

RB066 and RB067 recognize human CDKN2A-derived peptides by ELISA

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Abstract

The recombinant antibodies RB066 and RB067 detect by ELISA two synthetic peptides from the human CDKN2A protein.

Introduction

CDKN2A (UniProt #P42771) is a protein involved in cell cycle regulation through interaction with cyclin-dependent kinases 4 and 6 (Foulkes *et al.*, 1997). This paper reports the evaluation of 5 recombinant antibodies (RB066, RB067, RB068, RB069 and RB070) for the detection of human CDKN2A-derived peptides by ELISA.

RB066 exhibited specific recognition of a CDKN2A N-terminal peptide, while RB067 bound to a C-terminal peptide, both in a concentration-dependent manner.

Materials & Methods

Antibodies: ABCD_RB066, ABCD_RB067, ABCD_RB068, ABCD_RB069, and ABCD_RB070 antibodies (ABCD nomenclature, <http://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (<http://unige.ch/medecine/antibodies/>) as mini-antibodies with the antigen-binding 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 (GGGS)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 (~60 – 100 µg/mL) were collected after 4 days.

Antigen: All the antibodies were tested by ELISA against the biotinylated peptide antigens they were selected for. RB066 was assayed against an N-biotinylated peptide derived from residues 2-18 of the CDKN2A protein (peptide 1, sequence: EPAAGSSMEPSADWLAT). RB067, RB068, RB069, and RB070 were evaluated against an N-biotinylated peptide derived from residues 137-156 of the CDKN2A protein (peptide 2, sequence: TRGSNHARIDAAEGPSDIPD). An irrelevant N-biotinylated peptide from the human NDC80 protein (UniProt #O14777) was used as a negative control.

Protocol: MaxiSorp 96-well plates (Nunc #44-2404-21) were coated with 50 µL of streptavidin per well at a concentration of 1 µg/mL. The coating was performed overnight at 4°C. The rest of the procedure was carried out

at room temperature. As a saturation agent, 50 µL of PBS-BSA 3% (w/v) was added, followed by 20 minutes of incubation. The sample was then rinsed three times with 100 µL of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20). Biotinylated peptides were then added at a concentration of 0,2 nmol/mL in washing buffer for 30 minutes. After another 3 times rinsing, 50µl of serially diluted RB066-RB070 primary antibodies were added according to specifications described in figure 1. After three further washes, a secondary antibody (goat anti-rabbit IgG coupled to horseradish peroxidase, Sigma-Aldrich #A8275, dilution 1:1000, 50 µl per well) was incubated for 30 minutes. Finally, the wells were washed 5 times before exposure to 50 µl of tetramethylbenzidine (TMB) HRP substrate (Sigma #T5569). The reaction was stopped by the addition of 25 µl of 2 M H₂SO₄ and the absorbance (OD) was measured at 450 nm.

Results

As illustrated in Figure 1, antibodies RB066 and RB067 exhibited concentration-dependent binding to CDKN2A peptides 1 and 2, respectively. No binding was observed to the peptide derived from the human NDC80 protein (negative control). Despite being selected against this antigen, RB068, RB069, and RB070 did not recognize peptide 2 derived from the CDKN2A protein.

Although RB066 and RB067 antibodies specifically recognize CDKN2A peptides by ELISA, their ability to bind the full-length protein should be determined in future experiments.

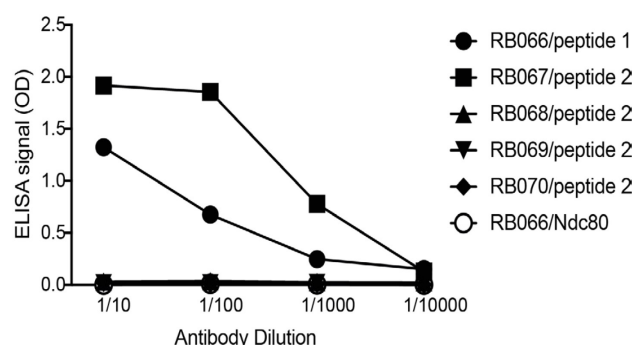


Fig. 1. RB066 and RB067 bound to CDKN2A peptide 1 and peptide 2, respectively, but not to the negative control peptide NDC80 (shown only for RB066; the other background curves were superimposed). RB068, RB069 and RB070 did not bind to CDKN2A peptide 2.

References

Foulkes, W. D., Flanders, T. Y., Pollock, P. M., & Hayward, N. K. (1997). The CDKN2A (p16) gene and human cancer. *Molecular medicine (Cambridge, Mass.)*, 3(1), 5–20.

Conflict of interest

Tania Jauslin is an editor of the Antibody Reports journal.

