**RB191, RB192, RB193, RB194 and RB195 antibodies recognize a fragment of the MeT-Y region of the hepatitis E virus ORF1 protein by ELISA**

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**Abstract**

The recombinant antibodies RB191, RB192, RB193, RB194 and RB195 detect by ELISA a fragment of the MeT-Y 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 five recombinant antibodies (RB191, RB192, RB193, RB194 and RB195) to detect by ELISA a GST-fused fragment of the MeT-Y region of the HEV (Kernow-C1 isolate, genotype 3) ORF1 protein.

**Materials & Methods**

**Antibodies:** ABCD_RB191, ABCD_RB192, ABCD_RB193, ABCD_RB194 and ABCD_RB195 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 (MRB191, MRB192, MRB193, MRB194 and MRB195). 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 51-102 (QPRQLVFRPEVLWHPQVIHNELEQYCRARAGRCLEVGAHPR3INP25) of the HEV ORF1 polyprotein. This chimeric GST-HEVMeT-Y was used as antigen for ELISA detection. GST was used as a 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$_2$SO$_4$. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

**Results**

Antibodies RB191, RB192, RB193, RB194 and RB195 bound in a concentration-dependent manner to the GST-HEVMeT-Y antigen, but not to the GST negative control (Fig. 1).

**Fig. 1.** Specific binding of RB antibodies to the target GST-HEVMeT-Y protein, as detected by ELISA. ‘Control’ indicates the binding of RB191 to GST (all other control curves were superimposed).

**References**

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547


**Conflict of interest**

The authors declare no conflict of interest.