

## Anti-PD-1 [5C4.B8 (Nivolumab)] Standard Size Ab00791-1.1

This chimeric mouse antibody was made using the variable domain sequences of the original human IgG4 (S228P) format, for improved compatibility with existing reagents, assays and techniques. NOT FOR THERAPEUTIC USE- This is a research-grade biosimilar. This is a chimeric antibody created for improved compatibility with existing reagents, assays and techniques.

**Isotype and Format:** Mouse IgG1, Kappa

**Clone Number:** 5C4.B8 (Nivolumab)

**Alternative Name(s) of Target:** CD279; programmed death-1; programmed death 1 ; PD1; PD 1; Programmed cell death protein 1; hPD-1; hPD1; hPD 1; BMS-936558; MDX-1106;ONO-4538

**UniProt Accession Number of Target Protein:** Q15116

**Published Application(s):** SPR, Block, FC, IHC

**Published Species Reactivity:** Cynomolgus, Human

**Immunogen:** mAb PD1.5 was prepared by immunizing IgH and IgK knock-out transgenic mice possessing a human immunoglobulin (heavy chain) minilocus with recombinant human PD-1-Fc protein consisting of the extracellular domain of PD-1 (amino acids 1-167) and the Fc portion of human IgG1, and Chinese hamster ovary (CHO) cells expressing human PD-1. Nivolumab was generated by grafting the variable regions of PD1.5 onto human kappa and IgG4 constant regions containing an S228P mutation (prevents Fab arm exchange with endogenous IgG4 antibodies).

**Specificity:** Nivolumab binds to the extracellular portion of human PD-1 (2.6 nM - Scatchard analysis and SPR) - the antibody also binds to cynomolgus PD-1 with a similar affinity (3.9 nM - SPR). The antibody does not bind to other immunoglobulin superfamily proteins such as CD28, CTLA-4, ICOS and BTLA. The epitope of Nivolumab for both human and cynomolgus PD-1 includes the sequences SFVLNWYR-MSPSNQTDKLAAPEDR (aa 29-53) and SGTYLCGAISLAPKAQIKE (aa 85-103), as shown by mass spectrometry of protease-treated fragments of PD-1 - these residues are thought to additionally be important for ligand binding to PD-1. PD-1 is an inhibitory receptor expressed on the surface of T cells. It is able to bind to its ligands PDL-1 and PDL-2 which results in an inhibitory signal leading to decreased T cell proliferation, cytokine production and cytotoxic activity. PDL-1 is often expressed in human tumors such as melanoma, lung and kidney where it is able to overactivate PD-1 and plays a role in the evasion of cancer cells from the immune system.

**Application Notes:** Nivolumab has been shown to bind to PD-1-expressing CHO cells (EC50 ~1.66nM). Nivolumab binds CD4+ T cells (EC50 ~0.64 nM) and stains only memory and effector, and not naive CD4+

or CD8+ T cells from human peripheral blood by FC. The antibody is able to block the interaction between PD-1 and its ligands PDL-1 and PDL-2 (IC50 ~2.52 nM and ~2.59 nM, respectively - determined by SPR) - these IC50 values are also similar to that measured by FACS to evaluate ligand binding to PD-1 expressed on CHO cells. In an allogenic T-cell/DC MLR, Nivolumab-mediated inhibition of PD-1 results in enhancement of IFN $\gamma$  release, and also enhances IL-2 secretion (97-139% over an isotype control) in response to the superantigen SEB using human peripheral blood mononuclear cells. The same is also observed in a CMV-restimulation assay. Nivolumab at very low concentrations (~1.5 ng/mL) is able to enhance T-cell reactivity in the presence of a T-cell receptor stimulus - nivolumab has no stimulatory effect in the absence of antigen or T-cell receptor stimulus. In the therapeutically used human IgG4 (S228P) format, this antibody is unable to mediate ADCC (antibody-dependent cell-mediated cytotoxicity) or CDC (complement-dependent cytotoxicity).

**Antibody First Published in:** Wang et al. In Vitro Characterization of the Anti-PD-1 Antibody Nivolumab, BMS-936558, and In Vivo Toxicology in Non-Human Primates Cancer Immunol Res. 2014 Sep;2(9):846-56.

[PMID:24872026](#)

**Note on publication:** Describes the generation and characterization of the anti-PD-1 antibody nivolumab, including its specificity, binding affinity and its in vivo and in vitro properties.

## Product Form

**Size:** 200  $\mu$ g Purified antibody.

**Purification:** Protein A affinity purified

**Supplied In:** PBS with 0.02% Proclin 300.

**Storage Recommendation:** Store at 4°C for up to 3 months. For longer storage, aliquot and store at -20°C.

**Concentration:** 1 mg/mL.

Important note – This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals.