**DESCRIPTION**

**Species Reactivity**  
Human

**Specificity**  
Detects human 4-1BB Ligand/TNFSF9 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant mouse 4-1BB Ligand, recombinant human (rh) APRIL, rhBAFF, rhEDA-A2, rhFas Ligand, rhGITR Ligand, rhLIGHT, rhOX40 Ligand, rhTNF-z, rhTRAIL, and rhTWEAK is observed.

**Source**  
Polyclonal Goat IgG

**Purification**  
Antigen Affinity-purified

**Immunogen**  
E. coli-derived recombinant human 4-1BB Ligand/TNFSF9  
Arg71-Glu254  
Accession # P41273

**Formulation**  
Lyophilized from a 0.2 µm 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 µm 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.

<table>
<thead>
<tr>
<th>APPLICATIONS</th>
<th>Recommended Concentration</th>
<th>Sample</th>
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</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>1 µg/mL</td>
<td>See Below</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>2.5 µg/10⁶ cells</td>
<td>Daudi human Burkitt's lymphoma cell line</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>1-15 µg/mL</td>
<td>See Below</td>
</tr>
<tr>
<td>CyTOF-ready</td>
<td></td>
<td>Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.</td>
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</tbody>
</table>

**DATA**

**Western Blot**  
Detection of Human 4-1BB Ligand/TNFSF9 by Western Blot. Western blot shows lysates of human placenta tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human 4-1BB Ligand/TNFSF9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2295) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for 4-1BB Ligand/TNFSF9 at approximately 26 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Immunohistochemistry**  
4-1BB Ligand/TNFSF9 in Human Placenta. 4-1BB Ligand/TNFSF9 was detected in immersion fixed paraffin-embedded sections of human placenta using Goat Anti-Human 4-1BB Ligand/TNFSF9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2295) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to syncytiotrophoblasts. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

**PREPARATION AND STORAGE**

**Reconstitution**  
Reconstitute at 0.2 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 shipped 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.
BACKGROUND

4-1BB ligand (4-1BBL; also CD137L) is a 32 kDa type II transmembrane protein that belongs to the TNF superfamily (TNFSF) molecules (1-4). The human 4-1BBL cDNA encodes a 254 amino acid (aa) protein that contains a 25 aa N-terminal cytoplasmic domain, a 23 aa transmembrane segment, and a 206 aa C-terminal extracellular region (5). The extracellular domain (ECD) of 4-1BBL has a jelly-roll, β-sandwich tertiary structure that is similar to other TNFSF members. There is only one cysteine in the human ECD, and no potential N-linked glycosylation sites. The potential exists, however, for O-linked glycosylation. The human 4-1BBL ECD shares 32% and 35% aa identity with mouse and rat ECD, respectively. In the cytoplasmic domain, human 4-1BBL is 55 aa shorter than the equivalent region in rodents. 4-1BBL is expressed by activated B cells, macrophages, dendritic cells, activated T cells, neurons and astrocytes (2, 3, 6). A 26 kDa soluble form of 4-1BBL is known to occur in humans. Although it is presumably generated by MMP activity, its amino acid size is currently unreported (4). The soluble form is bioactive. Human 4-1BBL signals through both CD137/4-1BB and itself. Its cytoplasmic tail participates in reverse signaling that induces apoptosis in T cells and cytokine secretion (IL-6; TNF-α) by monocytes (7, 8). 4-1BBL binding to CD137/4-1BB produces a number of effects. It seems to play a key role in the T cell recall response. It maintains T cell numbers at the end of a primary response, and induces CD4+ and CD8+ T cells to proliferate and secrete cytokines such as IL-2 and IFN-γ in CD4+ cells, and IFN-γ in CD8+ cells (9, 10).

References: