**DESCRIPTION**

**Species Reactivity**
Human

**Specificity**
Detects human CD27 in direct ELISAs and Western blots. In direct ELISAs, approximately 25% cross-reactivity with recombinant mouse CD27 is observed and less than 1% cross-reactivity with recombinant human (rh) CD30, rh4-1BB, rhCD40, rhDR3, rhDR6, rhFas, rhGITR, rhHVEM, rhRANK, rhNGFR, rhTNF R1, and rhTNF RII is observed.

**Source**
Polyclonal Goat IgG

**Purification**
Antigen Affinity-purified

**Immunogen**
S. frugiperda insect ovarian cell line Sf 21-derived recombinant human CD27 Thr21-Ile192

**Accession #**
P26842

**Endotoxin Level**
<0.10 EU per 1 µg of the antibody by the LAL method.

**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>Recommended Concentration</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>1 µg/mL See Below</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>0.25 µg/10^6 cells Human whole blood lymphocytes</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>3-15 µg/mL See Below</td>
</tr>
<tr>
<td>Simple Western</td>
<td>20 µg/mL See Below</td>
</tr>
</tbody>
</table>

**CyTOF-ready**
Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.

**Neutralization**
Measured by its ability to neutralize CD27/TNFRSF7-induced inhibition of proliferation in mouse splenic T cells. The Neutralization Dose (ND_{50}) is typically 2.25-9.0 µg/mL in the presence of 3 µg/mL Recombinant Human CD27/TNFRSF7 Fc Chimera, 10 µg/mL Recombinant Mouse CD27 Ligand/TNFSF7, and sub-optimal amounts of Mouse CD3ε Monoclonal Antibody.

**DATA**

**Western Blot**

Detection of Human CD27/TNFRSF7 by Western Blot. Western blot shows lysates of Raj human Burkitt's lymphoma cell line, Daudi human Burkitt's lymphoma cell line, Ramos human Burkitt's lymphoma cell line, and human tonsil tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human CD27/TNFRSF7 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF382) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for CD27/TNFRSF7 at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Immunohistochemistry**

CD27/TNFRSF7 in Human Tonsil. CD27/TNFRSF7 was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human CD27/TNFRSF7 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF382) at 3 µg/mL overnight at 4°C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes in germinal center. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.
Detection of Human CD27/TNFRSF7 by Simple Western™. Simple Western lane view shows lysates of human tonsil tissue, loaded at 0.2 mg/mL. A specific band was detected for CD27/TNFRSF7 at approximately 49 kDa (as indicated) using 20 µg/mL of Goat Anti-Human CD27/TNFRSF7 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF382) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Neutralization

CD27/TNFRSF7 Inhibition of Cell Proliferation and Neutralization by Human CD27/TNFRSF7 Antibody.

In the presence of sub-optimal amounts of Mouse CD3ε Monoclonal Antibody (Catalog # MAB484) and Recombinant Mouse CD27 Ligand (10 µg/mL, Catalog # 783-CL), Recombinant Human CD27 Fc Chimera (Catalog # 382-CD) inhibits proliferation in mouse splenic T cells in a dose-dependent manner (orange line). Under these conditions, inhibition of proliferation elicited by Recombinant Human CD27 Fc Chimera (3 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human CD27 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF382). The ND50 is typically 2.25-9.0 µg/mL.

References:


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

Human CD27 is a lymphocyte-specific member of the TNF receptor superfamily. CD27 is expressed on a subset of human thymocytes and on the majority of mature T cells. CD27 expression is up-regulated after TCR stimulation. Within the CD4+ compartment, it is preferentially expressed on CD45RA+ cells. In contrast, it is preferentially expressed on CD45RO+ cells in the CD8+ compartment. CD27 also appears to be a potential marker for memory B cells. It exists as both a disulfide-linked dimer on the cell surface and as a soluble protein found in serum. Human CD27 is a 260 amino acid (aa) protein with a 20 aa signal, a 173 aa extracellular domain, a 47 aa cytoplasmic domain. The ligand for CD27 is CD70. CD70 is expressed on thymic stromal cells and a small subset of activated T cells. Additionally a subset of activated B cells express CD70. The CD27/CD70 interaction appears to be a weak costimulatory pathway involved in T cell and B cell immune response. CD27/CD70 interactions may be more involved in controlling the expansion phase of an immune response. This would be in contrast to B7/CD28 interactions, which are important for the activation phase of immune responses.

References: