

# The pemphigus monoclonal antibody clone P5G6 (AX353) retains cell-surface staining by immunofluorescence and desmoglein binding by ELISA in an engineered scFv-Fc format

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## Abstract

The recombinant human antibody clone P5G6 (AX353) in an engineered scFv-Fc format detects by immunofluorescence cell-surface proteins on cryosections of monkey esophagus. The same antibody detects by ELISA recombinant human desmoglein 3 and desmoglein 1 protein.

## Introduction

The cadherins desmoglein (Dsg) 3 and Dsg1 (UniProt P32926 and Q02413) are structural transmembrane proteins mediating cell-cell adhesion in epidermis and mucous membranes (Hammers and Stanley, 2016). Binding of anti-Dsg antibodies to the extracellular domains can result in direct loss of adhesion and pemphigus disease. Here we describe a recombinant monoclonal scFv-Fc antibody previously cloned as a full IgG1 antibody from a human pemphigus patient (Cho *et al.*, 2019). This antibody recognizes both Dsg3 and Dsg1, and could be a useful reagent in basic and translational research (Burmester *et al.*, 2019).

## Materials & Methods

**Antibody:** ABCD\_AX353 (ABCD nomenclature, <https://web.expasy.org/abcd/>) with NCBI GeneBank accession numbers MN037555 and MN037556, was produced by the Geneva Antibody Facility (<http://www.unige.ch/medecine/antibodies/>) as a mini-antibody with the antigen-binding scFv fused to a human IgG1 Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clone P5G6 from patient ISD102 (Cho *et al.*, 2019; sequences obtained by single-cell expression cloning after single-cell sorting) joined by a peptide linker (GGGS)<sub>3</sub>. In between the scFv and the human IgG1 Fc (H1), an 8His tag and a HA tag (YPYDVPDYA) were integrated.

HEK293 cells (growing in HEK TF medium, Xcell #861-0001, supplemented with 0.1% Pluronic F-68, Sigma-Aldrich #P1300) were transiently transfected with the CMV promoter-based, pCDM8-derived pWA vector coding for the scFv-Fc P5G6-H1. Supernatants were collected after 4 days; the antibody showed high production yields (~120 µg/mL) as determined by reducing SDS-PAGE and comparison with a BSA standard of known concentration.

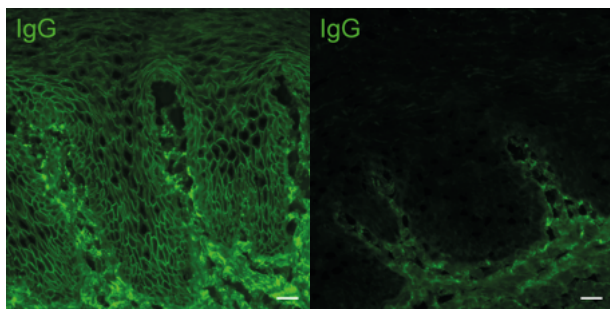
**Substrate and Antigens:** Monkey esophagus (obtained from the DPZ, Goettingen, Germany) was cryosectioned to 6 µm thickness and placed on Eprepia™ SuperFrost Plus™ glass slides for microscopy (Menzel, Braunschweig, Germany), serving as substrate for immunofluorescence. Dsg3/1-coated microplate wells were obtained from EUROIMMUN (Luebeck, Germany; #EA1496-4801G and #EA1495-4801G) as part of commercial ELISA kits.

**Protocol:** For immunofluorescence, consecutive dilutions of P5G6-H1 (stock concentration ~120 µg/mL) were prepared with PBS (1:10 to 1:40,960). Dilutions were incubated with esophagus sections for 30 min. After 2 washes in PBS (10 min each), sections were incubated with anti-human-IgG-FITC (Werfen, Barcelona, Spain; #504033) diluted 1:100 in PBS for 30 min. After 2 washes in PBS (10 min each), sections were mounted with 20% (v/v) glycerin in PBS, and pictures were taken using an OLYMPUS BX40 microscope (Olympus, Tokyo, Japan) and a Gryphax® NAOS camera with corresponding software (Jenoptic, Jena, Germany). For ELISA, two protocols were followed in parallel, with the same dilution series of P5G6-H1. Samples were diluted 1:10, 1:100, 1:1,000, and 1:10,000 in 1% (w/v) BSA/TBS/2 mM CaCl<sub>2</sub>. The first ELISA protocol followed the manufacturer's instructions, except for sample dilution (see above) and development: Dilutions were incubated on Dsg3/1 microplate wells for 60 min at RT, washed thrice (wash buffer from EUROIMMUN kits), then incubated for 60 min at RT with anti-IgG-HRP conjugate (ready-to-use, EUROIMMUN, #AE122-0112). After 3 washes, the ELISA was developed with TMB/H<sub>2</sub>O<sub>2</sub> chromogen and stopped by the addition of 0.5 M H<sub>2</sub>SO<sub>4</sub> stop solution after 3 min (all solutions from kits). Absorbance (OD) was measured at 450 nm - 620 nm reference wavelength. The second ELISA protocol made use of the HA-tag incorporated into the engineered protein, allowing for detection by an anti-HA-HRP conjugate. After incubation of above dilutions on Dsg3/1 microplate wells for 60 min at RT and three washes (wash buffer from EUROIMMUN kits), incubation with anti-HA-HRP clone 3F10 (Roche, #12013819001, stock concentration 25 U/mL, diluted 1:1,000 in 1% (w/v) BSA/TBS/2 mM CaCl<sub>2</sub>) for 60 min at RT followed. After three washes, the ELISA was developed with TMB/H<sub>2</sub>O<sub>2</sub> chromogen and stopped by the addition of 0.5 M H<sub>2</sub>SO<sub>4</sub> stop solution after 3 min (all solutions from EUROIMMUN kits). Absorbance (OD) was measured at 450 nm - 620 nm reference wavelength.

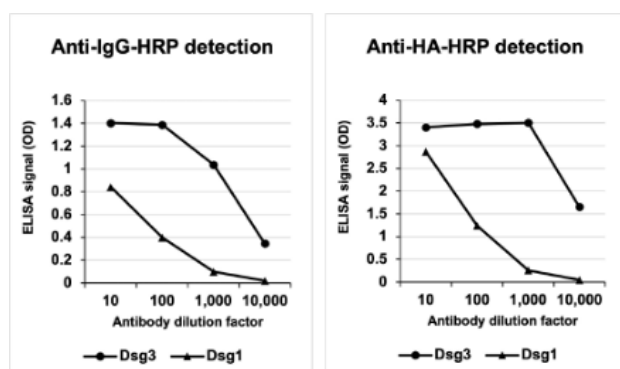
## Results

Immunofluorescence titration on monkey esophagus resulted in a titer of 1:10,240 for the scFv-Fc antibody P5G6-H1. The fluorescence pattern resembled that of polyclonal pemphigus serum antibodies, i.e., fishnet-like intercellular staining of epithelial cells (Fig. 1).

With regard to the ELISA testing, P5G6-H1 bound in a concentration-dependent manner to the antigens Dsg3 and Dsg1 (Fig. 2). Differences in OD readings for the development by anti-IgG-HRP and anti-HA-HRP conjugates may result from varying concentrations of these reagents (proprietary for the anti-IgG-HRP conjugate from EUROIMMUN, 25 mU/mL for the anti-HA-HRP conjugate from Roche). The successful detection via ELISA of Dsg3/1-bound P5G6-H1 via its HA tag confirms that the engineered recombinant antibody format retained its binding specificity, and it will allow for multiple experimental approaches, including immunofluorescence, using the HA tag in future experimentation (the 8His tag was not specifically tested in this work).



**Fig. 1.** P5G6-H1 antibody (left panel, 1:20 dilution) intercellularly labelled monkey esophagus epithelial cells that express desmosomal cadherins ortholog to human desmogleins by immunofluorescence. A negative control staining is shown in the right panel. Scale bar: 25 µm.



**Fig. 2.** P5G6-H1 antibody binds to antigens Dsg3 and Dsg1 by ELISA and can be detected by both anti-IgG-HRP and anti-HA-HRP conjugates.

## References

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## Conflict of interest

The authors declare no conflict of interest.