

DESCRIPTION	
Species Reactivity	Mouse
Specificity	Detects mouse PD-1 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 50% cross-reactivity with recombinant human PD-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> 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 CTL-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 # 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 # 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](#).

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 # 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](#).

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. <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 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.
2. Sharpe, A.H. and G.J. Freeman (2002) Nat. Rev. Immunol. **2**:116.
3. Coyle, A. and J. Gutierrez-Ramos (2001) Nat. Immunol. **2**:203.
4. Nishimura, H. and T. Honjo (2001) Trends in Immunol. **22**:265.
5. Latchman Y. *et al.* (2001) Nature Immun. **2**:261.
6. Tamura, H. *et al.* (2001) Blood **97**:1809.