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DESCRIPTION

| DESCRIPTION                     |                                                                                                                                                         |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| Source                          | Human embryonic kidney cell, HEK293-derived human CD40 Ligand/TNFSF5 protein<br>Gly116-Leu261, with an N-ter Avi-tag &6-His tag<br>Accession # P29965.1 |
| N-terminal Sequence<br>Analysis | Gly of Avi-tag                                                                                                                                          |
| Structure / Form                | Biotinylated via Avi-tag                                                                                                                                |
| Predicted Molecular<br>Mass     | 19 kDa                                                                                                                                                  |

| SPECIFICATIONS  |                                                                                                                                                                                                                                                                       |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SDS-PAGE        | 18 - 25 kDa, under reducing conditions                                                                                                                                                                                                                                |
| Activity        | Measured by its binding ability in a functional ELISA.<br>Biotinylated Recombinant Human CD40 Ligand/TNFSF5 Avi-tag His-tag (Catalog # AVI11596) binds Recombinant Human CD40/TNFRSF5 Fc<br>Chimera (Catalog # 1493-CDB) with an ED <sub>50</sub> of 2.50-30.0 ng/mL. |
| Endotoxin Level | <0.10 EU per 1 µg of the protein by the LAL method.                                                                                                                                                                                                                   |
| Purity          | >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.                                                                                                                                                          |
| Formulation     | Lyophilized from a 0.2 µm filtered solution in PBS and EDTA with Trehalose. See Certificate of Analysis for details.                                                                                                                                                  |

| PREPARATION AND STORAGE |                                                                                                                         |  |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------|--|
| Reconstitution          | Reconstitute at 250 μg/mL in sterile water.                                                                             |  |
| Shipping                | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. |  |
| Stability & Storage     | Use a manual defrost freezer and avoid repeated freeze-thaw cycles.                                                     |  |
|                         | <ul> <li>12 months from date of receipt, -20 to -70 °C as supplied.</li> </ul>                                          |  |
|                         |                                                                                                                         |  |

- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.



Biotinylated Recombinant Human CD40 Ligand/TNFSF5 Avi-tag His-tag Protein Binding Activity. Biotinylated Recombinant Human CD40 Ligand/TNFSF5 Avi-tag His-tag Protein (Catalog # AVI11596) binds Recombinant Human CD40/TNFRSF5 Fc Chimera (Catalog # 1493-CDB) with an ED<sub>50</sub> of 2.50-30.0 ng/mL.

### SDS-PAGE



Biotinylated Recombinant Human CD40 Ligand/TNFSF5 Avi-tag His-tag Protein SDS-PAGE. 2 µg/lane of Biotinylated Recombinant Human CD40 Ligand/TNFSF5 Avi-tag His-tag Protein (Catalog # AVI11596) was resolved with SDS-PAGE under reducing (R) condition and visualized by Coomassie® Blue staining, showing bands at 18-25 kDa.

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### BACKGROUND

CD40 Ligand, also known as TNFSF, CD154, TRAP, and gp39, is a 34-39 kDa type II transmembrane glycoprotein that belongs to the TNF superfamily (1-3). Mature human CD40 Ligand consists of a 22 amino acid (aa) cytoplasmic domain, a transmembrane segment, and an 215 aa extracellular region (4, 5). The extracellular domain of human CD40 Ligand shares 74% and 76% aa sequence identity with mouse and rat CD40 Ligand, respectively. Similar to other TNF superfamily members, CD40 Ligand forms a bioactive homotrimer, both as membrane bound and soluble forms (6-9). The 18 kDa soluble form (aa 113-261) arises from proteolytic processing. Mutation and alternative splicing generate additional forms of CD40 Ligand that are often truncated or non-trimerizable (8). CD40 Ligand is expressed on platelets, as well as on activated T cells and B cells, basophils, eosinophils, fibroblasts, mast cells, monocytes, natural killer cells, vascular endothelial cells, and smooth muscle cells. CD40 Ligand binds to CD40, which is expressed on the surface of B cells, dendritic cells, macrophages, monocytes, platelets, endothelial, and epithelial cells (10). The interaction of CD40 Ligand with CD40 initiates signaling in both CD40 and CD40 Ligand at presenting cells contributes to the immune deficiency associated with HIV infection and AIDS (14, 15). It is also implicated in the pathology of multiple cardiovascular diseases including atherosclerosis, atherothrombosis, and restenosis (16, 17). Our Avi-tag Biotinylated human CD40 Ligand features biotinylation at a single site contained within the Avi-tag, a unique 15 amino acid peptide. Protein orientation will be uniform when bound to streptavidin-coated surface due to the precise control of biotinylation and the rest of the protein is unchanged so there is no interference in the protein's bioactivity.

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