

PD-1 mAb (29F.1A12), InVivoPure+

Endotoxin level ≤ 1 EU/mg

Description:

Programmed death-1 (PD-1) is a cell surface receptor that functions as a T cell checkpoint and plays a central role in regulating T cell exhaustion. Binding of PD-1 to its ligand, programmed death-ligand 1 (PD-L1), activates downstream signaling pathways and inhibits T cell activation. Moreover abnormally high PD-L1 expression on tumor cells and antigen-presenting cells in the tumor microenvironment mediates tumor immune escape, and the development of anti-PD-1/PD-L1 antibodies has recently become a hot topic in cancer immunotherapy.[1]

The 29F.1A12 antibody is a monoclonal antibody directed against the mouse protein PD-1 (Programmed Death-1), also known as CD279. [2] It blocks the binding of PD-1 to its two ligands, PD-L1 and PD-L2. [3]

This antibody is produced exclusively under serum-free conditions from hybridoma and purified with Protein-A or Protein-G affinity chromatography.

Product-ID:	AK3613P+
Clone:	29F.1A12
Immunogen:	The antibody was raised by immunizing rats with plasmid DNA containing PD-1. Boost immunizations were done with PD-1-Ig-fusion proteins. [4]
Host:	Rat
Clonality:	Monoclonal
Isotype:	Rat IgG2a κ
Formulation:	Clear Liquid, PBS, pH 7.4, 0.2 μ m sterile filtered
Concentration:	≥ 1.00 mg/mL
Purity:	≥ 95 % (CGE, reducing conditions) ≤ 5 % aggregates (analytical SEC)
Endotoxin:	≤ 1 EU/mg (LAL test)
Storage:	2 - 8 °C
Recommended Isotype Control:	Rat IgG2a Isotype Control (AK3617P+)

The product is for research use only and not for use in diagnostic or therapeutic procedures.

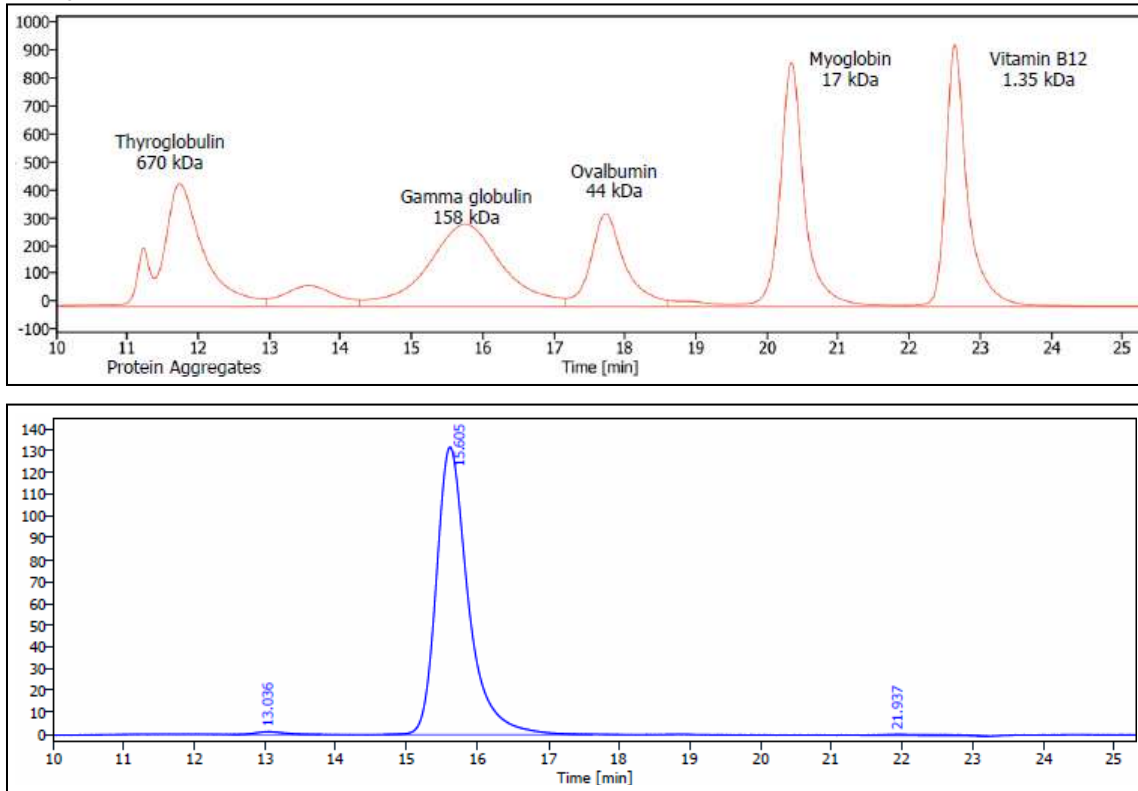
InVivo BioTech Services GmbH is certified to [ISO 9001](#) and [ISO 13485](#).

Literature:

- [1] Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. *Hum Vaccin Immunother.* 2019;15(5):1111-1122. doi: 10.1080/21645515.2019.1571892. Epub 2019 Mar 19. PMID: 30888929; PMCID: PMC6605868.
- [2] Chen L, Sham CW, Chan AM, Francisco LM, Wu Y, Mareninov S, Sharpe AH, Freeman GJ, Yang XJ, Braun J, Gordon LK. Role of the immune modulator programmed cell death-1 during development and apoptosis of mouse retinal ganglion cells. *Invest Ophthalmol Vis Sci.* 2009 Oct;50(10):4941-8. doi: 10.1167/iovs.09-3602. Epub 2009 May 6. PMID: 19420345; PMCID: PMC3222380.
- [3] Cooper ZA, Juneja VR, Sage PT, Frederick DT, Piris A, Mitra D, Lo JA, Hodi FS, Freeman GJ, Bosenberg MW, McMahon M, Flaherty KT, Fisher DE, Sharpe AH, Wargo JA. Response to BRAF inhibition in melanoma is enhanced when combined with immune checkpoint blockade. *Cancer Immunol Res.* 2014 Jul;2(7):643-54. doi: 10.1158/2326-6066.CIR-13-0215. Epub 2014 Apr 29. PMID: 24903021; PMCID: PMC4097121.
- [4] Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, Sharpe AH. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol.* 2003 Oct;33(10):2706-16. doi: 10.1002/eji.200324228. PMID: 14515254.

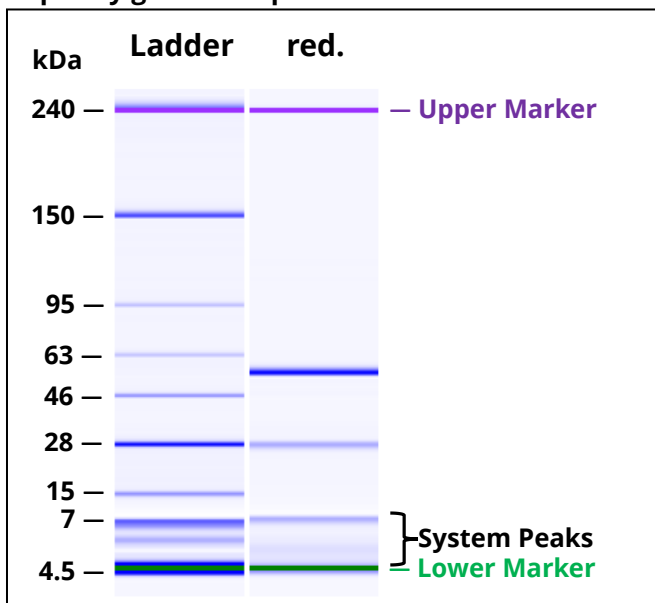
**PD-1 mAb (29F.1A12), InVivoPure+
— Supplementary Data**

Analytical SEC:



Analytical SEC of purified protein (blue) in comparison with gel filtration standard (red).

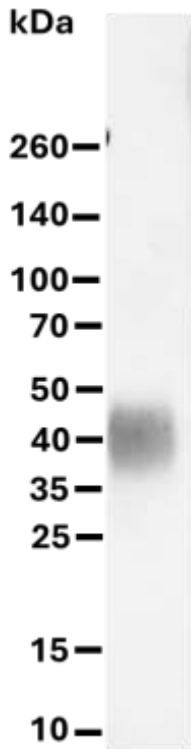
Capillary gel electrophoresis:



CGE of the purified protein under reducing (red.) conditions.

The following methods were used for the additional characterization of one exemplary batch:

Western blot analysis:



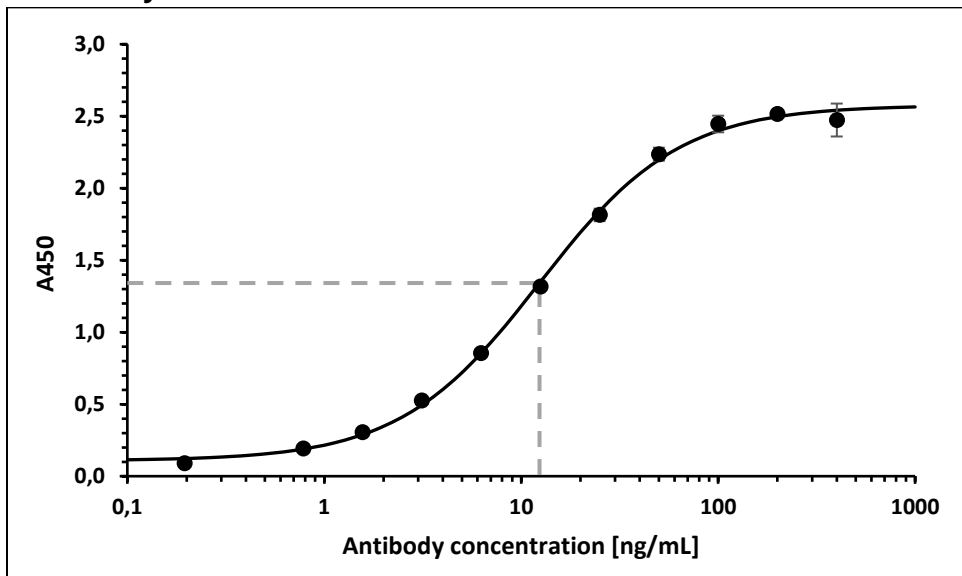
Lane 1: 1 µg reduced purified mouse PD-1 with C-terminal his-tag

Primary antibody: PD-L1 mAb (10F.9G2), InVivoPure+ (AK3613P+/02.2), 1:140 dilution

Secondary antibody: HRP labelled goat anti-rat IgG (H+L), 1:5.000 dilution

Predicted band size: ~35-45 kDa

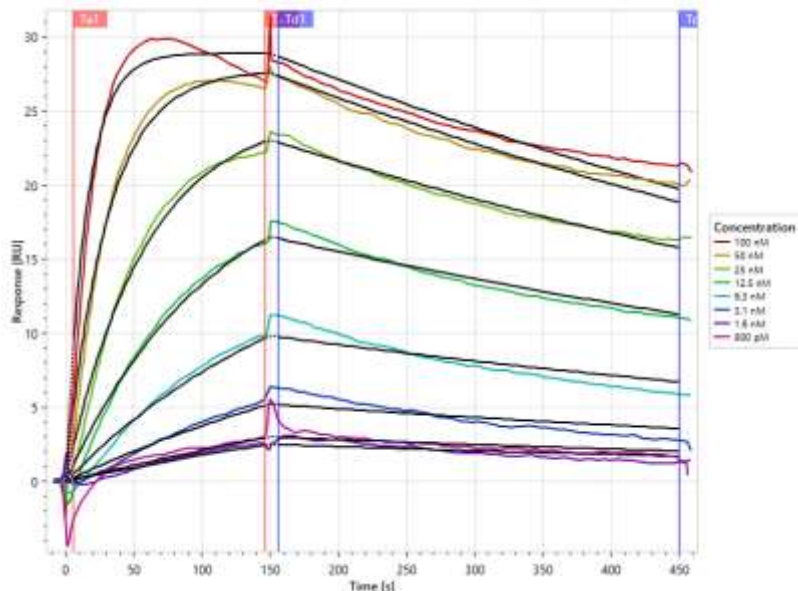
ELISA analysis:



ELISA analysis of PD-1 mAb (29F.1A12) (Product-ID: AK 3613P+/02.2). Coating antigen: Mouse PD-1 His-tag, Rec. Protein at 1 µg/mL. The EC50 of the antibody is 12,39 ng/mL.

SPR analysis:

Target	Captured Target	Analyte	K_D [M]	k_{on} [$M^{-1}s^{-1}$]	k_{off} [s^{-1}]
ProtA/G	PD-1 mAb (29F.1A12), AK 3613P+/02.2	PD-1	1.9E-09	6.5E+05	1.2E-03



A high capacity amine chip (Bruker Part No:1862614) was immobilized with Protein A/G. The antibody was captured at a concentration of 2.0 $\mu\text{g/mL}$. For the analyte, a concentration range of 0.8 to 100 nM is used in a multi-injection cycle kinetics assay on a Bruker SPR-32 to determine the K_D , k_{on} and k_{off} of the antibody-antigen-binding.