

RB712 and RB713 antibodies recognize the Spargel protein by ELISA

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Abstract

The recombinant antibodies RB712 and RB713 detect by ELISA the *Drosophila melanogaster* Spargel protein (or GCH) fused to a GST protein.

Introduction

Drosophila melanogaster Spargel protein (UniProt #Q8IPM1) is involved in mitochondria biogenesis. Spargel is a homolog of PGC-1 α , a human protein involved in Parkinson disease (Merzetti and Staveley 2015). Here we describe the ability of two recombinant antibodies (RB712 and RB713) to detect a GST-fused Spargel protein by ELISA.

Materials & Methods

Antibodies: ABCD_RB712 and ABCD_RB713 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc (MRB712 and MRB713). HEK293 suspension cells (growing in HEK TF medium, Xcell#861-0001, supplemented with 0.1% Pluronic F68, Sigma#P1300) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20 - 100 mg/l) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the RNA recognition motif (RRM, amino acids 916 to 999) of *Drosophila melanogaster* Spargel protein. This chimeric GST-Spargel 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 μ l of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 μ l of RRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 μ l washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-rabbit IgG (Bio-Rad #170-6516, dilution 1:1000, 50 μ l per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB)

substrate (Sigma #T5569) was added (50 μ l per well). The reaction was stopped by the addition of 25 μ l of 2 M H₂SO₄. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RRB712 and RRB713 bound in a concentration-dependent manner to the GST-Spargel antigen, but not to the GST negative control (Fig. 1).

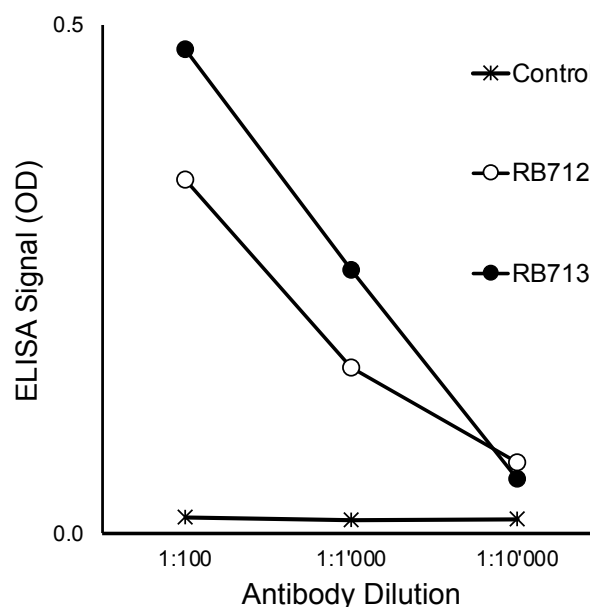


Fig. 1. Specific binding of RRB antibodies to the target GST-Spargel protein, but not to GST (shown only for MRB712; MRB713 background curve is superimposed), as detected by ELISA.

References

Merzetti, Eric M., and Brian E. Staveley. 2015. "Spargel, the PGC-1 α Homologue, in Models of Parkinson Disease in *Drosophila Melanogaster*." *BMC Neuroscience* 16 (1): 70. PMID: 26502946.

Conflict of interest

The authors declare no conflict of interest.