RB217, RB218 and RB219 antibodies recognize Protoporphyrin IX by ELISA

Philippe Hammel, Norbert Lange

School of Pharmaceutical Sciences, University of Geneva, Rue Michel Servet 1, 1211, Geneva, Switzerland
Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Rue Michel Servet 1, 1211, Geneva, Switzerland

Abstract
The recombinant antibodies RB217, RB218 and RB219 detect protoporphyrin IX by ELISA.

Introduction
Protoporphyrin IX (PpIX, ChEBI 15430), a heterocyclic organic compound with four pyrrole rings, is the biosynthetic precursor of hemes and chlorophylls. Heme (ChEBI 30413) consists of PpIX with a Fe(II) center (Sitte and Senge, 2020). PpIX is also used as photosensitizer in photodynamic therapy (PDT) (Sansaloni-Pastor *et al.*, 2019). Here we describe the ability of three recombinant antibodies (RB217, RB218 and RB219) to detect PpIX by ELISA.

Materials & Methods
Antibodies: ABCD_RB217, ABCD_RB218 and ABCD_RB219 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies) as mini-antibodies with the antigen-binding scFv portion fused to a rabbit Fc. HEK293T cells (growing in DMEM GlutaMAXTM (Gibco 31966) supplemented with 8% Fetal Bovine Serum (Gibco 10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~5 mg/L) were collected after 3 days.

Antigen: The antibodies were raised against biotinylated protoporphyrin IX. As a control, biotinylated heme (PpIX with Fe^{2+}) and an irrelevant biotinylated peptide (`LWSFTPSKCSGPYGE`) were used.

Protocol: The whole procedure was carried out at room temperature. Biotinylated PpIX and heme at 4 µg/ml were immobilized on streptavidin-coated ELISA plates (Pierce 15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of antibody-containing supernatant diluted in washing buffer. 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 3 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, and the absorbance at 570 nm was subtracted.

Results
Antibodies RB217, RB218 and RB219 bound in a concentration-dependent manner to protoporphyrin IX (PP) (Fig. 1). Antibodies RB218 and RB219 also bound to heme, but not RB217 (Fig. 1). The antibodies did not bind to an irrelevant control peptide (Fig. 1).

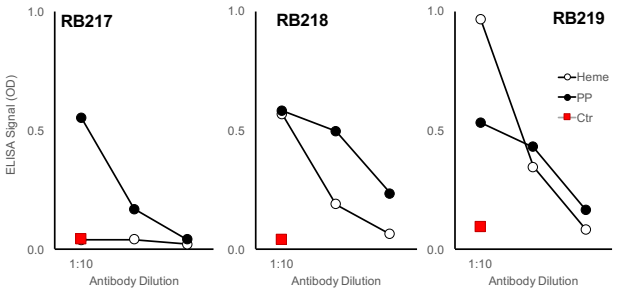

Fig. 1. Binding of RB antibodies to Protoporphyrin IX (PP), Heme and a control peptide (Ctr, values shown only for the 1:10 antibody dilution), as detected by ELISA.

References
Sansaloni-Pastor S, Bouilloux J, Lange N. The Dark Side: Photosensitizer Prodrugs. Pharmaceuticals (Basel). 2019; 12(4):148. PMID: 31590223.

Sitte E, Senge MO. The red color of life transformed - synthetic advances and emerging applications of protoporphyrin IX in chemical biology. European J Org Chem. 2020; 2020:3171-3191. PMID:32612451

Conflict of interest
The authors declare no conflict of interest.