Human PD-1 Antibody

Monoclonal Mouse IgG_1 Clone # 1015846

Catalog Number: MAB10864

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human PD-1 in direct ELISAs.
Source	Monoclonal Mouse IgG_1 Clone # 1015846
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Human embryonic kidney cell HEK293-derived human PD-1 Leu25-Thr168 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.

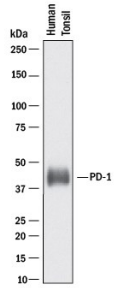
APPLICATIONS

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	2 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below
Blockade of Receptor-ligand Interaction	In a functional ELISA, 0.09-0.72 µg/mL of this antibody will block 50% of the binding of 5 µg/mL of Recombinant Human PD-L1/B7-H1 Fc Chimera (Catalog # 156-B7) to immobilized Recombinant Human PD-1 His-tagged Protein (Catalog # 8986-PD) coated at 1 µg/mL (100 µL/well). At 5 µg/mL, this antibody will block >90% of the binding.	
ELISA	This antibody functions as an ELISA detection antibody when paired with Rabbit Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10863). *This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human PD-1 DuoSet ELISA Kit (Catalog # DY1086) for convenient development of a sandwich ELISA.*	

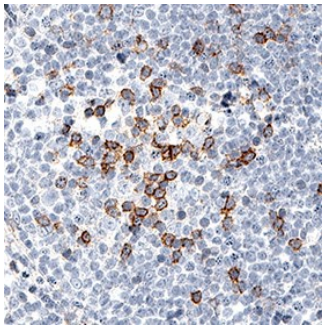
DATA

Western Blot



Detection of Human PD-1 by Western Blot. Western blot shows lysates of human tonsil tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10864) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for PD-1 at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



PD-1 in Human Tonsil. PD-1 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10864) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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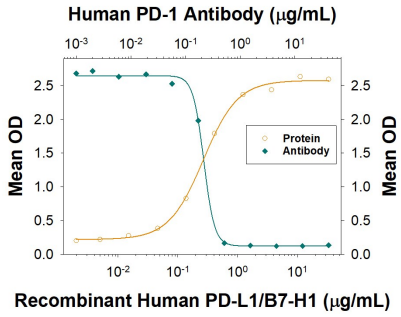
Human PD-1 Antibody

Monoclonal Mouse IgG_1 Clone # 1015846

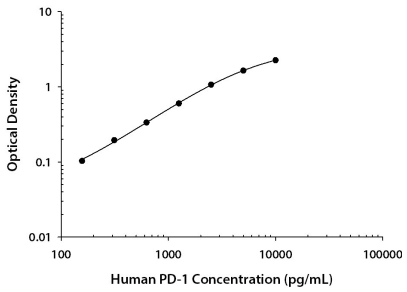
Catalog Number: MAB10864

Blockade of Receptor-ligand Interaction



PD-L1/B7-H1 Binding to PD-1 Blocked by Human PD-1 Antibody. In a functional ELISA, 0.09-0.72 µg/mL of this antibody (green line) will block 50% of the binding of 5 µg/mL of Recombinant Human PD-L1/B7-H1 Fc Chimera (orange line, Catalog # 156-B7) to immobilized Recombinant Human PD-1 His-tagged Protein (Catalog # 8986-PD) coated at 1 µg/mL (100 µL/well). At 5 µg/mL, this antibody will block >90% of the binding.

ELISA



Human PD-1 ELISA Standard Curve. Recombinant Human PD-1 protein was serially diluted 2-fold and captured by Rabbit Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10863) coated on a Clear Polystyrene Microplate (Catalog # DY990). Mouse Anti-Human PD-1 Monoclonal Antibody (Catalog # MAB10864) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # DY998) followed by Substrate Solution (Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # DY994).

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.** • 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 receptor (PD-1), also known as CD279, is type I transmembrane protein belonging to the CD28 family of immune regulatory receptors (1). Other members of this family include CD28, CTLA-4, ICOS, and BTLA (2-5). Mature human PD-1 consists of a 148 amino acid (aa) extracellular region (ECD) with one immunoglobulin-like V-type domain, a 24 aa transmembrane domain, and a 95 aa cytoplasmic region. The human PD-1 ECD shares 65% aa sequence identity with the mouse PD-1 ECD. 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 for mediating PD-1 signaling. PD-1 acts as a monomeric receptor and interacts in a 1:1 stoichiometric ratio with its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) (6, 7). PD-1 is expressed on activated T cells, B cells, monocytes, and dendritic cells while PD-L1 expression is constitutive on the same cells and also on nonhematopoietic cells such as lung endothelial cells and hepatocytes (8, 9). Ligation of PD-L1 with PD-1 induces co-inhibitory signals on T cells promoting their apoptosis, anergy, and functional exhaustion (10). Thus, the PD-1: PD-L1 interaction is a key regulator of the threshold of immune response and peripheral immune tolerance (11). Finally, blockade of the PD-1: PD-L1 interaction by either antibodies or genetic manipulation accelerates tumor eradication and shows potential for improving cancer immunotherapy (12, 13, 14).

References:

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