

## DESCRIPTION

<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human Fas Ligand in direct ELISAs and Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant mouse Fas Ligand is observed, approximately 7% cross-reactivity with recombinant rat Fas Ligand and recombinant human (rh) BAFF, and less than 1% cross-reactivity with rhTRAIL, rhTNF- $\alpha$ , rhGITR Ligand, and rhAPRIL is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	Chinese hamster ovary cell line CHO-derived recombinant human Fas Ligand/TNFSF6 Pro134-Leu281 Accession # Q53ZZ1
<b>Endotoxin Level</b>	<0.10 EU per 1 $\mu$ g of the antibody by the LAL method.
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

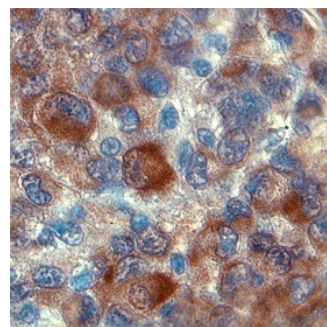
## APPLICATIONS

**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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.1 $\mu$ g/mL	Recombinant Human Fas Ligand/TNFSF6 (Catalog # 126-FL)
<b>Immunohistochemistry</b>	5-15 $\mu$ g/mL	See Below
<b>Neutralization</b>	Measured by its ability to neutralize Fas Ligand/TNFSF6-induced apoptosis in the Jurkat human acute T cell leukemia cell line. Cifone, M.G. <i>et al.</i> (1994) J. Exp. Med. <b>180</b> :1547. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.012-0.072 $\mu$ g/mL in the presence of 2 ng/mL Recombinant Human Fas Ligand/TNFSF6 and 10 $\mu$ g/mL of a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody.	

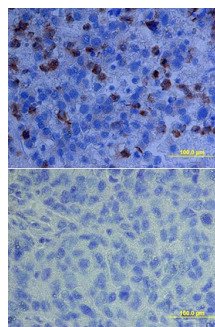
## DATA

### Immunohistochemistry



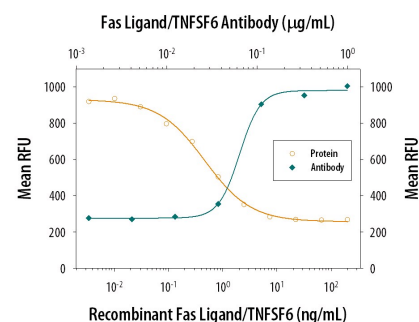
**Fas Ligand/TNFSF6 in Human Melanoma.** Fas Ligand/TNFSF6 was detected in immersion fixed paraffin-embedded sections of human melanoma tissue using Goat Anti-Human Fas Ligand/TNFSF6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF126) at 15  $\mu$ g/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for [Chromogenic IHC Staining of immersion fixed paraffin-embedded Tissue Sections](#).

### Immunohistochemistry



**Fas Ligand/TNFSF6 in Human Melanoma.** Fas Ligand/TNFSF6 was detected in immersion fixed paraffin-embedded sections of human melanoma using Goat Anti-Human Fas Ligand/TNFSF6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF126) at 15  $\mu$ g/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). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

### Neutralization



### Apoptosis Induced by Fas Ligand/TNFSF6 and Neutralization by Human Fas Ligand/TNFSF6 Antibody

In the presence of a cross-linking antibody, Mouse polyHistidine Monoclonal Antibody (10  $\mu$ g/mL, Catalog # MAB050), Recombinant Human Fas Ligand/TNFSF6 (Catalog # 126-FL) induces apoptosis in the Jurkat human acute T cell leukemia cell line in a dose-dependent manner (orange line). Apoptosis elicited by Recombinant Human Fas Ligand/TNFSF6 (2 ng/mL) is neutralized (green line) by increasing concentrations of Human Fas Ligand/TNFSF6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF126). The ND<sub>50</sub> is typically 0.12-0.072  $\mu$ g/mL.

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

Fas Ligand (FasL), also known as CD178, CD95L, or TNFSF6, is a 40 kDa type II transmembrane member of the TNF superfamily of proteins. Its ability to induce apoptosis in target cells plays an important role in the development, homeostasis, and function of the immune system (1). Mature human Fas Ligand consists of a 179 amino acid (aa) extracellular domain (ECD), a 22 aa transmembrane segment, and a 80 aa cytoplasmic domain (2). Within the ECD, human Fas Ligand shares 81% and 78% aa sequence identity with mouse and rat Fas Ligand, respectively. Both mouse and human Fas Ligand are active on mouse and human cells (2, 3). Fas Ligand is expressed on the cell surface as a nondisulfide-linked homotrimer on activated CD4<sup>+</sup> Th1 cells, CD8<sup>+</sup> cytotoxic T cells, and NK cells (1). Fas Ligand binding to Fas/CD95 on an adjacent cell triggers apoptosis in the Fas-expressing cell (2, 4). Fas Ligand also binds DcR3 which is a soluble decoy receptor that interferes with Fas Ligand-induced apoptosis (5). Fas Ligand can be released from the cell surface by metalloproteinases as a 26 kDa soluble molecule which remains trimeric (6, 7). Shed Fas Ligand retains the ability to bind Fas, although its ability to trigger apoptosis is dramatically reduced (6, 7). In the absence of TGF- $\beta$ , however, Fas Ligand/Fas interactions instead promote neutrophil-mediated inflammatory responses (3, 8). Fas Ligand itself transmits reverse signals that costimulate the proliferation of freshly antigen-stimulated T cells (9). Fas Ligand-induced apoptosis plays a central role in the development of immune tolerance and the maintenance of immune privileged sites (10). This function is exploited by tumor cells which evade immune surveillance by upregulating Fas Ligand to kill tumor infiltrating lymphocytes (8, 11). In gld mice, a Fas Ligand point mutation is the cause of severe lymphoproliferation and systemic autoimmunity (12, 13).

## References:

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3. Seino, K-I. *et al.* (1998) *J. Immunol.* **161**:4484.
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