Recombinant antigen E for *West Nile virus* (WNV)

CATALOG NUMBER: RAG0065 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: West Nile virus envelope antigen (Beasley *et al*., 2004).

DESCRIPTION: the structural domain III of the WNV envelope glycoprotein has been prepared as a recombinant antigen fused to a his-tag at the C-terminal. It is produced from the envelope protein of the West Nile virus.

PRESENTATION: liquid protein solution

SOURCE: *Pichia pastoris*

MOLECULAR WEIGHT: SDS-PAGE analysis determines that the protein band is between the molecular markers of 18,400 Da and 14,400 Da due to the glycosylation pattern, while relative molecular mass, calculated from amino acid sequence and without glycosylation, is 14,676.4 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-E	recombinant antigen with a his-tag in its C-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.1 M KCl and 5mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.17
$A_{0.1\,\%}$ (=1 g/l) = 0.789
CONCENTRATION*: 1.48 mg/ml

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and vonHippel, 1989. It is recommended that the users carry out their absorbance determinations to avoid equipment variabilities regarding final concentration, mainly in reproducibility analysis.

2. PURITY CONTROL IN SDS-PAGE: 15 %

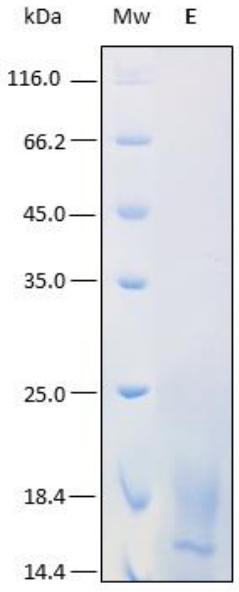

Figure 1. SDS-PAGE analysis (15%) of 10 µl of recombinant E. Purity is > 95% as determined by gel electrophoresis.

3. POSITIVE AND NEGATIVE SERA DISCRIMINATION BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech over the first lot obtained. ELISA assays were performed with two IgG positive pre-validated serum samples obtained from Biomnis and 5 negative specimen sera from general population.

Each end user should carry out his own titration for his particular application.

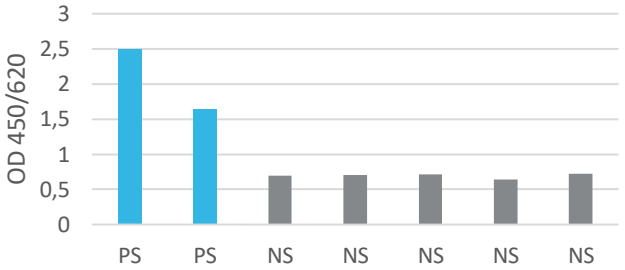

Figure 2. This plot shows an indirect IgG ELISA assay performed with the DIIIE recombinant antigen. The optical density at 450/620 nm for positive sera (light blue) and negative sera (gray) are displayed in the graphic. **For specimen discrimination sera, the coating was performed with 1 µg/ml of protein in microtiter plates, 12.5 µg of anti-CCD sorbent (Ref. Rekom SOR0001) was added to the sera 10 min before incubation with the coated antigen.**

4. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.48 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.708 ml

4. SUGGESTED TITER BY ELISA: up to 1:1,480, which corresponds to 1 µg/ml of protein concentration in plates for IgG detection.

5. STORAGE: Protein is shipped with dry ice. Upon arrival, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation.

6. TESTED APPLICATIONS: ELISA.

7. POSIBLE APPLICATIONS: WB, DB, Indirect ELISA, positive control in direct ELISA, CLIA, lateral-flow. Where this product has not been tested for use in a particular technique, this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. **Due to the nonspecific interaction with anti-CCDs present in normal human or animal sera, we strongly recommend using 12.5 µg of our sorbent SOR0001 on the analyzed sera in any immunoassay.** It is recommended that the user titrates.

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8. OBSERVATIONS: proteins should be maintained frozen at high concentrations. The dilution to be performed for ELISA assays should be made with a small quantity of protein, the same day of the experiment. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation. Prior making test dilutions and after defrosting the protein, is recommended to remove possible protein aggregates by centrifuging the stock solution, avoiding alterations in the immobilization of the biomolecule to the solid surface.

RELATED PRODUCTS:

E (RAG0001).

BIBLIOGRAPHY:

Beasley *et al*., 2004. Use of a recombinant envelope protein subunit antigen for specific serological diagnosis of West Nile virus infection. *The Journal of Clinical Microbiology,* 42:2759-2765.

Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. *Anal Biochem.* 1989 Nov 1;182(2):319-26.

Important Notes: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µl or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the containers cap.

Although recombinant antigens are expressed in non-pathogenic *P. pastoris* and bacterial integrity is destroyed during purification, the antigen preparation should be handled as potentially infectious.

FOR RESEARCH AND COMMERCIAL USE *IN VITRO*: not for human *in vivo* or therapeutic use.

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