

## Anti-PD-1 [5C4.B8 (Nivolumab)] Standard Size Ab00791-15.0

This antibody does not have a J-chain and therefore presents as a hexamer, rather than a pentamer.

This reformatted human antibody was made using the variable domain sequences of the original Human IgG4 format, for improved compatibility with existing reagents, assays and techniques.

**Isotype and Format:** Human IgM, 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 (EC<sub>50</sub> ~1.66nM). Nivolumab binds CD4<sup>+</sup> T cells (EC<sub>50</sub> ~0.64 nM) and stains only memory and effector, and not naive CD4<sup>+</sup> or CD8<sup>+</sup> 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:** 50  $\mu$ g Purified antibody.

**Purification:** Affinity Purified using a recombinant lectin column

**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.