Immunol. 2001 Jun 1;166(11):6812-9.

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[4]. Mikolajczak SA, et al. The modulation of CD40 ligand signaling by transmembrane CD28 splice variant in human T cells. J Exp Med. 2004 Apr 5;199(7):1025-31.

[5]. Takada YK, et al. Soluble CD40L activates soluble and cell-surface integrin αvβ3, α5β1, and α4β1 by binding to the allosteric ligand-binding site (site 2). J Biol Chem. 2021 Jan-Jun;296:100399.

[6]. Pietravalle F, et al. Human native soluble CD40L is a biologically active trimer, processed inside microsomes. J Biol Chem. 1996 Mar 15;271(11):5965-7.

[7]. Kotowicz K, et al. Biological function of CD40 on human endothelial cells: costimulation with CD40 ligand and interleukin-4 selectively induces expression of vascular cell adhesion molecule-1 and P-selectin resulting in preferential adhesion of lymphocytes. Immunology. 2000 Aug;100(4):441-8.

[8]. Schönbeck U, et al. Soluble CD40L and cardiovascular risk in women. Circulation. 2001 Nov 6;104(19):2266-8.

[9]. Lee N, et al. Comparison of Flow-cytometric Antibody Secreting Cell Assay and Mabtech Immunoglobulin ELISpot Assay. Transplant Proc. 2017 Jun;49(5):963-966.

Caution: Product has not been fully validated for medical applications. For research use only.

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