# *RB250 and RB251 antibodies recognize the human MitoNEET/CISD1 protein by ELISA*

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## Abstract

The recombinant antibodies RB250 and RB251 detect by ELISA the human MitoNEET/CISD1 fused to a GST protein.

## Introduction

MitoNEET/CISD1 (CDGSH iron-sulfur domain-containing protein 1, UniProt #Q9NZ45) is a human transmembrane protein localized in the outer mitochondrial membrane (Vernay *et al.*, 2017). Here we describe the ability of two recombinant antibodies (RB250 and RB251) to detect by ELISA a GST-fused MitoNEET protein.

## Materials & Methods

**Antibodies:** ABCD_RB250 and ABCD_RB251 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 fused to a mouse Fc (MRB250 and MRB251). 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 almost full-length MitoNEET protein (missing the initial 31 residues). This chimeric GST-MitoNEET 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 MRB250 and MRB251 bound in a concentration-dependent manner to the GST-MitoNEET antigen, but not to the GST negative control (Fig. 1).

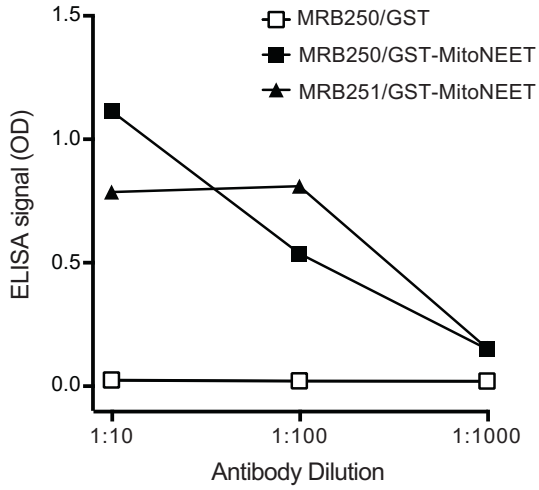


**Fig. 1.** Specific binding of MRB antibodies to the target GST-MitoNEET protein, but not to GST (shown only for MRB250; MRB251 background curve is superimposed), as detected by ELISA.

## References

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## Conflict of interest

The authors declare no conflict of interest.