RB690, RB691, RB692 and RB693 antibodies recognize a Drosophila melanogaster Nop56 peptide by ELISA

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Abstract

The recombinant antibodies RB690, RB691, RB692 and RB693 detect by ELISA a synthetic peptide from the *Drosophila melanogaster* Nop56 protein.

Introduction

Nop56 (UniProt #Q8IGT5) is a *Drosophila melanogaster* protein involved in ribosome biogenesis (Baral *et al*., 2020). Here we describe the ability of four recombinant antibodies (RB690, RB691, RB692 and RB693) to detect by ELISA a synthetic biotinylated peptide from the *Drosophila melanogaster* Nop56 protein.

Materials & Methods

Antibodies: ABCD_RB690, ABCD_RB691, ABCD_RB692 and ABCD_RB693 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding VHH portion fused to a rabbit IgG Fc (RRB690, RRB691, RRB692 and RRB693). HEK293 suspension cells (growing in HEK TF medium, Xell#861-0001, supplemented with 0.1% Pluronic F68, Sigma#P1300) were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (~80 - 110 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to residues 391 to 411 (TSVFGETLKQQVEDRLKFYES) of the Nop56 protein (Uniprot #Q8IGT5). As a negative control, an irrelevant N-biotinylated peptide (RAKFDHRKSRKSK) from PeterPan protein (UniProt #Q9VDE5) was used.

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma #A8275, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB690, RB691, RB692 and RB693 bound in a concentration-dependent manner to the Nop56 peptide against which they were raised, but not to the negative control peptide (Fig. 1).

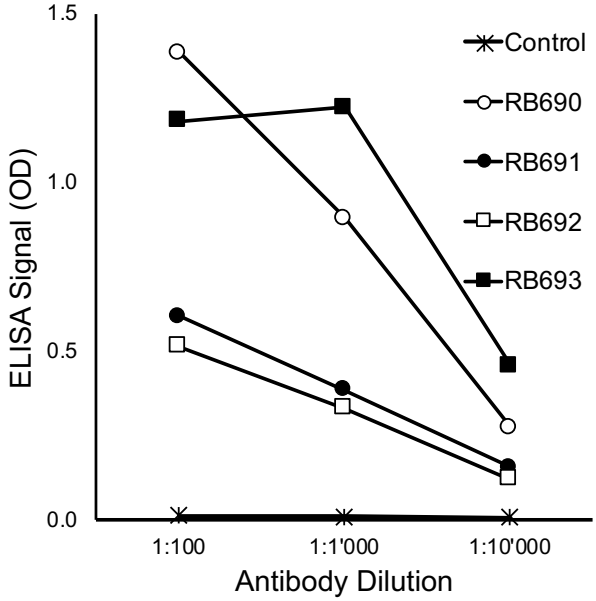

Fig. 1. Specific binding of RRB antibodies to the target Nop56 peptide, as detected by ELISA. 'Control' indicates the binding of RRB690 to the negative control peptide (all other control curves were superimposed).

References

Baral SS, Lieux ME, DiMario PJ. Nucleolar stress in Drosophila neuroblasts, a model for human ribosomopathies. Biol Open. 2020 Apr 13;9(4): bio046565.PMID: 32184230.

Conflict of interest

The authors declare no conflict of interest.