# biotechne

## Human TL1A/TNFSF15 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2116A Catalog Number: MAB74422

### RDSYSTEMS

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human TL1A/TNFSF15 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2116A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human TL1A/TNFSF15 Leu72-Leu251 Accession # O95150
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.

#### 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	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 μg/10 <sup>6</sup> cells	See Below
Immunohistochemistry	0.3-25 μg/mL	See Below
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 TL1A/TNFSF15-induced apoptosis in the TF-1 human erythroleukemic cell line. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.04-0.2 μg/mL in the presence of 80 ng/mL Recombinant Human TL1A/TNFSF15.	

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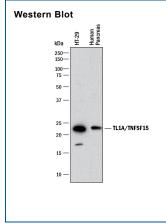
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**R**DSYSTEMS

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#### DATA



Detection of Human TL1A/TNFSF15 by Western Blot. Western blot shows lysates of HT-29 human colon adenocarcinoma cell line and human pancreas tissue. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human TL1A/TNFSF15 Monoclonal Antibody (Catalog # MAB74422) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for TL1A/TNFSF15 at approximately 22 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

TL1A/TNFSF15 in Human

TL1A/TNFSF15 was detected in

Prostate Cancer Tissue.

immersion fixed paraffin-

Rabbit Anti-Human

embedded sections of human

prostate cancer tissue using

TL1A/TNFSF15 Monoclonal

with the Anti-Rabbit IgG

VisUCyte™ HRP Polymer

Antibody (Catalog # VC003)

Basic (Catalog # CTS013). Tissue was stained using DAB

Reagents.

Antibody (Catalog # MAB74422)

at 0.3 µg/mL for 1 hour at room

temperature followed by incubation

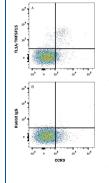
Before incubation with the primary

antibody, tissue was subjected to heat-induced epitope retrieval

using Antigen Retrieval Reagent-

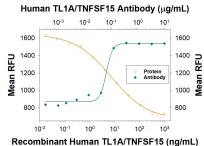
(brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in epithelial cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection

#### Flow Cytometry



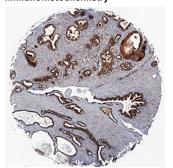
Detection of TL1A/TNFSF15 in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) were stained with (A) Rabbit Anti-Human TL1A/TNFSF15 Monoclonal Antibody (Catalog # MAB74422) or (B) Rabbit IgG control antibody (Catalog # MAB1050) followed by Goat anti-Rabbit IgG PEconjugated Secondary Antibody (Catalog # F0110) and Mouse anti-Human CCR9 APCconjugated Monoclonal Antibody (Catalog # FAB1791A). Cells were gated on CD4+ lymphocytes using Mouse anti-Human CD4 FITC-conjugated Monoclonal Antibody (Catalog # FAB3791F). View our protocol for Staining Membrane-associated Proteins

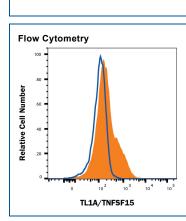
#### Neutralization



Apoptosis Induced by TL1A/TNFSF15 and Neutralization by Human TL1A/TNFSF15 Antibody. Recombinant Human TL1A/TNFSF15 (Catalog # 1319-TL) induces apoptosis in the TF-1 human erythroleukemic cell line in a dose-dependent manner (orange line), as measured by Resazurin (Catalog # AR002). Apoptosis elicited by Recombinant Human TL1A/TNFSF15 (80 ng/mL) is neutralized (green line) by increasing concentrations of Rabbit Anti-Human TL1A/TNFSF15 Monoclonal Antibody (Catalog # MAB74422). The ND<sub>50</sub> is typically 0.04-0.2 µg/mL.

#### Immunohistochemistry





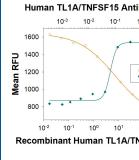
Detection of TL1A/TNFSF15 in HT-29 cells by Flow Cytometry. HT-29 cells were stained with Rabbit Anti-Human TL1A/TNFSF15 Monoclonal Antibody (Catalog # MAB74422, filled histogram) or isotype control antibody (Catalog # AB-105-C, open histogram), followed by Allophycocyanin-conjugated Anti-

Rabbit IgG Secondary Antibody (Catalog # F0111). View our protocol for Staining Membraneassociated Proteins

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### **R**Dsystems

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 shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C	
Stability & Storage	<ul> <li>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</li> <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> </ul>	

• 6 months, -20 to -70 °C under sterile conditions after reconstitution.

#### BACKGROUND

TL1A is a type II transmembrane protein belonging to the TNF superfamily and has been designated TNF superfamily member 15 (TNFSF15). Human TL1A is a 251 aa protein consisting of a 35 aa cytoplasmic domain, a 24 aa transmembrane region and a 192 aa C-terminal extracellular domain. It is a longer variant of the previously cloned TL1 (also known as VEGI) that is possibly a cloning artifact. TL1A is predominantly expressed in endothelial cells and its expression is inducible by TNF-α and IL-1α. TL1A binds with high affinity to death receptor 3 (DR3), which is now designated TNF receptor superfamily member 25 (TNFRSF25). DR3 was formerly designated TNFRSF12 when it was thought to be the receptor for TWEAK/TNFSF12. DR3 is expressed primarily on activated T cells. Depending on the cell context, ligation of DR3 by TL1A can trigger one of two signaling pathways, activation of the transcription factor NF-kappa-B or activation of caspases and apoptosis. On primary T cells, TL1A induces NF-kappa-B activation and a costimulatory signal to increase IL-2 responsiveness and the secretion of proinflammatory cytokines. However, in a tumor cell line, TF-1, TL1A has been shown to induce caspase activity and apoptosis. These effects of TL1A are blocked by the secreted, soluble decoy receptor 3 (DcR3), also known as TR6 and TNFRSF6B, which compete with DR3 for binding to TL1A. Consistent with the observed in vitro activities, TL1A promotes *ex vivo* splenocyte expansion and enhances *in vivo* graft-versus-host-response.

#### References:

1. Migone, T.S. et al. (2002) Immunity 16:479

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