RB196 and RB197 antibodies recognize a fragment of the RdRp region of the hepatitis E virus ORF1 protein by ELISA

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Abstract

The recombinant antibodies RB196 and RB197 detect by ELISA a fragment of the RdRp region of the hepatitis E virus ORF1 protein fused to a GST protein.

Introduction

Hepatitis E virus (HEV) ORF1 protein (UniProt #H9E9C7) is the viral replicase responsible for the neosynthesis of viral RNA genomes during infection (Debing *et al.*, 2016). Here we describe the ability of two recombinant antibodies (RB196 and RB196) to detect by ELISA a GST-fused fragment of the RdRp region of the HEV (Kernow-C1 isolate, genotype 3) ORF1 protein.

Materials & Methods

Antibodies: ABCD_RB196 and ABCD_RB197 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 (MRB196 and MRB197). 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 1414-1474 (QATTCELYELVEAMVEKGQDGSAVLELDLCNRDVSRITFFQKDCNKFTTGETIAHGKVGQG) of the HEV ORF1 polyprotein. This chimeric GST-HEVRdRp 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 RB196 and RB197 bound in a concentration-dependent manner to the GST-HEVRdRp antigen, but not to the GST negative control (Fig. 1).

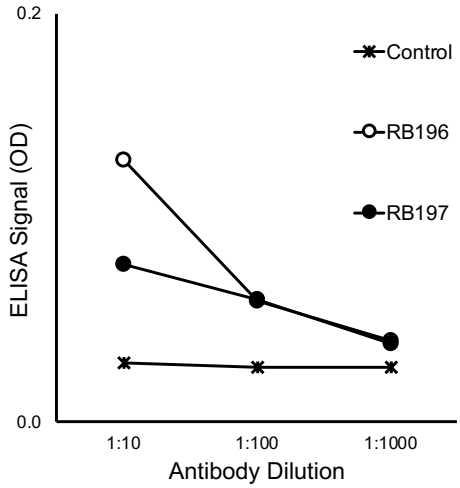

Fig. 1. Specific binding of RB antibodies to the target GST-HEVRdRp protein, as detected by ELISA. 'Control' indicates the binding of RB196 to GST (RB197 curve is superimposed).

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Conflict of interest

The authors declare no conflict of interest.