**DESCRIPTION**

**Species Reactivity**  
Human

**Specificity**  
Detects human CD40 Ligand in direct ELISAs.

**Source**  
Monoclonal Mouse IgG\(_2\B\) Clone # 938601

**Purification**  
Protein A or G purified from hybridoma culture supernatant

**Immunogen**  
E. coli-derived recombinant human CD40 Ligand  
Glu108-Leu261  
Accession # P29965

**Formulation**  
Lyophilized from a 0.2 \(\mu\)m filtered solution in PBS with Trehalose. See Certificate of Analysis for details.  
*Small pack size (-SP) is supplied either lyophilized or as a 0.2 \(\mu\)m filtered solution in PBS.

**APPLICATIONS**

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

**Recommended Concentration**  
Western Blot  
1 \(\mu\)g/mL  
See Below

**DATA**

**Western Blot**

Detection of Human CD40 Ligand/TNFSF5 by Western Blot.  
Western blot shows lysates of human CD4\(^+\) cells untreated (+) or treated (+) with immobilized Mouse Anti-Human CD23v Monoclonal Antibody (Catalog # MAB100) and Mouse Anti-Human CD28 Monoclonal Antibody (Catalog # MAB342). PVDF membrane was probed with 1 \(\mu\)g/mL of Mouse Anti-Human CD40 Ligand/TNFSF5 Monoclonal Antibody (Catalog # MAB6171) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). Specific bands were detected for CD40 Ligand/TNFSF5 at approximately 15-18 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**PREPARATION AND STORAGE**

**Reconstitution**  
Reconstitute at 0.5 mg/mL in sterile PBS.

**Shipping**  
The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.  
*Small pack size (-SP) is supplied with polar packs. Upon receipt, store it immediately at -20 to -70 °C.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  
- 12 months from date of receipt, -20 to -70 °C as supplied.  
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.  
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.
CD40 Ligand (CD40L), now renamed TNFSF5, but also known as CD154, TRAP and gp39, is a 34-39 kDa type II transmembrane glycoprotein that belongs to the TNF superfamily (1-3). Human CD40L is 261 amino acids (aa) in length and consists of a 22 aa cytoplasmic domain, a 24 aa transmembrane segment, and a 215 aa extracellular region that consists of multiple β-strands and one N-linked glycosylation site (4, 5). Although carbohydrates are present, they are not necessary for activity (6). As with other TNF superfamily members, CD40L will exist as a trimer, both as a membrane bound and soluble form (6-8). The soluble form is 18 kDa in size and about 150 aa in length, and arises from intracellular proteolytic processing. As a trimer, the soluble form is bioactive (7-9). Multiple mutations and alternate splice forms of CD40L exist, often associated with pathology and leading to truncated or nontrimerizable forms of CD40L (8). While CD40L is a ligand for CD40, the ligation of CD40L by CD40 initiates bidirectional signaling in both CD40 and CD40L expressing cells (10). The extracellular region of human CD40L is 99%, 88%, 86%, 82%, 75% and 75% aa identical to the extracellular regions of CD40L in rhesus monkey, bovine, porcine, canine, mouse and rat, respectively. CD40L binds to both CD40 and to integrin αIIbβ3 (CD41) (3, 11). In the cell membrane, it also associates with a unique splice variant of CD28 (CD28i) that may facilitate CD40L signal transduction (12). CD40L is expressed by monocytes, NK cells, mast cells, basophils, smooth muscle cells, endothelial cells, dendritic cells, activated and resting B cells, plus activated platelets and CD4+ T cells (13, 14). CD40L ligation of CD40 on dendritic cells (DC) initiates DC maturation and differentiation. CD40L signaling into naïve B cells promotes germinal center formation and isotope switching. With IL-21, CD40L generates IgA plus IgG3; with IL-4, CD40L generates IgG1 secretion (14, 15).

References: