

ABCD_AQ776, ABCD_AS298, ABCD_AS299 and ABCD_AS300 antibodies label the human PD-1 protein by western blot

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Abstract

The recombinant antibodies ABCD_AQ776, ABCD_AS298, ABCD_AS299 and ABCD_AS300 detect by western blot the human protein PD-1.

Introduction

The Programmed cell death protein 1 (PD-1, UniProt #Q15116) is a transmembrane receptor expressed on activated T, B, and NK cells. Upon engagement with its ligands PD-L1 (CD274) or PD-L2 (CD273), PD-1 transmits inhibitory signals that suppress T-cell activation and cytokine production, thereby contributing to the maintenance of peripheral immune tolerance (Keir *et al.*, 2008). Tumor cells can exploit this pathway to escape immune surveillance, making PD-1 a central target for immune checkpoint blockade therapies (Topalian *et al.*, 2012). In this study, we selected seven anti-PD-1 antibodies from the ABCD database (Lima *et al.*, 2020) for testing by western blot. The clone names, formats, and original references for these antibodies are described in Table 1. One of these antibodies, ABCD_AA679 (pidilizumab), was annotated in the ABCD database as an anti-PD-1 antibody at the time of selection. A re-examination of the available literature indicated that the evidence supporting PD-1 as its target was inconclusive. More recent studies have instead suggested that pidilizumab binds delta-like protein 1 (DLL1) (Albuquerque *et al.*, 2022).

This paper reports the ability of the antibodies ABCD_AQ776, ABCD_AS298, ABCD_AS299 and ABCD_AS300 to detect the human protein PD-1 by western blot. Antibodies ABCD_AA679, ABCD_AF632 and ABCD_AQ775 did not recognize the human protein PD-1 by western blot.

Materials & Methods

Antibodies: ABCD_AA679 (AA679), ABCD_AF632 (AF632), ABCD_AQ775 (AQ775), ABCD_AQ776 (AQ776), ABCD_AS298 (AS298), ABCD_AS299 (AS299) and ABCD_AS300 (AS300) (ABCD

nomenclature, <http://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<http://unige.ch/medecine/antibodies/>) and produced as minibodies with the antigen-binding scFv portion fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker (GGGGS)₃. HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius), supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants containing antibodies (~5 to 100 mg/L) were collected after 4 days.

Table 1: Clone names, formats and references and of the antibodies used in this study

ABCD	name	Format	Reference
AA679	pidilizumab	scFv	Hardy <i>et al.</i> , 2008
AF632	m107	scFv	Dimitrov <i>et al.</i> , 2017
AQ775	GY-5	scFv	Chen <i>et al.</i> , 2019
AQ776	GY-14	scFv	Chen <i>et al.</i> , 2019
AS298	MH8	scFv	Finlay <i>et al.</i> , 2019
AS299	MH4	scFv	Finlay <i>et al.</i> , 2019
AS300	MH12	scFv	Finlay <i>et al.</i> , 2019

Antigen: We used a fusion protein composed of the extracellular domain of the human PD-1 protein fused to the transmembrane domain of the interleukin 2 receptor alpha subunit (UniProt #P01589), with a C-terminal HA epitope TAG (YPYDVDPDYASLRS). The fusion protein was produced in transiently transfected HeLa cells. Non-transfected (NT) HeLa cells were used as a negative control.

Protocol: 1x10⁵ HeLa cells were pelleted, washed once in PBS and lysed in PBS containing 0.5% (v/v) Triton X-100. A centrifugation step (5 min at 12'000 g) allowed to discard the pelleted nuclei and to recover the supernatants containing PD-1 which were mixed with reducing or non-reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, +/- 6% (v/v) β-mercaptoethanol). Each

sample was separated by electrophoresis on an acrylamide gel 4-20% (SurePAGE Bis-Tris, Genscript #M00655) at 200 V for 30 min. Transfer was performed onto a nitrocellulose membrane using a dry transfer system for 10 min (iBlot gel transfer device, Invitrogen #IB1001EU). Membranes were blocked 20 minutes in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk (PBS-Tween-milk) and were then incubated overnight with primary antibody at a concentration of 5 mg/L diluted in PBS-Tween-milk at 4°C. The membranes were then washed three times for 5 min with PBS-Tween and incubated for 20 min with the horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich #A8275, dilution 1:3000 in PBS-Tween-milk). After 5 washes for 5 min in PBS-Tween, the signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging system (Syngene).

Results & Discussion

As shown in Figure 1, immunoblotting with AQ776, AS298, AS299, and AS300 revealed two bands under non-reducing conditions at approximately 38 and 45 kDa, consistent with previous reports for human PD-1 expressed in mammalian cells (Sun *et al.*, 2020). Previous studies using PNGase F, an enzyme that removes N-linked glycans from glycoproteins, showed that the 45 kDa band corresponds to the fully glycosylated form of PD-1, whereas the 38 kDa band represents a partially glycosylated form (Sun *et al.*, 2020). Under reducing conditions, the two bands detected with AS299 were lost, suggesting that its epitope is conformation-dependent and relies on one or more disulfide bonds. For AQ776, AS298, and AS300, the band intensities were markedly reduced upon reduction, indicating partial denaturation of conformation-sensitive epitopes by the action of β -mercaptoethanol on disulfide bonds. No bands were detected with the antibodies AA679, AF631, or AQ775. Importantly, no signal was observed in non-transfected cells, confirming the specificity of the detected signals.

The seven anti-PD-1 antibodies evaluated in the present study were also assessed using ELISA (Schaffner *et al.*, 2026), immuno-cytometry (Fedosova *et al.*, 2026), and immunofluorescence (Almeida Gomes *et al.*, 2026) techniques. A comparative summary of their performance across the different immunodetection techniques is provided in Table 2. Four antibodies (AQ776, AS298, AS299, and AS300) exhibited consistent reactivity across all tested methodologies, indicating that their antigen recognition is not affected by the physicochemical conditions associated with the different assays. In contrast, antibodies AF632 and AQ775 demonstrated reactivity in ELISA, immunofluorescence, and immuno-cytometry (only tested for AF632), but failed to detect its target in western blot analyses. This pattern suggests that AF632 and AQ775 recognize a conformational epitope that is disrupted under the denaturing conditions inherent to

western blotting. Finally, antibody AA679 did not exhibit detectable activity in any of the tested methods, confirming recent reports that this antibody is not an anti-PD-1 antibody.

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Conflict of interest

Tania Jauslin is an associate-editor of the journal Antibody Reports.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

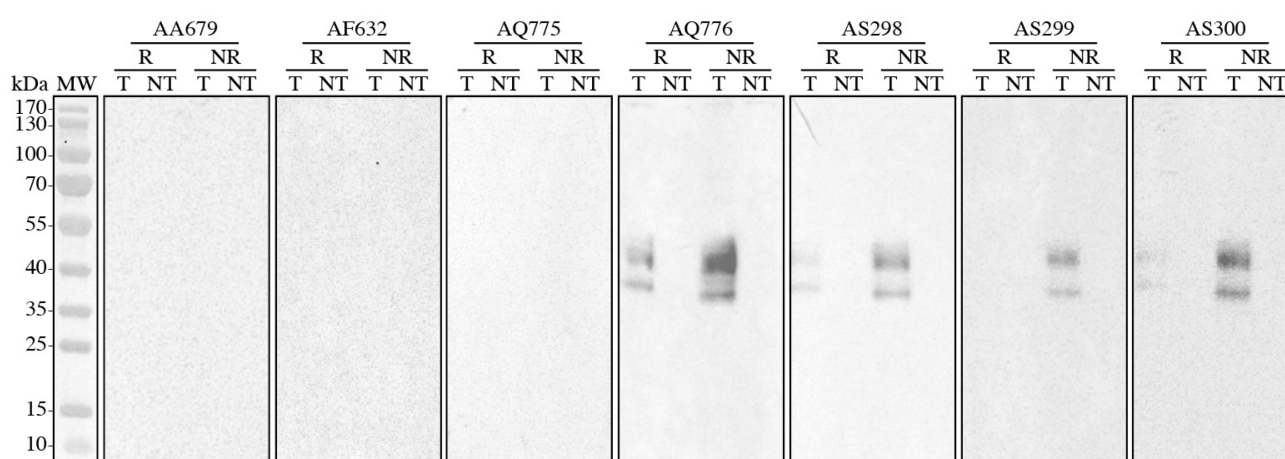


Fig.1. Specific detection of PD-1 by AQ776, AS298, AS299, and AS300 antibodies in HeLa cells transfected with a PD-1 expression plasmid under reducing (R) and non-reducing (NR) conditions. No bands were detected in non-transfected (NT) cells. No signal was detected with AA679, AF632, or AQ775.

	AA679	AF632	AQ775	AQ776	AS298	AS299	AS300
ELISA	-	+	+	+	+	+	+
Immunofluorescence	-	+	+	+	+	+	+
Immuno-cytometry	-	+	ND	+	+	+	+
Western blot	-	-	-	+	+	+	+

Table.2. Summary of experimental results for seven anti-PD-1 antibodies evaluated by ELISA, immunofluorescence, immuno-cytometry, and Western blot analyses. Antibodies demonstrating reactivity are indicated in green (+), whereas non-reactive antibodies are indicated in red (-) (ND: Not Determined).