RB252, RB253, RB254 and RB255 antibodies recognize the human Miner1/CISD2 protein by ELISA

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Abstract

The recombinant antibodies RB252, RB253, RB254 and RB255 detect by ELISA the human Miner1/CISD2 fused to a GST protein.

Introduction

Miner1/CISD2 (CDGSH iron-sulfur domain-containing protein 2, MitoNEET-related 1 protein, UniProt #Q8N5K1) is an integral protein of the endoplasmic reticulum. Here we describe the ability of four recombinant antibodies (RB252, RB253, RB254 and RB255) to detect by ELISA a GST-fused Miner1 protein.

Materials & Methods

Antibodies: ABCD_RB252, ABCD_RB253, ABCD_RB254 and ABCD_RB255 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 IgG2A Fc (MRB252, MRB253, MRB254 and MRB255). HEK293T cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~5 mg/L) were collected after 3 days.

Antigen: The antibodies were originally raised against a GST protein fused to the 75 C-terminal residues of Miner1 protein (61-135), corresponding to the entire presumptive cytosolic domain of the protein. This chimeric GST-Miner1 protein was used as antigen for ELISA detection. GST was used as negative control.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies MRB252, MRB253, MRB254 and MRB255 bound in a concentration-dependent manner to the GST-Miner1 antigen, but not to the GST negative control (Fig. 1).

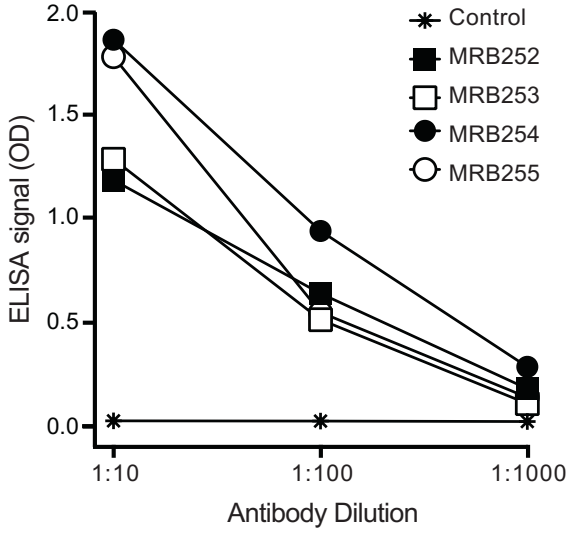

Fig. 1. Specific binding of MRB antibodies to the target GST-Miner1 protein, as detected by ELISA. 'Control' indicates the binding of MRB252 to GST (all other control curves were superimposed).

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

The authors declare no conflict of interest.