# *RB198, RB199 and RB200 antibodies recognize a fragment of the hepatitis E virus ORF3 protein by ELISA*

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## Abstract

The recombinant antibodies RB198, RB199 and RB200 detect by ELISA a fragment of the hepatitis E virus ORF3 protein fused to a GST protein.

## Introduction

Hepatitis E virus (HEV) ORF3 protein (UniProt #E9N3C1) is a small palmitoylated protein required for the secretion of infectious viral particle (Gouttenoire *et al*., 2018). Here we describe the ability of three recombinant antibodies (RB198, RB199 and RB200) to detect by ELISA a GST-fused fragment of the HEV (Kernow-C1 isolate, genotype 3) ORF3 protein.

## Materials & Methods

**Antibodies:** ABCD_RB198, ABCD_RB199 and ABCD_RB200 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB198, MRB199 and MRB200). HEK293 adherent cells (growing in DMEM, Gibco #11960044 supplied with 8%FBS) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~1-5 mg/l) were collected after 5 days.

**Antigen:** The antibodies were originally raised against a GST protein fused to the residues 62-113 (QPTPSPPISFHNPGLELALGSRPAPLAPLGVTSPSAPPLPPAVDLPQLGLRR) of the HEV ORF3 protein. This chimeric GST-ORF3 was used as antigen for ELISA detection. GST was used as negative control.

**Protocol:** The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 MRB antibody-containing supernatant diluted in washing buffer (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 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 RB198, RB199 and RB200 bound in a concentration-dependent manner to the GST-ORF3 antigen, but not to the GST negative control (Fig. 1). RB198 and RB200 recognize the native antigen in HEV-infected cells by immunofluorescence and immunoblotting (Gouttenoire *et al*., 2018).

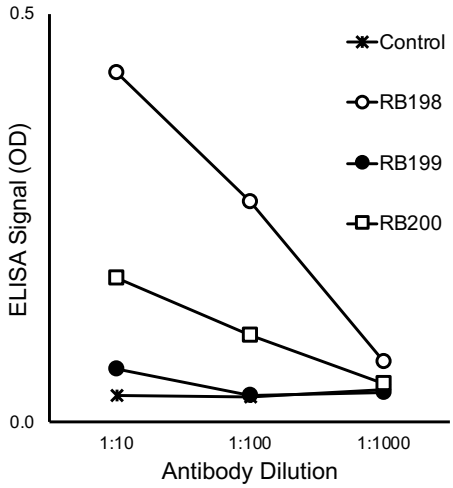


**Fig. 1.** Specific binding of RB antibodies to the target GST-ORF3 protein, as detected by ELISA. 'Control' indicates the binding of RB198 to GST (all the other background curves are superimposed).

## References

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## Conflict of interest

The authors declare no conflict of interest.