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.

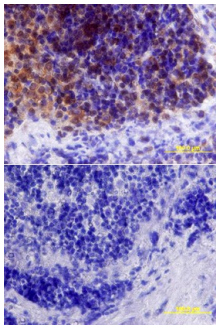
APPLICATIONS

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	0.1 µg/mL	Recombinant Human PD-1 Fc Chimera (Catalog # 1086-PD)
Flow Cytometry	2.5 µg/10^6 cells	Human T cells treated with PHA
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)
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

Immunohistochemistry



PD-1 in Human Lymph Node. PD-1 was detected in immersion fixed paraffin-embedded sections of human lymph node using Human PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1086) at 15 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). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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.
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 from date of receipt, 2 to 8 °C, reconstituted. ● 6 months from date of receipt, -20 to -70 °C, reconstituted.

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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.