AF394, AF395, AF397, AK142, AK652, AN193 and AV441 antibodies label the GFP protein by western blot

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Abstract
The recombinant antibodies AF394, AF395, AF397, AK142, AK652, AN193 and AV441 detect the GFP protein by western blot.

Introduction
The green fluorescent protein (GFP) (Uniprot P42212) is a large (~235 aa) protein tag, widely used as a fluorescent reporter to detect and visualize GFP-fused proteins (Tsien, 1998). Here, we described the ability of 7 recombinant antibodies to detect the GFP protein by western blot. Three other tested antibodies (AF396, AN712 and AR464) did not.

Materials & Methods
Antibodies: ABCD_AF394, ABCD_AF395, ABCD_AF396, ABCD_AF397, ABCD_AK142, ABCD_AK652, ABCD_AN193, ABCD_AN712, ABCD_AR464 and ABCD_AV441 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding portion fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions joined by a peptide linker $(GGGGS)_3$ (see Table 1 for clone names and references). 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 (see Table 1 for individual yields) were collected after 4 days.

Table 1: Clone number, reference and production yields for the antibodies used in this study.

ABCD	Clone	Reference	Yield (mg/L)
AF394	GBP1	Kirchhofer *et al* ., 2010	120
AF395	GBP4		80
AF396	VHH	Rothbauer *et al* ., 2006	100
AF397	LaG-2	Fridy *et al* ., 2014	140
AK142	cAbGFP4	Saerens *et al* ., 2008	<5
AK652	GBP2	Pellis *et al* ., 2012	120
AN193	3G86.32	Brauchle *et al* ., 2014	60
AN712	3C3/64	Cosson, personal communication	100
AR464	B9	Fang *et al* ., 2020	120
AV441	N86/20	Andrews *et al* ., 2019	20

Antigen: *Klebsiella pneumoniae* bacteria transformed with a GFP-encoding plasmid were used to detect the GFP protein. Non-transformed bacteria (WT) were used as a negative control.

Protocol: 5 µL of overnight cultured *Klebsiella pneumoniae* bacteria (WT or transformed with the GFP-encoding plasmid) were pelleted, lysed in 20 µL of 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) and boiled at 95°C for 5 minutes. 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 min (iBlot gel transfer device, Invitrogen IB1001EU). The membranes were blocked overnight in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk and washed three times for 5 min in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with the recombinant antibodies (5 mg/L in PBS-Tween-milk) for 30 min at room temperature and washed three times for 5 min. The membranes were then incubated for 30 min with horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich A8275, dilution 1:3000 in PBS-Tween-milk) and washed 5 times for 5 min in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging system (Syngene).

Results
Antibodies AF394, AF395, AF397, AK142, AK652, AN193 and AV441 recognized the GFP protein expressed in *Klebsiella pneumoniae* bacteria by western blot (Fig. 1). One band was observed at approximatively 35 kDa, which is slightly higher than the expected theoretical size (27 kDa). This may be due to post-translational modifications, such as glycosylation, which also occur in bacteria (Nothaft and Szymanski, 2010). No signal was detected in WT bacteria (Fig. 1). In these experimental conditions, a very weak signal was obtained with the antibody AR464, and no signal was detected with antibodies AF396 and AN712. Data obtained with AF394, AF395 and AF396 are consistent with previously published data (Lamrabet, 2019).

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Conflict of interest

The authors declare no conflict of interest.

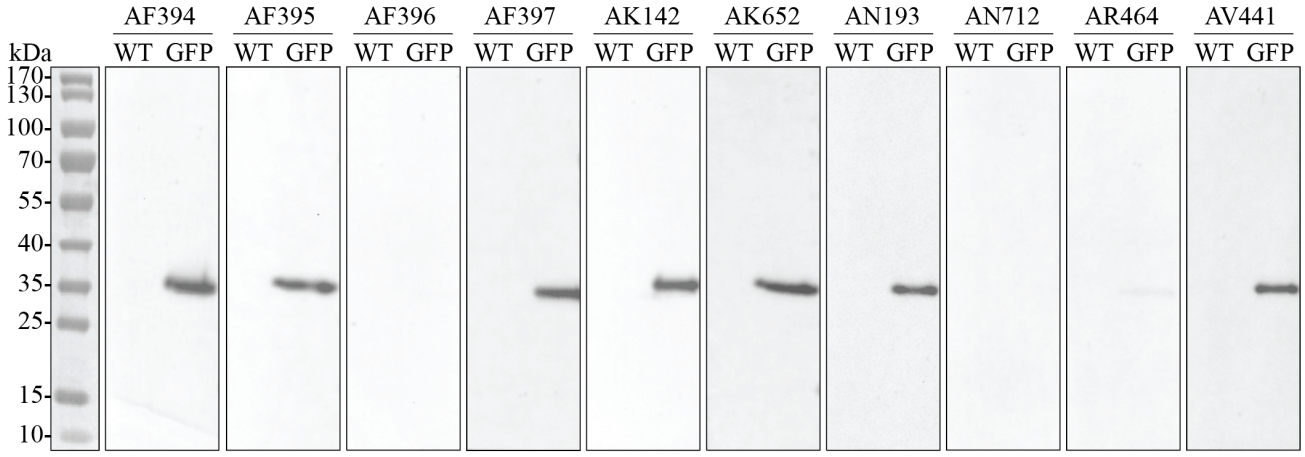

Fig. 1. Specific binding of AF394, AF395, AF397, AK142, AK652, AN193 and AV441 antibodies to the GFP protein in *Klebsiella pneumonia* harboring a GFP-encoding plasmid (GFP). No band was observed in non-transformed (WT) bacteria. A very weak signal was obtained with the antibody AR464, and no signal was obtained with AF396 and AN712.