AF291 and AE391 antibodies recognize a Dictyostelium HA-tagged protein by Western blot

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Abstract

The AF291 and AE391 antibodies detect an HA-tagged *Dictyostelium discoideum* protein by Western blot.

Introduction

The HA tag is a commonly used epitope tag derived from the human influenza virus hemagglutinin protein. AF291 and AE391 have been shown to recognize an HA-tagged protein in HEK293T cells by Western blot (Keszei and Picard, 2019). Here, we describe the ability of AF291 and AE391 antibodies to recognize an HA-tagged *D. discoideum* protein by Western blot.

Materials & Methods

Antibodies: ABCD_AF291 and ABCD_AE391 (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2019) were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/) as mini-antibodies with the antigen-binding scFv fused to the Fc region of a mouse IgG (IgG2b for AF291 and IgG2a for AE391). The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the monoclonal anti-HA clones 12CA5 (for AF291, Niman *et al.*, 1983) and 26/9 (for AE391, Churchill *et al.*, 1994) joined by a peptide linker $(GGGGS)_3$. HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (70 and 10 mg/L, respectively) were collected after 5 days.

Antigen: *D. discoideum alyL* KO cells were stably transfected with an expression vector derived from pDXA-3C expressing a C-terminally HA-tagged AlyA protein (Amoeba LYsozyme, DDB_G0275123, residues 1-138). The HA sequence used was YPYDVPDYA. *alyA*-KO cells were used as negative control, and anti-AlyA MRB376 antibody was used as positive control to confirm overexpression of the tagged protein (Lamrabet, 2019).

Protocol: $5x10^6$ *D. discoideum* cells were pelleted and resuspended in 200 µL of 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). 20 µL of each sample was migrated (200 V, 30 min) on a 4-20% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Genscript #00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked during 2 hours in PBS containing 0.1% (v/v) Tween 20 and 7% (w/v) milk, and washed three times for 5 minutes in PBS + 0.1% (v/v) Tween 20. The membranes were then incubated with the different antibodies (non-diluted, dilution 1:10, and 1:2 in PBS-Tween for AE391, AF291, and MRB376, respectively), for 90 minutes at room temperature and washed three times for 5 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) and washed twice for 5 minutes and once for 15 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging systems (Syngene).

Results

Antibodies AF291 and AE391 specifically recognize the HA-tagged AlyA protein (Fig. 1). The protein was also detected with an anti-AlyA antibody (MRB376): a band of the correct size (~19 kDa) can be detected on the overexpressing cells, but is absent in non-transfected *alyL*-KO cells. As previously described, MRB376 does not recognize endogenous AlyA (presumably because the endogenous level of this protein is too low). The antibodies also detect several bands unrelated to the HA-tagged protein.

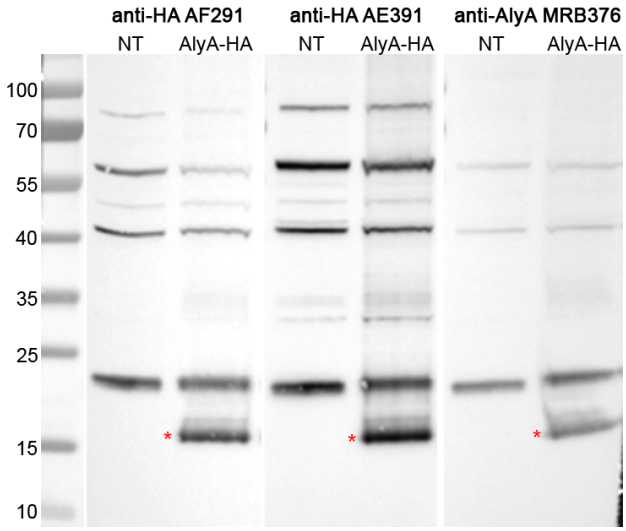

Fig. 1. Specific binding of AF291 and AE391 antibodies to *alyL* KO cells overexpressing AlyA-HA (position indicated by an asterisk). Non-transfected *alyL* KO cells (NT) were used as negative control; the endogenous AlyA protein is not detected. MRB376 was used as a positive control for AlyA-HA detection.

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Conflict of interest

The authors declare no conflict of interest.

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