

AG275, AG294 and AG274 antibodies recognize the hen egg-white lysozyme by ELISA

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Abstract

The recombinant antibodies AG275, AG294 and AG274 detect the hen egg-white lysozyme by ELISA.

Introduction

Lysozyme is an antibacterial enzyme widely distributed in vertebrates, invertebrates, plants, and microbes. It is also found in a large variety of animal secretions and tissues such as saliva, mucus, or avian eggs, and it is secreted by polymorphonuclear leukocytes among other cells (Mason and Taylor, 1975). Lysozyme hydrolyzes the 1,4-beta-linkages between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of Gram-positive bacteria (Chipman and Sharon, 1969). Three recombinant antibodies (AG275, AG294 and AG274) detect the hen egg-white lysozyme (HEWL) (Uniprot P00698) by ELISA. Two other tested antibodies (AE913 and AE915) did not.

Materials & Methods

Antibodies: ABCD_AE913, ABCD_AE915, ABCD_AG274, ABCD_AG275 and ABCD_AG294 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 (GGGS)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)
AE913	L2,5	Davies <i>et al.</i> , 1995	100
AE915	L1		10
AG274	D44.1	Braden <i>et al.</i> , 1994	5
AG275	HyHEL-63	Li <i>et al.</i> , 2000	70
AG294	D1.3	Holmes <i>et al.</i> , 1998	80

Protocol: The whole procedure was carried out at room temperature. HEWL (5 µg/mL; Sigma L6876) was incubated in high protein-binding capacity 96-well plates (50 µL/well) (Thermo Fisher Scientific 44-2404) for 30 minutes. The support was blocked 20 minutes in PBS containing 4% (w/v) BSA. Each well was rinsed three times with 100 µL of washing buffer (PBS + 0.1% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 30 minutes 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-rabbit IgG (Sigma A8275, 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 AG275, AG294 and AG274 bound in a concentration-dependent manner to the HEWL, but not to the BSA negative control (Fig. 1). AE913 and AE915 did not recognize the HEWL by ELISA.

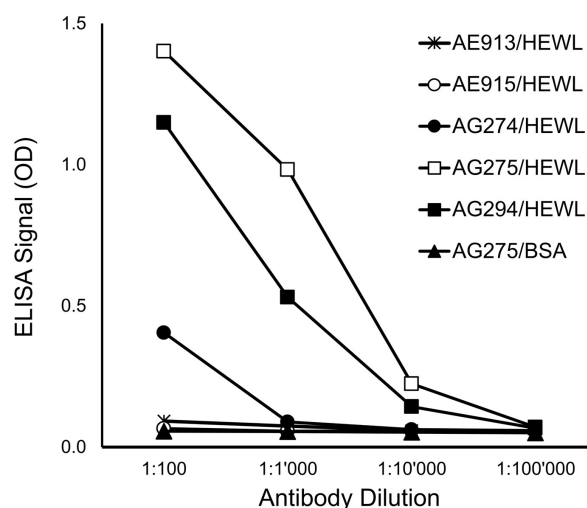


Fig. 1. AG275, AG294 and AG274 bound specifically to the hen egg-white lysozyme (HEWL), but not to the BSA control (shown only for AG275; the other background curves were superimposed), as detected by ELISA.

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Conflict of interest

The authors declare no conflict of interest.