PD-1 (D4W2J) XP® Rabbit mAb

For Research Use Only. Not For Use In Diagnostic Procedures.

**Applications:** W, IP, IHC-P, F

**Species Cross-Reactivity:**

- H

**Molecular Wt.:** 52-65 kDa

**Isotype:** Rabbit IgG **

**Recommended Antibody Dilutions:**

- Western blotting: 1:1000
- Immunoprecipitation: 1:200
- Immunohistochemistry (Paraffin): 1:100-1:400
- Flow Cytometry: 1:100-1:200

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at −20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

**Background:** The programmed cell death 1 protein (PD-1, PDCD1, CD279) is a member of the CD28 family of immunoreceptors that regulate T cell activation and immune responses (1-3). The PD-1 protein contains an extracellular Ig V domain, a transmembrane domain, and a cytoplasmic tail that includes an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 is activated by the cell surface ligands PD-L1 and PD-L2 (4).

Upon activation, PD-1 ITIM and ITSM phosphorylation leads to the recruitment of the protein tyrosine phosphatases SHP-1 and SHP-2, which suppress TCR signaling (5-7). In addition to activated T-cells, PD-1 is expressed in activated B-cells and monocytes, although its function in these cell types has not been fully characterized (8). The PD-1 pathway plays an important role in immune tolerance (3); however, research studies show that cancer cells often adopt this pathway to escape immune surveillance (9). Consequently, blockade of PD-1 and its ligands is proving to be a sound strategy for neoplastic intervention (10).

**Specificity/Sensitivity:** PD-1 (D4W2J) XP® Rabbit mAb recognizes endogenous levels of total PD-1 protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala274 of human PD-1 protein.

**Immunohistochemical analysis of paraffin-embedded human colon carcinoma using PD-1 (D4W2J) XP® Rabbit mAb.**

Western blot analysis of extracts from human CD4+ T cells, MOLT-4, and Jurkat cells using PD-1 (D4W2J) XP® Rabbit mAb (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower). CD4+ T cells were purified from human blood and stimulated for 9 days using beads coated with CD3 and CD28 antibodies in the presence of human interleukin-2 (NIL-2) #8607 (6.7 ng/ml).

**IMPORTANT:** For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.
Immunohistochemical analysis of paraffin-embedded 293 cell pellets, control (left) or PD-1 transfected (right), using PD-1 (D4W2J) XP® Rabbit mAb.

Immunoprecipitation of PD-1 protein from Molt-4 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is PD-1 (D4W2J) XP® Rabbit mAb. Western blot analysis was performed using PD-1 (D4W2J) XP® Rabbit mAb.


Immunohistochemical analysis of paraffin-embedded human infiltrating papillary carcinoma of the breast using PD-1 (D4W2J) XP® Rabbit mAb performed on the Leica® Bond™ Rx.

Flow cytometric analysis of human peripheral blood mononuclear cells, untreated (left column) or CD3/CD28-treated (72 hr; right column), using PD-1 (D4W2J) XP® Rabbit mAb #86163 (top row) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (bottom row), and co-stained with a CD3 antibody. Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.