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

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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). 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 H2SO4. The absorbance (OD) was measured at 450 nm.

Results
Antibodies AI239, RB94 and RB95 bind 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.

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.