RB599, RB600, RB601, RB602, RB603 and RB605 antibodies recognize a mouse Claudin-9 peptide by ELISA

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Abstract
The recombinant antibodies RB599, RB600, RB601, RB602, RB603 and RB605 detect by ELISA a synthetic peptide from the mouse Claudin-9 protein.

Introduction
Claudins are transmembrane proteins involved in tight junctions between epithelial cells (Tsukita *et al*., 2019). Here we describe the ability of six recombinant antibodies (RB599, RB600, RB601, RB602, RB603 and RB605) to detect by ELISA a synthetic biotinylated peptide from the mouse Claudin-9 protein (Uniprot #Q9Z0S7).

Materials & Methods
Antibodies: ABCD_RB599, ABCD_RB600, ABCD_RB601, ABCD_RB602, ABCD_RB603 and ABCD_RB605 nanobodies (ABCD nomenclature, http://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/). They were produced as mini-antibodies with the antigen-binding VHH portion fused to a mouse IgG2A Fc. HEK293 suspension cells (growing in HEK TF medium, Xell#861-0001, supplemented with 0.1% Pluronic F68, Sigma #P1300) were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (see Table 1 for individual yields) were collected after 4 days.

Name	*Yield (mg/L)*
RB599	30
RB600	30
RB601	20
RB602	50
RB603	10
RB605	30

Table 1: Production yields of the antibodies used in this study.

Antigen: The antibodies were raised against an N-biotinylated synthetic peptide corresponding to residues 189 to 217 (HFERPRGPRLGYSIPSRSGASGLDKRDY V) from the Claudin-9 protein (Uniprot #Q9Z0S7). An irrelevant N-biotinylated antimicrobial peptide LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTE S, UniProt #P49913) was used as a negative control.

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides (saturating concentration of 10 pmol/well) were immobilized on streptavidin-coated 8-well ELISA plate (Pierce #15120) for 30 min. 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-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 H_2SO_4. The absorbance (OD) was measured at 450 nm.

Results
Antibodies RB599, RB600, RB601, RB602, RB603 and RB605 bound in a concentration-dependent manner to the Claudin-9 peptide (CLD9), but not to the LL-37 peptide (Fig. 1). Although these antibodies recognize specifically the Claudin-9 peptide by ELISA, their ability to bind the full-length protein should be determined in future experiments.

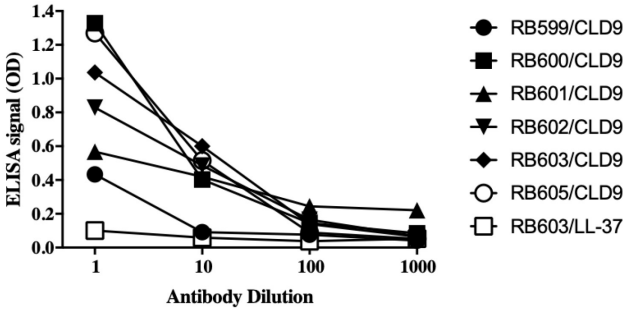

Fig. 1. RB599, RB600, RB601, RB602, RB603 and RB605 bound specifically to the Claudin-9 peptide (CLD9), but not to the negative control peptide (LL-37) (shown only for RB603; the other background curves were superimposed), as detected by ELISA.

References
Tsukita, S., Tanaka, H., and Tamura, A. (2019). The Claudins: From Tight Junctions to Biological Systems. Trends in Biochemical Sciences *44*, 141–152. PMID: 30665499.

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Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.

Tania Jauslin is an associate editor of the journal Antibody Reports.

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