Mouse 4-1BB/TNFRSF9/CD137 Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF937

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse 4-1BB/TNFRSF9/CD137 in direct ELISAs and Western blots. In direct ELISAs, approximately 100% cross-reactivity with recombinant rat 4-1BB/TNFRSF9 is observed. In Western blots, less than 5% cross-reactivity with recombinant human 4-1BB and recombinant mouse EDAR is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse 4-1BB/TNFRSF9/CD137 Val24-Leu187 Accession # P20334
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.

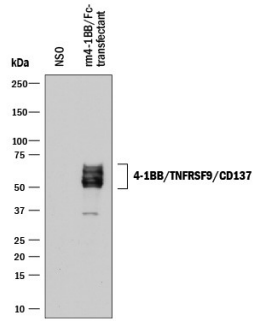
APPLICATIONS

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.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	1-15 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.	
Agonist Activity	Measured by its ability to co-stimulate IFN-γ secretion by mouse splenic T cells in the presence of anti-CD3. Saoulli, K. *et al.* (1998) J. Exp. Med. **187(11)**:1849 and Cannons, J. *et al.* (2001) J. Immunol. **167**:1313. The ED_{50} for this effect is typically 0.3 - 1 µg/mL.	

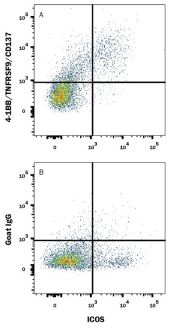
DATA

Western Blot



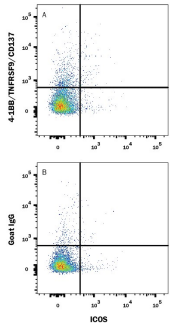
Detection of Mouse 4-1BB/TNFRSF9/CD137 by Western Blot. Western blot shows lysates of NS0 mouse myeloma cell line either mock transfected or transfected with recombinant mouse 4-1BB/Fc chimera. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Mouse 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF937) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for 4-1BB/TNFRSF9/CD137 at approximately 50-60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



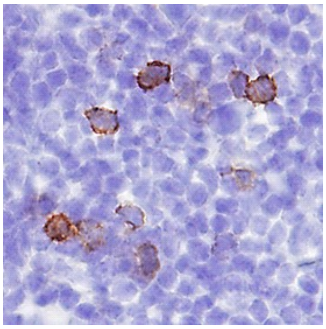
Detection of 4-1BB/TNFRSF9/CD137 in Activated Mouse Splenocytes by Flow Cytometry. Activated mouse splenocytes were stained with Rat Anti-Mouse ICOS APC-conjugated Monoclonal Antibody (Catalog # FAB168A) and either (A) Goat Anti-Mouse 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF937) or (B) Normal Goat IgG Control (Catalog # AB-108-C) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Flow Cytometry



Detection of 4-1BB/TNFRSF9/CD137 in Resting Mouse Splenocytes by Flow Cytometry. Resting mouse splenocytes were stained with Rat Anti-Mouse ICOS APC-conjugated Monoclonal Antibody (Catalog # FAB168A) and either (A) Goat Anti-Mouse 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF937) or (B) Normal Goat IgG Control (Catalog # AB-108-C) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



4-1BB/TNFRSF9/CD137 in Mouse Spleen. 4-1BB/TNFRSF9/CD137 was detected in perfusion fixed frozen sections of mouse spleen using Goat Anti-Mouse 4-1BB/TNFRSF9/CD137 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF937) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS009) and counterstained with hematoxylin (blue). Specific staining was localized to cell surfaces. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

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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

4-1BB, also known as CD137 and ILA (induced by lymphocyte activation), is a TNF receptor superfamily member and has been designated TNFRSF9. Mouse 4-1BB cDNA encodes a 256 amino acid (aa) residues type I transmembrane protein with a putative 23 aa signal peptide, a 164 aa extracellular domain, a 21 aa transmembrane domain and a 48 aa cytoplasmic region (1-3). A soluble 4-1BB is released from surfaces of cells expressing the transmembrane protein (4). Mouse 4-1BB shares approximately 60% aa sequence identity with its human counterpart. 4-1BB is expressed on activated $CD4^+$ and $CD8^+$ T cells, thymocytes, and NK cells. It is also expressed on monocytes, neutrophils, DCs and eosinophils (5). The ligand for 4-1BB (4-1BBL), also named TNFSF9, belongs to the TNF ligand superfamily. 4-1BBL is predominantly expressed on activated antigen presenting cells (APCs) such as B cells, macrophages and dendritic cells (DCs). It is also expressed on most T and B lymphoma cell lines. In response to 4-1BBL binding, 4-1BB transduce a T cell costimulatory signal in both $CD4^+$ and $CD8^+$ T cells to promote survival and enhance proliferation, cytokine production and effector function. *In vivo,* the costimulatory activity of 4-1BB has been shown to be important in graft-v s-host disease and antiviral CTL responses. On dendritic cells, 4-1BB is a DC-activating molecules that enhances cytokine production and up-regulates expression of B7-1 and B7-2 costimulatory molecules, resulting in an improved ability to stimulate T cell responses (1-5).

References:

1. Goodwin, R.G. *et al.* (1993) Eur. J. Immunol. **23**:2631.
2. Alderson, M.R. *et al.* (1994) Eur. J. Immunol. **24**:2219.
3. Kwon, B.S. and S.M. Weissman (1989) Proc. Nat. Acad. Sci. USA **86**:1963.
4. Wilcox, R.A. *et al.* (2002) J. Immunol. **168**:4262.
5. Kwon, B., H.W. Lee and B.S. Kwon, 2002, TRENDS in Immunology **23**:378.

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