The RB487 antibody recognizes a yeast WBP1 peptide by ELISA

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Abstract

The recombinant antibody RB487 detects by ELISA a synthetic peptide from the yeast WBP1 protein.

Introduction

The *Saccharomyces cerevisiae* WBP1 (UniProt #P33767) is a subunit of the oligosaccharyl transferase (OST) complex involved in asparagine-linked N-glycosylation (te Heesen *et al.*, 1992). Here we describe the ability of the recombinant antibody RB487 to detect by ELISA a synthetic peptide from the WBP1 protein.

Materials & Methods

Antibodies: The ABCD_RB487 antibody (ABCD nomenclature, https://web.expasy.org/abcd/; Lima *et al.*, 2020) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibody with the antigen-binding scFv portion fused to a rabbit IgG Fc (RRB487). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (~50 mg/L) was collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to the last 10 C-terminal cytosolic amino acids of WBP1 (KKLETFKKTN). As a negative control, an N-biotinylated modified peptide (KKLETFSSTN) 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

The antibody RB487 bound in a concentration-dependent manner to the WBP1 peptide against which they were raised (KKLETFKKTN), but not to the negative control peptide (KKLETFSSTN) (Fig. 1).

The antigen used is a short cytosolic domain. It presumably does not fold into a complex structure, nor contains post-translational modifications. Accordingly, it is likely that the RB487 antibody will also recognize the full-length protein. Further experiments will be necessary to determine if this is the case, and in which experimental procedures RB487 can be used.

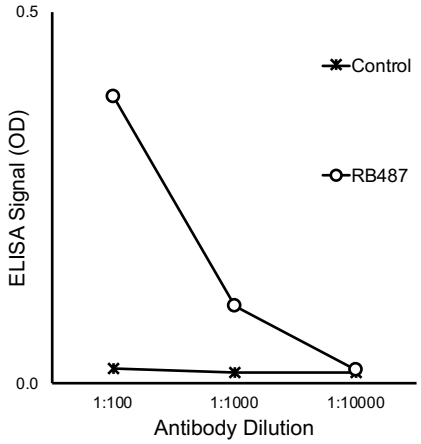

Fig. 1. Specific binding of the RRB487 antibody to the target WBP1 peptide (KKLETFKKTN), as detected by ELISA. 'Control' indicates the binding of RRB87 to the negative control peptide (KKLETFSSTN).

References

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Conflict of interest

The authors declare no conflict of interest.