The AY165 antibody recognizes the V5-tag by immunofluorescence

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Abstract

Recombinant anti-V5 tag antibodies AE408 and AY165 were tested for their ability to recognize an overexpressed V5-tagged protein by immunofluorescence. Only AY165 labeled efficiently cells overexpressing a V5-tagged protein.

Introduction

The V5-tag is a short peptide sequence derived from the P and V protein of the simian virus 5 (Hanke *et al*., 1992). It can be fused to a recombinant protein and used for detection in many applications using specific antibodies. This study aimed to assess the efficacy of antibodies AE408 and AY165 in detecting V5-tagged IL2RA protein by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AE408, ABCD_AF291, ABCD_AJ519, ABCD_AN703 and ABCD_AY165 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/). Details and origin of the variable and VHH sequences are indicated in Table 1. All the antibodies were designed as minibodies with the single-chain variable fragment (scFv) or Variable Heavy chain of nanobodies (VHH) antigen-binding portions fused to the indicated Fc region (Table 1). Antibodies were produced in HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius), supplemented with 0.1% Pluronic F68 (Sigma #P1300). Cells were transiently transfected using FectoPro® transfection reagent (Polyplus #101000014) with the vector coding for the VHH/scFv-Fc of each antibody and supernatants were collected after 3 days.

Antigen: Adherent HEK293T cells were grown on glass coverslips at 37 °C and 5% CO2 in Dulbecco's modified Eagle's medium (Gibco #31966) containing 10% fetal bovine serum (Gibco #10270) and 100 µg/mL of penicillin–streptomycin (Sigma #15140). Two days prior to the experiment, cells were transfected using polyethylenimine (PEI, Polysciences #23966-2) with an expression vector encoding the extracellular, transmembrane, and cytosolic domains of the interleukin-2 receptor alpha subunit (human IL2RA; amino acids 1 to 272, UniProt #P01589) fused at its cytosolic C-terminus to either a V5-tag (GKPIPNPLLGLDST) or an HA-tag (YPYDVPDYASLRS).

Table 1: Clone name, reference, target and Fc domain of the recombinant antibodies used in this study.

ABCD	Clone	Reference	Target	Fc
AE408	Pk1	Hanke and Randall., 1995	V5-tag	Rabbit IgG
AF291	12CA5	Arimori *et al*., 2017	HA-tag	Rabbit IgG
AJ519	7G7/B6	Rubin *et al*., 1985	Uniprot P01589	Mouse IgG2A
AN703	221-42-1	Monnat *et al*., 1997	Uniprot Q86IA3	Rabbit IgG
AY165	NbA1	Zeghal *et al*., 2023	V5-tag	Rabbit IgG

Protocol: Cells were fixed for 30 min at room temperature in PBS containing 4% paraformaldehyde (PanReac Applichem #A3013,1000), then washed with PBS containing 40 mM NH4Cl (PanReac Applichem #A3661,0500), and permeabilized for 5 min in PBS containing 0.2% saponin (Sigma #S-7900). After blocking for 5 min with PBS containing 0.2% BSA (Sigma #A3912) (PBS-BSA), fixed and permeabilized cells were incubated for 30 min at room temperature in PBS-BSA and the indicated primary antibodies at a concentration of 5 µg/mL. After three washes with PBS-BSA, cells were further incubated for 30 min with fluorescent secondary antibodies: Alexa-Fluor-647-coupled anti-rabbit-immunoglobulin G (IgG) antibodies (Life Technologies #A21235, dilution 1/400) and Alexa-Fluor-546-coupled anti-mouse-IgG antibodies (Life Technologies #A11030, dilution 1/400). Cells were then washed three times with PBS-BSA, once with PBS and mounted in Möwiol (Plüss-StauferAG #cmB339161). Pictures were taken using a Zeiss LSM800 confocal microscope with a 63× oil immersion objective. The signals generated by each anti-tag antibody and by the anti-IL2RA antibody were quantified with imageJ software (http://rsb.info.nih.gov/ij/). For each condition, three pictures were analyzed. In each picture, 20 regions of 30 × 30 pixels were selected and the mean gray value in the two fluorescence channels was measured. The raw values obtained were plotted, and linear regression was used to calculate the efficiency with which the anti-tag antibody recognized its epitope.

Results

We used a previously described strategy to quantify the signal generated by different anti-tag antibodies (Marchetti *et al*., 2023). The transfected IL2RA-tagged protein was simultaneously detected using an anti-IL2RA antibody (AJ519) and an anti-tag antibody (HA or V5, Fig. 1). The previously characterized anti-HA antibody AF291 was used as a positive control (Marchetti *et al.,* 2023), and the antibody AN703, recognizing an irrelevant antigen, as a negative control. AY165 labeled the cells expressing IL2RA-V5, and the signal co-localized with that obtained using the anti-IL2RA AJ519 antibody (Fig. 1). No labeling was observed with the anti-V5 antibody AE408.

The signals generated by each anti-tag antibody and the anti-IL2RA antibody were quantified, plotted, and subjected to linear regression analysis to determine the ratio of the two signals and the binding efficiency (Fig. 2). The signal generated by AY165 was comparable to that generated by the anti-HA AF291 antibody, one of the best anti-tag antibodies identified previously in a comparative study (Marchetti *et al*., 2023). The signal observed with AE408 was comparable to the background signal. Based on these results, we conclude that AY165 specifically and efficiently recognizes the V5-tag by immunofluorescence. Since the V5 epitope is a short linear peptide, it is likely that the AY165 antibody also recognizes it by western blot, but this remains to be established.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability Statement

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

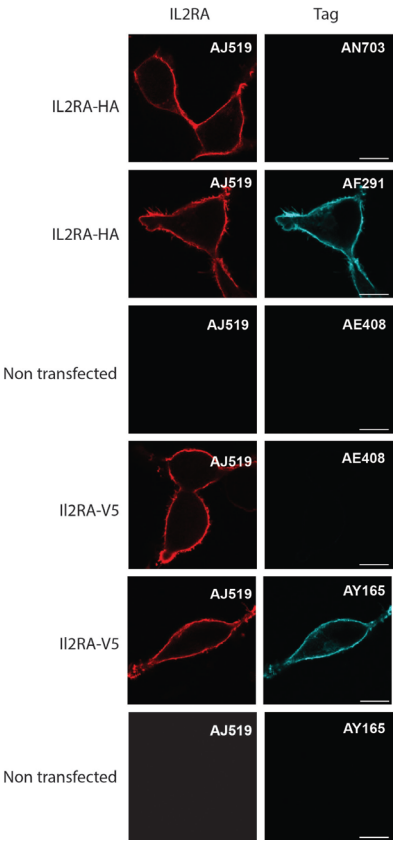

Fig. 1. Specific binding of AY165 to IL2RA-V5 detected by immunofluorescence. IL2RA-tag fusion proteins were detected with an anti-IL2RA antibody (AJ519, red) and anti-tag antibodies (blue). Anti-V5 AY165 specifically labeled the surface of cells expressing IL2RA-V5 and the signal co-localized with that obtained using the anti-IL2RA AJ519 antibody. No labeling was observed with the anti-V5 antibody AE408. Scale bar: 10 μm.

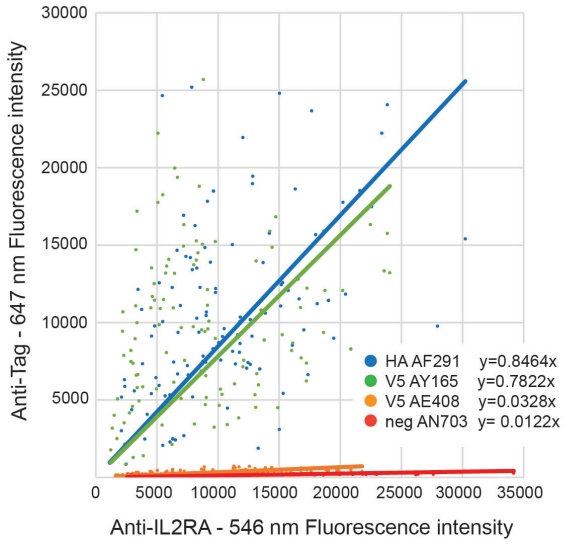

Fig. 2. AY165 efficiently recognizes the V5-tag by immunofluorescence. Fluorescence signals generated by each anti-tag antibody and the anti-IL2RA antibody were quantified on multiple areas of the picture, plotted, and subjected to linear regression analysis to determine the ratio of the two signals and the relative binding efficiency.