RB94, RB95, AI239 and AF209 antibodies recognize the Glutathione S-transferase protein by Western blot

Otmane Lamrabet

Cell Physiology and Metabolism Dpt, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The recombinant antibodies RB94, RB95, AI239 and AF209 detect by Western blot the Glutathione S-transferase (GST) protein; AF212 does not.

Introduction

Glutathione S-transferase (GST) (Uniprot #P08515) is an enzyme often used to purify GST-fused recombinant proteins, given its ability to bind its glutathione substrate with high affinity. Four recombinant antibodies (RB94, RB95, AI239 and AF209) detect the GST protein by Western blot; one (AF212) does not.

Materials & Methods

Antibodies: RB94, RB95, AI239 and AF209 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 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; AF209 and AF212 have a low production yield in this system (<5 mg/L).

Antigen: *E. coli* AVB101 bacteria expressing a GST protein (220 amino acids) fused to an N-terminal biotinylation tag (GLNDIFEAQKIEWHE) were used to detect the GST-tag protein.

Protocol: Recombinant protein expression was induced in *E. coli* bacteria growing exponentially (OD_{600}, 0.5) at 37°C (in Luria-Bertani (LB) medium containing 100 µM ampicillin) by addition of 1 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (PBS + 1% Triton X100 + aprotinin 10 µg/ml + leupeptin 20 µg/ml + iodoacetamide 1.8 mg/ ml + PMSF 18 µg/ml) and lysed in the presence of 1 g of Lysing Matrix B beads (MP Biomedicals #6540-428) using a bead beater (Fastprep 5G, MP Biomedicals). GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 200 µl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 20 µL of each sample was migrated (200 V, 30 min) in a 4-20% acrylamide gel (SurePAGE Bis-Tris, Genscript #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked during 2 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed three times for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (dilution 1:10 in PBS-Tween), overnight at 4 °C, then washed three times in PBS-Tween for 15 minutes. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences, #WBLUC0500) using a PXi-4 gel imaging systems (Syngene).

Results

Antibodies RB94, RB95 and AF209 specifically recognize the GST protein (Fig. 1). The signal obtained with AI239 is very weak. AF212 does not recognize GST; this is most probably due to the fact that it is poorly produced.

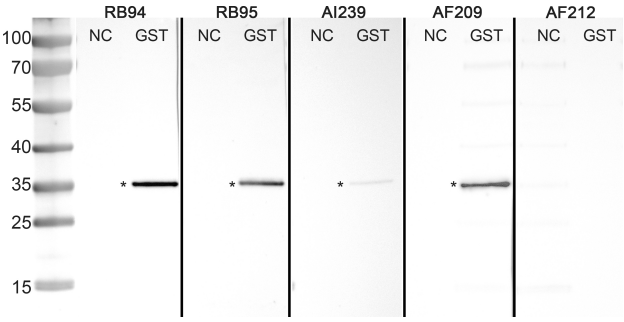

Fig. 1. Specific binding of GST antibodies to the GST protein. GST was successfully detected by RB94, RB95, AI239 and AF209 (positions indicated by asterisks), but not by AF212. No band was observed in non-transfected *E. coli* bacteria (negative control, NC).

References

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Conflict of interest

The authors declare no conflict of interest.