Mouse PD-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1021

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse PD-1 in direct ELISAs and Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant human PD-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*S. frugiperda* insect ovarian cell line *Sf* 21-derived recombinant mouse PD-1 Leu25-Gln167 Accession # Q02242
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.

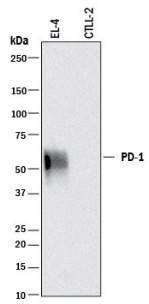
APPLICATIONS

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	0.2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	Mouse splenocytes treated with PHA
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

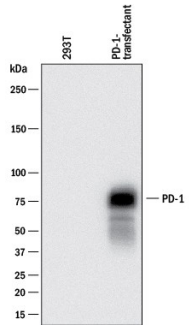
DATA

Western Blot



Detection of Mouse PD-1 by Western Blot. Western blot shows lysates of EL-4 mouse lymphoblast cell line and CTLL-2 mouse cytotoxic T cell line (negative control). PVDF membrane was probed with 0.2 µg/mL of Goat Anti-Mouse PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1021) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for PD-1 at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



Detection of Mouse PD-1 by Western Blot. Western blot shows lysates of 293T human embryonic kidney cell line mock transfected or transfected with mouse PD-1. PVDF membrane was probed with 0.2 µg/mL of Goat Anti-Mouse PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1021) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for PD-1 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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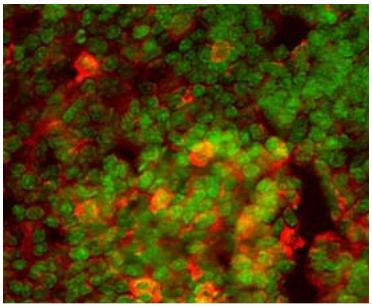
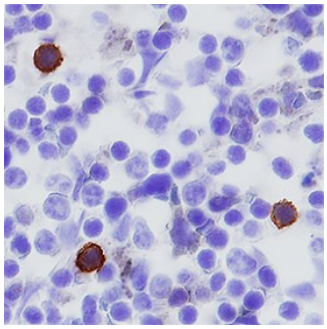
Immunohistochemistry



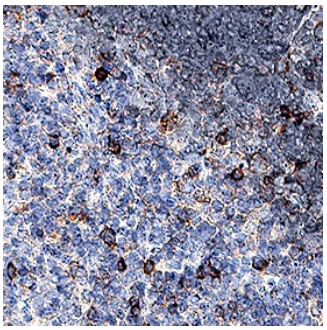
PD-1 in Mouse Thymus. PD-1 was detected in perfusion fixed frozen sections of mouse thymus using 15 µg/mL Mouse PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1021) overnight at 4 °C. Tissue was stained (red) and counterstained (green). View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.

Immunohistochemistry



PD-1 in Mouse Spleen. PD-1 was detected in perfusion fixed frozen sections of mouse spleen using Goat Anti-Mouse PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1021) at 5 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 cytoplasm in splenocytes. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Immunohistochemistry



PD-1 in Mouse Thymus. PD-1 was detected in perfusion fixed paraffin-embedded sections of mouse thymus using Goat Anti-Mouse PD-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1021) at 1.7 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell membranes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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.

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BACKGROUND

Programmed Death-1 (PD-1) is type I transmembrane protein belonging to the CD28/CTLA-4 family of immunoreceptors that mediate signals for regulating immune responses (1). Other members of this family include CD28, CTLA-4, and ICOS (2-4). PD-1 is most closely related to CTLA-4 and shares approximately 24% amino acid (aa) sequence identity. The mouse PD-1 gene encodes a 288 aa protein with a putative 20 aa signal peptide, a 149 aa extracellular region with one immunoglobulin-like V-type domain, a 21 aa transmembrane domain, and a 98 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 69% aa sequence identity. Two B7 family proteins, PD-L1 (also called B7-H1) and PD-L2, have been identified as PD-1 ligands (5, 6). PD-1 is expressed on activated T cells, B cells, myeloid cells, and on a subset of thymocytes. PD-1 deficient mice have a defect in peripheral tolerance and spontaneously develop autoimmune diseases. Binding of PD-1 to PD-L1 or PD-L2 results in the inhibition of TCR-mediated proliferation and cytokine production as well as BCR-mediated signaling. PD-1 likely has an inhibitory role in regulating immune responses (1-4).

References:

1. Ishida, Y. *et al.* (1992) EMBO J. **11**:3887.
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4. Nishimura, H. and T. Honjo (2001) Trends in Immunol. **22**:265.
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6. Tamura, H. *et al.* (2001) Blood **97**:1809.

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