RB792, RB793, RB794, RB795, RB796 and RB797 antibodies recognize a human NDC80 phosphorylated peptide by ELISA

Philippe Hammel[1], Keith DeLuca[2], Jeanne E. Mick[2], Jennifer G. DeLuca[2]

[1]*Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland*
[2] *Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado 80523, USA*

Abstract

The recombinant antibodies RB792, RB793, RB794, RB795, RB796 and RB797 recognize by ELISA a synthetic phosphorylated peptide from the human NDC80 protein.

Introduction

NDC80 (UniProt #Q05DQ6) is a kinetochore protein which is essential for efficient linkage between kinetochores and spindle microtubules (DeLuca *et al*., 2012). Here we describe the ability of six recombinant antibodies (RB792, RB793, RB794, RB795, RB796 and RB797) to detect by ELISA a synthetic biotinylated phosphorylated peptide from the NDC80 protein.

Materials & Methods

Antibodies: ABCD_RB792, ABCD_RB793, ABCD_RB794, ABCD_RB795, ABCD_RB796 and ABCD_RB797 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding scFv portion fused to a human IgG1 Fc (HRB792, HRB793, HRB794, HRB795, HRB796 and HRB797). 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 scFv-Fc of each antibody. Supernatants (~80 to 100 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to residues 38 to 51 of the NDC80 protein, which includes a phosphorylated serine residue at position 44 (PTFGKLpSINKPTSE). The non-phosphorylated version of the same N-biotinylated peptide (PTFGKLSINKPTSE) 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 HRB 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-human IgG (BioRad #1721050, 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 RB792, RB793, RB794, RB795, RB796 and RB797 bound in a concentration-dependent manner to the phosphorylated NDC80 peptide against which they were raised, but not to the non-phosphorylated control peptide (Fig. 1). Although these antibodies recognized specifically the S44 phosphorylated NDC80 peptide by ELISA, their ability to bind the full-length protein should be determined in future experiments.

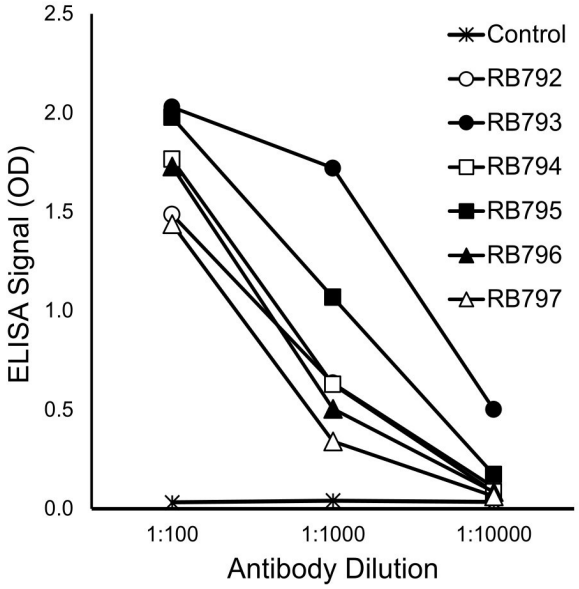

Fig. 1. Specific binding of HRB antibodies to the target S44 phosphorylated NDC80 peptide, as detected by ELISA. 'Control' indicates the binding of HRB792 to the non-phosphorylated control peptide (all other control curves were superimposed).

References

DeLuca JG, Musacchio A. Structural organization of the kinetochore-microtubule interface. Curr Opin Cell Biol. 2012 Feb;24(1):48-56. PMID: 22154944

Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.