RB702, RB703, RB704 and RB705 antibodies recognize a human ROR1 peptide by ELISA

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Abstract

The recombinant antibodies RB702, RB703, RB704 and RB705 detect by ELISA a synthetic peptide from the ROR1 protein.

Introduction

Tyrosine-protein kinase transmembrane receptor ROR1, also known as neurotrophic tyrosine kinase, receptor-related 1 (NTRKR1) is a receptor tyrosine kinase that plays an essential role in embryogenesis and is overexpressed in many types of malignant tumors (Borcherding *et al*., 2014). Here we describe the ability of four recombinant antibodies (RB702, RB703, RB704 and RB705) to detect by ELISA a synthetic biotinylated peptide from the ROR1 protein.

Materials & Methods

Antibodies: ABCD_RB702, ABCD_RB703, ABCD_RB704 and ABCD_RB705 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 VHH portion fused to a human IgG1 Fc (HRB702, HRB703, HRB704 and HRB705). 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 (~30 – 100 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to amino acid residues 185-198 of ROR1 (UniProt #Q01973, RTVYMESLHMQGEI). As a negative control, an irrelevant N-biotinylated peptide (THSPNHNFQDDYHE) from ROR2 protein (UniProt # Q01974) 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 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 RB702, RB703, RB704 and RB705 bound in a concentration-dependent manner to the ROR1 peptide against which they were raised, but not to the negative control peptide (Fig. 1).

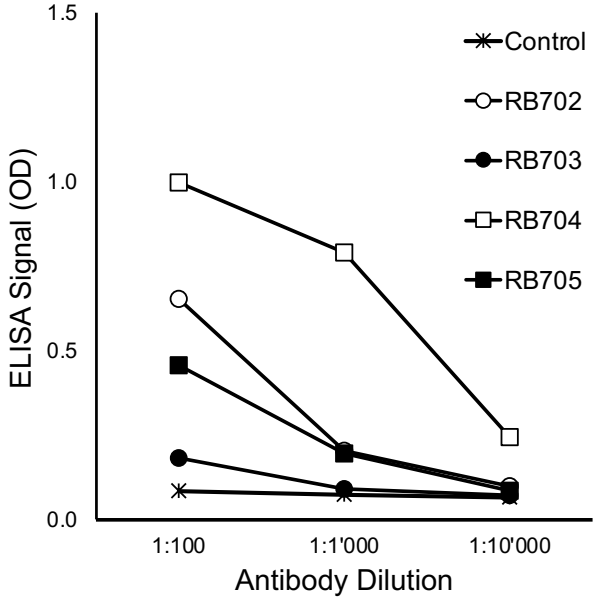

Fig. 1. Specific binding of HRB antibodies to the target ROR1 peptide, as detected by ELISA. 'Control' indicates the binding of HRB702 to the negative control peptide (all other control curves were superimposed).

References

Borcherding, Nicholas, David Kusner, Guang-Hui Liu, and Weizhou Zhang. 2014. "ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology." Protein & Cell 5 (7): 496–502. https://doi.org/10.1007/s13238-014-0059-7.

Conflict of interest

The authors declare no conflict of interest.