Human PD-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1086

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PD-1 in ELISAs and Western blots. In sandwich ELISAs, less than 2% cross-reactivity with recombinant mouse PD-1 and less than 0.2% cross-reactivity with recombinant human (rh) CD28, rhICOS, and rhCTLA-4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human PD-1 Leu25-Gln167 Accession # Q8IX89
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.

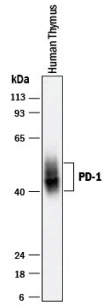
APPLICATIONS

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	0.5-2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Human PD-1 Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Human PD-1 Antibody (Catalog # AF1086)
ELISA Detection	0.1-0.4 µg/mL	Human PD-1 Biotinylated Antibody (Catalog # BAF1086)
Standard		Recombinant Human PD-1 Fc Chimera (Catalog # 1086-PD)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 3-12 µg/mL of this antibody will block 50% of the binding of 500 ng/mL of Recombinant Human B7-H1 Fc Chimera (Catalog # 156-B7) to immobilized Recombinant Human PD-1 Fc Chimera (Catalog # 1086-PD) coated at 1 µg/mL (100 µL/well). At 30 µg/mL, this antibody will block >90% of the binding.	

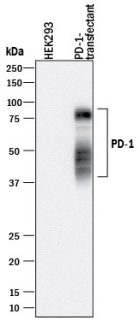
DATA

Western Blot



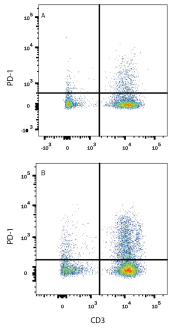
Detection of Human PD-1 by Western Blot. Western blot shows lysate of human thymus tissue. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for PD-1 at approximately 40-50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



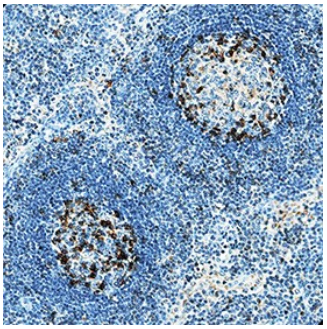
Detection of Human PD-1 by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line either mock transfected or transfected with human PD-1. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for PD-1 at approximately 40-80 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of PD-1 in Human PBMCs treated with PHA by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) treated with 5 µg/mL PHA overnight were stained with Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107) and Mouse Anti-Human CD3ε APC-conjugated Monoclonal Antibody (Catalog # FAB100A). Quadrant markers were set based on control antibody staining (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



PD-1 in Human Lymph Node. PD-1 was detected in immersion fixed paraffin-embedded sections of human lymph node using Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) 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. View our protocol for Chromogenic IHC Staining of Paraffin-embedded 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

Programmed Death-1 (PD-1) is a type I transmembrane protein belonging to the CD28/CTLA-4 family of immunoreceptors that mediate signals for regulating immune responses (1). Members of the CD28/CTLA-4 family have been shown to either promote T cell activation (CD28 and ICOS) or down-regulate T cell activation (CTLA-4 and PD-1) (2). PD-1 is expressed on activated T cells, B cells, myeloid cells, and on a subset of thymocytes. *In vitro*, ligation of PD-1 inhibits TCR-mediated T-cell proliferation and production of IL-1, IL-4, IL-10, and IFN-γ. In addition, PD-1 ligation also inhibits BCR mediated signaling. PD-1 deficient mice have a defect in peripheral tolerance and spontaneously develop autoimmune diseases (2, 3).

Two B7 family proteins, PD-L1 (also called B7-H1) and PD-L2 (also known as B7-DC), have been identified as PD-1 ligands. Unlike other B7 family proteins, both PD-L1 and PD-L2 are expressed in a wide variety of normal tissues including heart, placenta, and activated spleens (4). The wide expression of PD-L1 and PD-L2 and the inhibitor effects on PD-1 ligation indicate that PD-1 might be involved in the regulation of peripheral tolerance and may help prevent autoimmune diseases (2).

The human PD-1 gene encodes a 288 amino acid (aa) protein with a putative 20 aa signal peptide, a 148 aa extracellular region with one immunoglobulin-like V-type domain, a 24 aa transmembrane domain, and a 95 aa cytoplasmic region. The cytoplasmic tail contains two tyrosine residues that form the immuno-receptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) that are important in mediating PD-1 signaling. Mouse and human PD-1 share approximately 60% aa sequence identity (4).

References:

1. Ishida, Y. *et al*. (1992) EMBO J. **11**:3887.
2. Nishimura, H. and T. Honjo (2001) Trends in Immunol. **22**:265.
3. Latchman, Y. *et al*. (2001) Nature Immun. **2**:261.
4. Carreno, B.M. and M. Collins (2002) Annu. Rev. Immunol. **20**:29.

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