The AD946 antibody recognizes a 6xHis-tagged recombinant protein by Western blot

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Abstract

The recombinant antibody AD946 detects by Western blot the 6xHis-tagged human myelin oligodendrocyte glycoprotein (MOG).

Introduction

A polyhistidine-tag consists of at least six histidine (6xHis) residues, fused at the N- or C-terminus of a protein. Here, we describe the ability of the AD946 antibody to detect the 6xHis-tagged human MOG-Fc recombinant protein by Western blot.

Materials & Methods

Antibodies: ABCD_AD946 antibody (ABCD nomenclature, https://web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/ antibodies; Blanc *et al*., 2014) as a mini-antibody with the antigen-binding scFv fused to a mouse Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the monoclonal anti-6xHis clone 3D5 (Lindner *et al.*, 1997) joined by a peptide linker $(GGGS)_3$. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of AD946. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: The recombinant human MOG-Fc-Avi-6xHis protein (a gift from Prof. Walter Reith, University of Geneva, Switzerland) was used as a target protein. It comprises 354 amino acids from the human MOG protein (myelin oligodendrocyte glycoprotein, Uniprot #Q16653), fused at its C-terminus to the human IgG1 (Uniprot #P01857), an Avi-tag (GLNDIFEAQKIEWHE) and a 6xHis tag.

Protocol: The recombinant human MOG-Fc-Avi-6xHis protein was purified from HEK293 cells (560 ng/µL) and diluted in 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). Three different amounts of the protein were used in this study (225, 22.5 and 2.25 ng). 20 µL of each sample was migrated (200 V, 30 min) on a 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1086), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were incubated with AD946 (dilution 1:2 in PBS-Tween) overnight at 4°C, then washed three times for 15 minutes. The membranes were then incubated 1 hour with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

The AD946 antibody specifically recognizes, in a dose-dependent manner, the 6xHis-tagged recombinant human MOG-Fc protein (Fig. 1).

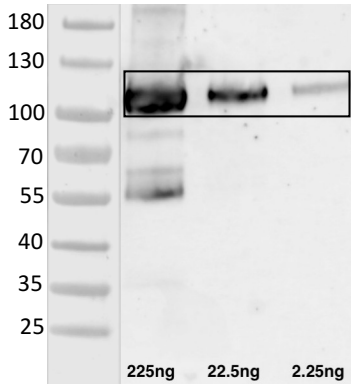

Fig. 1. Specific binding of the AD946 antibody to 6xHis-tag fused to the MOG-Fc-Avi protein (predicted molecular mass: ~100kD in reducing conditions).

References

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Conflict of interest

The authors declare no conflict of interest.