

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PD-1 in direct ELISAs.
Source	Recombinant Monoclonal Mouse IgG ₁ Clone # 913429
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human PD-1 Met1-Gln167 Accession # Q15116
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.

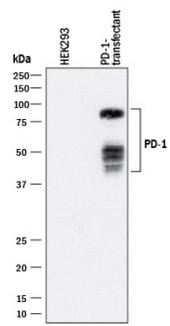
APPLICATIONS

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	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
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 flow cytometry test, biotinylated recombinant human B7-H1 (10 ng/mL, Catalog # 9049-B7) binds to HEK293 human embryonic kidney cell line transfected with human PD-1. Binding is blocked by 2.5 µg/mL of Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10861)	

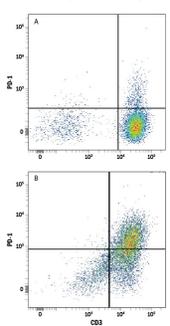
DATA

Western Blot



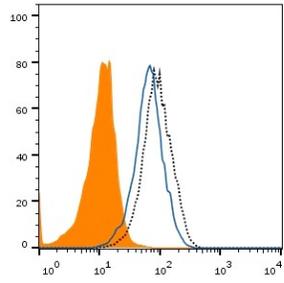
Detection of Human PD-1 by Western Blot. Western blot shows lysates of HEK293 human embryonic kidney cell line either mock transfected or transfected with human PD-1. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10861) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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 by Flow Cytometry. Human peripheral blood mononuclear cells (PBMCs) either (A) untreated or (B) treated with 5 ng/mL PHA for 2 days were stained with Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10861) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B) and Mouse Anti-Human CD3ε PE-conjugated Monoclonal Antibody (Catalog # FAB100P). Quadrant markers were set based on control antibody staining (Catalog # MAB002).

Blockade of Receptor-ligand Interaction



PD-1 Binding to B7-H1-transfected HEK293 Human Cell Line is Blocked by Human PD-1 Antibody. In a functional flow cytometry test, biotinylated recombinant human B7-H1 (10 ng/mL, Catalog # 9049-B7) binds to HEK293 human embryonic kidney cell line transfected with human PD-1 (black dotted line). Binding is completely blocked (orange histogram) by 2.5 µg/mL of Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10861). Mouse IgG2B Isotype Control (Catalog # MAB004) at 2.5 µg/mL was used as a control (blue line). Cells were stained with Streptavidin-APC (Catalog # F0050).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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. <ul style="list-style-type: none"> ● 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 downregulate 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 Immunoreceptor 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.