

RB438, RB440, RB441 and RB442 antibodies recognize a *Dictyostelium* Nup133 peptide by ELISA

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Abstract

The recombinant antibodies RB438, RB440, RB441 and RB442 detect by ELISA a synthetic peptide from the *Dictyostelium* Nup133 protein.

Introduction

Nup133 (NUcleoPorin 133 kD; DDB_G0287497, UniProt #Q54KA3) is a component of the nuclear pore complex of the amoeba *Dictyostelium discoideum*. Here we describe the ability of four recombinant antibodies (RB438, RB440, RB441 and RB442) to detect by ELISA a synthetic biotinylated peptide from the Nup133 protein.

Materials & Methods

Antibodies: ABCD_RB438, ABCD_RB440, ABCD_RB441 and ABCD_RB442 antibodies (ABCD nomenclature, <https://web.expasy.org/abcd/>) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies; Blanc *et al.*, 2014) as mini-antibodies with the antigen-binding scFv portion fused to a mouse Fc (MRB438, MRB440, MRB441 and MRB442). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to the residues 520 to 544 of the Nup133 protein (LKQHTRVDQFVKICKGYNDFDQVIQ). A N-biotinylated peptide (KRQFEISVARLLELSKLEIGQKQI) corresponding to the last 24 C-terminal residues of the Nup133 protein was used as a negative control.

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 MRB 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-mouse IgG (Bio-Rad #170-6516, 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 H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies MRB436, MRB437 and MRB439 bound in a concentration-dependent manner to the Nup133 peptide against which they were raised, but not to the negative control peptide (Fig. 1).

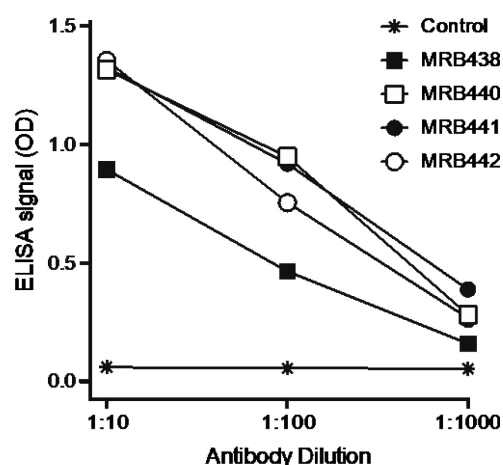


Fig. 1. Specific binding of MRB antibodies to the target Nup133 peptide, as detected by ELISA. 'Control' indicates the binding of MRB438 to the negative control peptide (all other control curves were superimposed).

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Conflict of interest

Wanessa Cristina Lima is an editor of the Antibody Reports journal.