# biotechne

## Human 4-1BB/TNFRSF9/CD137 Antibody

Antigen Affinity-purified Polyclonal Goat IgG Catalog Number: AF838

### DESCRIPTION

**R**Dsystems

DESCRIPTION		
Species Reactivity	Human	
Specificity	Detects 4-1BB/TNFRSF9/CD137 in direct ELISAs and Western blots.	
Source	Polyclonal Goat IgG	
Purification	Antigen Affinity-purified	
Immunogen	Mouse myeloma cell line NS0-derived recombinant human 4-1BB/TNFRSF9/CD137 Leu24-Gln186 Accession # Q07011	
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 deter	mined by each laboratory for each applica	ation. General Protocols are available in the Technical Information section on our website.	
	Recommended Concentration	Sample	
Western Blot	0.5 µg/mL	See Below	
Flow Cytometry	0.25 μg/10 <sup>6</sup> cells	See Below	
Immunocytochemistry	5-15 µg/mL	Immersion fixed human peripheral blood mononuclear cells	
Immunohistochemistry	5-25 µg/mL	See Below	
Simple Western	20 µg/mL	HDLM-2 human Hodgkin's lymphoma cells	
Human 4-1BB/TNFRSF9/CD137 Sandwich Immunoassay		Reagent	
ELISA Capture	0.2-0.8 μg/mL	Human 4-1BB/TNFRSF9/CD137 Antibody (Catalog # AF838)	
ELISA Detection	0.1-0.4 μg/mL	Human 4-1BB/TNFRSF9/CD137 Biotinylated Antibody (Catalog # BAF838)	
Standard		Recombinant Human 4-1BB/TNFRSF9/CD137 Fc Chimera (Catalog # 838-4B)	
CyTOF-ready	Ready to be labeled conjugation.	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Agonist Activity	,	Measured by its ability to co-stimulate IFN- $\gamma$ secretion by human T cells in the presence of anti-CD3. The ED <sub>50</sub> for this effect is typically ≤ 20 µg/mL.	

Rev. 7/5/2023 Page 1 of 2



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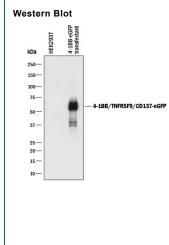
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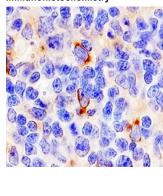
## **R**DSYSTEMS

Data



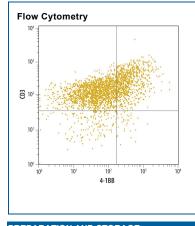
Detection of Human 4-1BB/TNFRSF9/ CD137 by Western Blot, Western blot shows lysates of HEK293T human embryonic kidney cell line either mock transfected or transfected with human 4-1BB/TNFRSF9/ CD137-eGFP Fusion. PVDF membrane was probed with 0.5 ug/mL of Goat Anti-Human 4-1BB/ TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF838) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody ([catalogNumber:HAF017]]). A specific band was detected for 4-. 1BB/ TNFRSF9/CD137-eGFP Fusion at approximately 50-65 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

## Immunohistochemistry

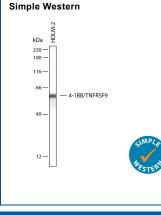


#### 4-1BB/TNFRSF9/CD137 in Human Tonsil. 4-

1BB/TNFRSF9/CD137 was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF838) 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 lymphocytes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections



Detection of 4-1BB/TNFRSF9/CD137 in PHA-treated Human T Cells by Flow Cytometry. Human T cells were treated for 48 hours with 5 µg/mL PHA then stained with Goat Anti-Human 4-1BB/TNFRSF9/ CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF838) followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108) and PEconjugated anti-human CD3 (Catalog # FAB100P). Quad marker was set based on control antibody (Catalog # AB-108-C) staining



Detection of Human 4-1BB/TNFRSF9/CD137 by Simple Western<sup>™</sup>. Simple Western lane view shows lysates of HDLM-2 human Hodgkin's lymphoma cells, loaded at 0.2 mg/mL. A specific band was detected for 4-1BB/TNFRSF9/CD137 at approximately 58 kDa (as indicated) using 20 µg/mL of Goat Anti-Human 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF838) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system

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	<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> <li>6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>	

## BACKGROUND

4-1BB is an inducible T cell surface protein belonging to the TNF receptor superfamily. It is alternatively known as TNFRSF9, CD137, and ILA. The 255 amino acid human 4-1BB is a type I transmembrane protein having in its extracellular domain four of the cysteine-rich motifs that are characteristic of the TNF receptor superfamily. The 30 kDa glycoprotein exists both as a monomer and as a dimer on T cells. The human and mouse proteins share 60% amino acid identity. 4-1BB is absent from naive T cells, but it is upregulated and continually expressed following T cell activation. The natural ligand, 4-1BBL, is a member of the TNF superfamily and is expressed on activated antigen presenting cells including dendritic cells, macrophages, and B cells. Cross-linking of 4-1BB by 4-1BBL or by agonistic antibodies transmits a potent co-stimulatory signal that enhances the effect of other activating signals such as PHA or anti-CD3 antibodies. 4-1BB signals through the TFAF2-NIK pathway resulting in activation of NF-κB and ultimately promoting proliferation and survival of T cells.

### References:

- 1. Vinay, D. and B. Kwon (1998) Semin. Immunol. 10:481.
- 2. Sica, G. and L. Chen (2000) Adv. Exp. Med. Biol. 465:355

Rev. 7/5/2023 Page 2 of 2



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