doi:10.24450/journals/abrep.2019.e25 **Antibody Reports**, 2019, vol. 2, e25

AF291 and AE391 single-chain antibodies recognize the HA tag by Western blotting

Zsofia Keszei and Didier Picard

Département de Biologie Cellulaire, Université de Genève, Sciences III, CH - 1211 Genève 4, Switzerland

Abstract

The recombinant antibodies AF291 and AE391 against the standard HA tag detect an HA-tagged protein exogenously expressed in human cells by Western blotting. However, similarly to one of the original monoclonal antibodies, they also recognize a number of unrelated endogenous proteins.

Introduction

A short peptide sequence from the human influenza virus hemagglutinin is widely used as a protein tag and typically referred to as HA tag (Field *et al.*, 1988). Here we describe the ability of the single-chain variable antibodies (scFv) AF291 and AE391, derived from commonly used monoclonal antibodies, to detect an HA-tagged marker protein by Western blotting.

Materials & Methods

Antibodies: ABCD_AF291 and ABCD_AE391 (ABCD nomenclature, https://web.expasy.org/abcd/) target the overlapping epitopes YPYDVPDYA and YPYDVPDYASLRS, respectively. The antibodies were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/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 sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the anti-HA monoclonal antibody 12CA5 (Niman *et al.*, 1983) for AF291 and anti-HA monoclonal antibody 26/9 (Churchill *et al.*, 1994) for AE391. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20-100 mg/l) were collected after 5 days. As positive controls, supernatant of the hybridoma 12CA5 and the mouse monoclonal antibody HA.11 (BioLegend #11071) were used.

Antigen: HEK293T cells, grown in Dulbecco's Modified Eagle's Medium supplemented with GlutaMAX, 10% fetal bovine serum and penicillin/streptomycin (100 U/ml), were transiently transfected with the expression vector pcDNA3.1-HOP wt-HA (obtained from Adrienne Edkins, Rhodes University, Grahamstown, South Africa), coding for the full-length Hop protein (UniProt #P31948) with the C-terminal HA tag YPYDVPDYA. Cells transfected with an unrelated expression vector were used as negative control.

Protocol: Two days after transfection, cells were pelleted and lysed in 25 mM Tris-HCl pH 7.4, 250 mM sucrose, 5 mM $MgCl_2$, 1% IGEPAL CA-630 (Sigma-Aldrich #I30211), 1 mM DTT, protease inhibitor cocktail. After 10 min of incubation at room temperature, extracts were centrifuged at 16'100 g for 10 min, and the pellet was discarded. Samples were diluted 1:2 and 1:10 using an identically prepared cell lysate of HEK293T transfected with the control vector. 30 µg of proteins of the undiluted samples and the corresponding diluted samples were separated on a 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. All steps of blocking, staining and washing of the membranes were performed either with Tris-buffered saline (TBS) containing 0.2% Tween 20 or with TBS containing 0.2% IGEPAL. The membranes were blocked for 30 minutes in 5% w/v non-fat dry milk in TBS with detergent, then incubated for 1 hour at room temperature with the different antibody dilutions in TBS with detergent. As a loading control, corresponding sections of the same membranes were probed with an anti-GAPDH antibody (HyTest, #5G4, dilution 1:5000) After incubation, the membranes were washed three times for 15 minutes with TBS containing detergent. Finally, they were incubated with horseradish peroxidase-coupled goat anti-mouse antibody (Invitrogen #31430, dilution 1:10'000 in TBS with detergent) and washed again three times for 15 minutes. Images were taken with a LI-COR Odyssey Fc Imaging System.

Results

AF291 and AE391 antibodies detect the HA-tagged Hop protein by Western blotting with similar sensitivity as the monoclonal antibody 12CA5, from which AF291 was generated. These three antibodies also detect several proteins unrelated to the HA-tagged protein, whereas the HA.11 antibody does not (Figure 1). The use of IGEPAL instead of Tween 20 as detergent did not have a visible effect on the recognition of the antigen or of the unrelated proteins (Figure 1B).

This work is licensed under a Creative Commons Attribution 4.0 International License.

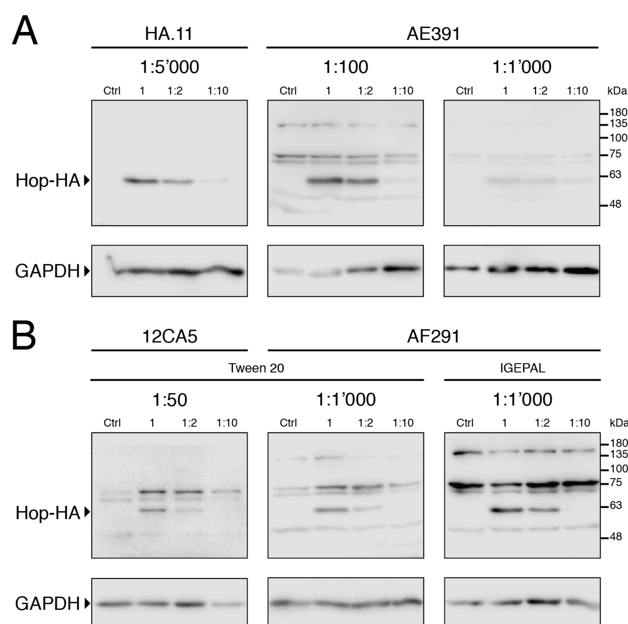

Fig. 1. AF291 and AE391 both detect Hop-HA. Western blots of undiluted (1) and of the diluted (1:2 and 1:10) samples. A lysate of cells overexpressing an unrelated protein without HA tag was used as a negative control (Ctrl), and GAPDH as loading control. (A) Western blot with AE391 and control antibody HA.11, done with TBS containing Tween 20. (B) Western blot with AF291 and control antibody 12CA5, done with TBS containing either Tween 20 or IGEPAL.

References

Churchill ME, Stura EA, Pinilla C, *et al*. Crystal structure of a peptide complex of anti-influenza peptide antibody Fab 26/9. Comparison of two different antibodies bound to the same peptide antigen. J Mol Biol. 1994; 241(4):534-56. PMID:7520084

Field J, Nikawa J, Broek D, *et al*. Purification of a RAS-responsive adenylyl cyclase complex from Saccharomyces cerevisiae by use of an epitope addition method. Mol Cell Biol. 1988; 8(5):2159-65. PMID:2455217

Niman HL, Houghten RA, Walker LE, *et al*. Generation of protein-reactive antibodies by short peptides is an event of high frequency: implications for the structural basis of immune recognition. Proc Natl Acad Sci USA. 1983; 80(16):4949-53. PMID:6192445

Acknowledgments

This work was supported by the Swiss National Science Foundation and the Canton de Genève.

Conflict of interest

The authors declare no conflict of interest.

This work is licensed under a Creative Commons Attribution 4.0 International License.