Human CD27/TNFRSF7 Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF382

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, rhNGF R, rhsTNF RI, and rhsTNF 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 as a 0.2 µm filtered solution in PBS.

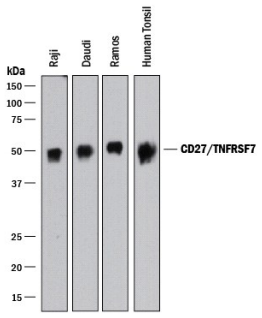
APPLICATIONS

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.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	Human whole blood lymphocytes
Immunohistochemistry	3-15 µg/mL	See Below
Simple Western	20 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 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.	

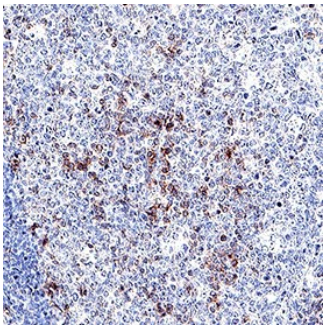
DATA

Western Blot



Detection of Human CD27/TNFRSF7 by Western Blot. Western blot shows lysates of Raji 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.

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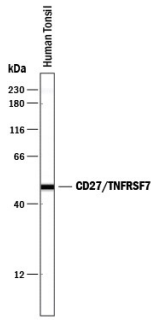
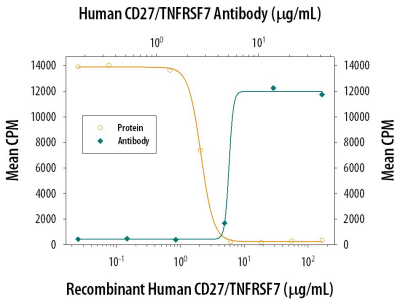
Simple Western



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 ND_{50} is typically 2.25-9.0 µg/mL.

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 appeaars 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 20 aa transmembrane domain, and 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:

1. Camerini, D. *et al.* (1991) J. Immunol. **147**:3165.
2. Loenen, W.A. *et al.* (1992) J. Immunol. **149**:3937.
3. Lens, S.M.A. *et al.* (1998) Sem. Immunol. **10**:491.

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