**DESCRIPTION**

**Species Reactivity:** Human

**Specificity:** Detects human TRAIL R1/TNFRSF10A in direct ELISAs and Western blots. In Western blots, approximately 10% cross-reactivity with recombinant human (rh) TRAIL R2 is observed and less than 1% cross-reactivity with rhTRAIL R3 and rhTRAIL R4 is observed.

**Source:** Polyclonal Goat IgG

**Purification:** Antigen Affinity-purified

**Immunogen:** *S. frugiperda* insect ovarian cell line Sf 21-derived recombinant human TRAIL R1/TNFRSF10A Ala24-Asn239

**Accession #** AAC51226

**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>Sample</th>
<th>Recommended Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>1 μg/mL</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>5-15 μg/mL</td>
</tr>
</tbody>
</table>

**Neutralization**

Measured by its ability to neutralize TRAIL R1/TNFRSF10A-mediated inhibition of cytotoxicity in the L-929 mouse fibroblast cell line. The Neutralization Dose (ND_{50}) is typically 0.02-0.055 μg/mL in the presence of 10 ng/mL Recombinant Human TRAIL R1/TNFRSF10A Fc Chimera, 12 ng/mL Recombinant Human TRAIL/TNFSF10, a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody, and 1 μg/mL actinomycin D.

**DATA**

**Western Blot**

Detection of Human TRAIL R1/TNFRSF10A by Western Blot. Western blot shows lysates of TF-1 human erythroleukemic cell line. PVDF membrane was probed with 1 μg/mL of Goat Anti-Human TRAIL R1/TNFRSF10A Antibody (Catalog # AF347) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for TRAIL R1/TNFRSF10A at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions.

**Neutralization**

TRAIL R1/TNFRSF10A Inhibition of TRAIL/TNFSF10-induced Cytotoxicity and Neutralization by Human TRAIL R1/TNFRSF10A Antibody. In the presence of a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody (Catalog # MAB050) and the metabolic inhibitor actinomycin D (1 μg/mL), Recombinant Human TRAIL R1/TNFRSF10A Fc Chimera (Catalog # 347-DR) inhibits Recombinant Human TRAIL/TNFSF10 (Catalog # 375-TR) induced cytotoxicity in the L-929 mouse fibroblast cell line in a dose-dependent manner (orange line), as measured by crystal violet staining. Under these conditions, inhibition of Recombinant Human TRAIL/TNFSF10 (12 ng/mL) activity elicited by Recombinant Human TRAIL R1/TNFRSF10A Fc Chimera (10 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human TRAIL R1/TNFRSF10A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF347). The ND_{50} is typically 0.02-0.055 μg/mL.
Immunohistochemistry

TRAIL R1/TNFRSF10A in Human Brain.
TRAIL R1/TNFRSF10A was detected in
immersion fixed paraffin-embedded sections
of human brain using Goat Anti-Human
TRAIL R1/TNFRSF10A Antigen Affinity-
purified Polyclonal Antibody (Catalog #
AF347) at 10 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
cytoplasm in neurons. View our protocol for
Chromogenic IHC Staining of Paraffin-
embedded Tissue Sections.

PREPARATION AND STORAGE

<table>
<thead>
<tr>
<th>Component</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reconstitution</strong></td>
<td>Reconstitute at 0.2 mg/mL in sterile PBS.</td>
</tr>
<tr>
<td><strong>Shipping</strong></td>
<td>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</td>
</tr>
<tr>
<td><strong>Stability &amp; Storage</strong></td>
<td>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</td>
</tr>
<tr>
<td></td>
<td>12 months from date of receipt, -20 to -70 °C as supplied.</td>
</tr>
<tr>
<td></td>
<td>1 month, 2 to 8 °C under sterile conditions after reconstitution.</td>
</tr>
<tr>
<td></td>
<td>6 months, -20 to -70 °C under sterile conditions after reconstitution.</td>
</tr>
</tbody>
</table>

BACKGROUND

Human TRAIL R1, also called DR4, is a type 1, TNF R family, membrane protein which is a receptor for TRAIL (APo2 ligand). In the TNF superfamily nomenclature, TRAIL R1 is referred to as TNFRSF10A. TRAIL R1 cDNA encodes a 468 amino acid residue precursor protein containing extracellular cysteine-rich domains, a transmembrane domain and a cytoplasmic death domain. Among the TNF receptor family proteins, TRAIL R1 is most closely related to TRAIL R2/DR5, sharing 55% amino acid sequence identity. Binding of trimeric TRAIL to TRAIL R1 induces apoptosis. The induction of apoptosis likely requires oligomerization of the receptor. The human TRAIL R1/Fc chimera neutralizes the ability of TRAIL to induce apoptosis. Besides TRAIL R1, an additional TRAIL R2/DR5, which transduces apoptosis signal, and two TRAIL decoy receptors, which antagonize TRAIL-induced apoptosis, have been reported.

References: