AI239, RB94 and RB95 antibodies recognize the Glutathione S-transferase protein by ELISA

Margaux Verdon, Sébastien Spedaliero, Maxime Richard, Cyril Pirek, Orlane L. Maxit, Arec Manoukian, Mélissa Longepierre, Alexandra Laszlo, Sara Hussami, Mustafa Haraj, Boris R. Gueorguiev, Teresa Maria Giusti, Vincent Gaspoz, Elisa Domingos, Alessia G. D'Esposito, Aurélie Cino, David Celeny, Eliott Bosshard, David Arsimoles, Ilhan Ameti, Monica Bulla, Cyril Guilhen

Bachelor en Sciences Biomédicales, Département de Physiologie Cellulaire et Métabolisme, Faculté de Médecine, CMU, 1211 Genève 4

Abstract

The recombinant antibodies AI239, RB94 and RB95 detect by ELISA the Glutathione S-transferase (GST) protein.

Introduction

Glutathione S-transferase (GST) (Uniprot #P08515) is an enzyme often used to purify GST-fused recombinant proteins. High affinity binding of GST to its glutathione substrate allows easy purification. Three recombinant antibodies (AI239, RB94 and RB95) detect the GST protein by ELISA; two (AF209, AF212) do not, presumably due to the fact that these antibodies are poorly produced.

Materials & Methods

Antibodies: ABCD_RB094, ABCD_RB095, ABCD_AI239, ABCD_AF209 and ABCD_AF212 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/; Lima *et al.*, 2019) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) for AI239, AF209 and AF212 correspond to the sequences of the variable regions of the VHH, VHii and VHG64 clones (Lin *et al.*, 2018; O'Brien *et al.*, 1999) joined by a peptide linker $(GGGGS)_3$. RB94 and RB95 were originally selected against a GST fusion protein (Blanc *et al*., 2014). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium; Gibco, #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (for RB94, RB95, AI239, ~100 mg/L) were collected after 4 days; production of AF209 and AF212 was undetectable in this system, indicating a low production yield (<5 mg/L).

Antigen: *E. coli* AVB101 bacteria expressing a GST protein (220 amino acids) fused to an N-terminal biotinylation tag (GLNDIFEAQKIEWHE) (pAN4-GST vector) (Blanc *et al.*, 2014) were used to produce the GST protein.

Protocol: The whole procedure was carried out at room temperature. Biotinylated BSA or whole bacterial lysates containing biotinylated GST proteins were incubated in a glutathione-coated 8-well plate (Pierce #15120) 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 antibody-containing supernatant diluted in washing buffer as indicated (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 5 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.

Results

Antibodies AI239, RB94 and RB95 bound in a concentration-dependent manner to the GST protein, but not to the BSA negative control (Fig. 1). AF209 and AF212 did not recognize GST by ELISA; this is most probably due to the fact that these antibodies are poorly produced.

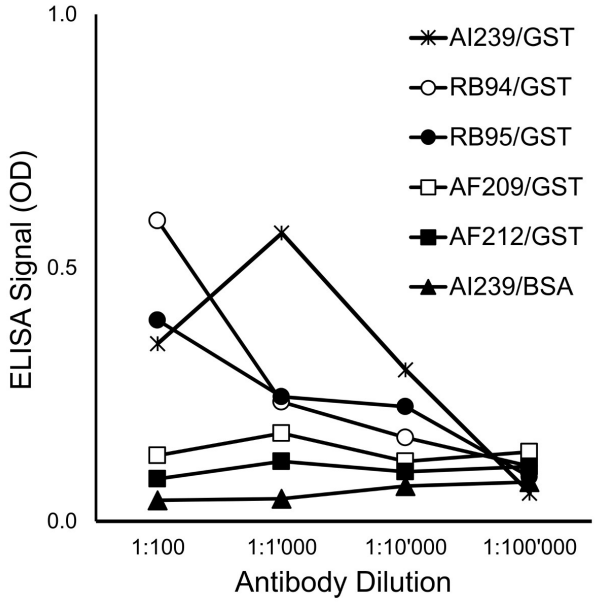

Fig. 1. AI239, RB94 and RB95 bind specifically to the GST protein, but not to the BSA control (shown only for AI239; RB94, RB95, AF209 and AF212 background curves are superimposed), as detected by ELISA.

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Conflict of interest

The authors declare no conflict of interest.