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
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.

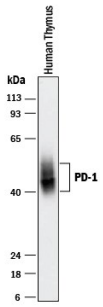
APPLICATIONS

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
Dual RNAscope ISH-IHC Compatible	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil
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.	

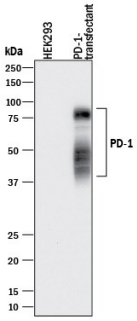
DATA

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 # 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 # 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.

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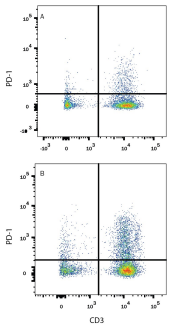
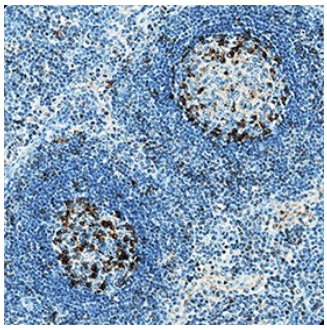
Flow Cytometry



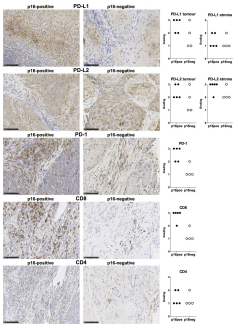
Detection of PD-1 in Human PBMCs treated with PHA by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) treated with 5 µg/mL PHA overnight were stained with Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0107) and Mouse Anti-Human CD3e APC-conjugated Monoclonal Antibody (Catalog # Catalog # FAB100A). Quadrant markers were set based on control antibody staining (Catalog # Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



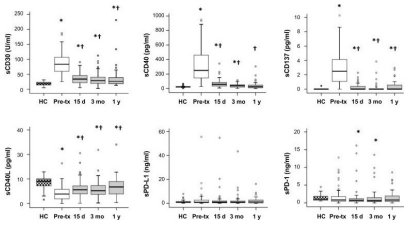
PD-1 in Human Lymph Node. PD-1 was detected in immersion fixed paraffin-embedded sections of human lymph node using Goat Anti-Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) at 3 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # 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.

Immunohistochemistry



Detection of Human PD-1 by Immunohistochemistry PD-L1, PD-L2, PD-1, CD8, and CD4 expression in p16-positive and p16-negative HNSCC. PD-L1, PD-L2, PD-1, CD8, and CD4 expression was assessed in tumor biopsy tissue from five p16-positive and four p16-negative HNSCC patients using immuno-histochemistry (details in Methods). Representative staining (scale bars, 100 µm) and cumulative data of marker expression (grading scale PD-L1/PD-L2: 1, low; 2, moderate; 3 high expression; grading scale PD-1, CD8, CD4: 1, <50 cells/field; 2, 50–150 cells/field; 3, >150 cells/field). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31379843), licensed under a CC-BY license. Not internally tested by R&D Systems.

ELISA



Detection of Human PD-1 by ELISA Modulation of soluble co-signaling molecules in kidney-transplanted patients over time.The levels of the soluble co-signaling molecules CD30, CD40, CD137, CD40L, PD-1 and PD-L1 were assayed by ELISA in serum samples of healthy controls (n=25) and kidney-transplanted patients (n=59) obtained at different times: just before transplantation, and 15 days, 3 months and 1 year after transplantation. Data are shown as box-plots, in which the horizontal line within each box represents the median, the bottom and top of each box represent the 25th and 75th percentiles, the bars represent the 10th and 90th percentiles and circles indicate outliers. Unpaired and paired Wilcoxon tests were used to compare distributions between independent and dependent groups, respectively. * indicates statistically significant differences between healthy controls and kidney-transplanted patient samples, and † indicates statistically significant differences between patients samples obtained at different pre- and post-transplantation times. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/25478957), licensed under a CC-BY license. Not internally tested by R&D Systems.

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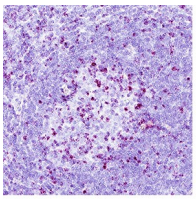
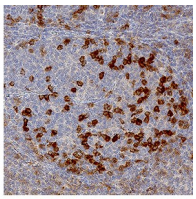
In-situ Hybridization



In Situ Hybridization (ISH)



Immunohistochemistry (IHC)

Detection of PD-1 in Human Tonsil. Formalin-fixed paraffin-embedded tissue sections of human tonsil were probed for PD1 mRNA (ACD RNAScope Probe, catalog #602021; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using goat anti-human PD1 polyclonal antibody (R&D Systems catalog # Catalog # AF1086) at 1ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-goat IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC004) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to cell surface.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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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