

Recombinant antigen E for West Nile virus (WNV)

CATALOG NUMBER: RAG0001

LOT NUMBER: #

RECOMBINANT ANTIGEN: West Nile virus antigen E (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 N-terminal. It is produced from the envelope protein of the West Nile virus.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 25,000-18,400 Da, while relative molecular mass calculated from amino acid sequence is 19,582.7 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-E	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl, 0.1% polyoxyethylene (10) tridecyl ether and 6 M urea

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 1.01
 $A_{0.1\%}$ (=1 g/l) = 0.585
 CONCENTRATION*: 1.762 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 %

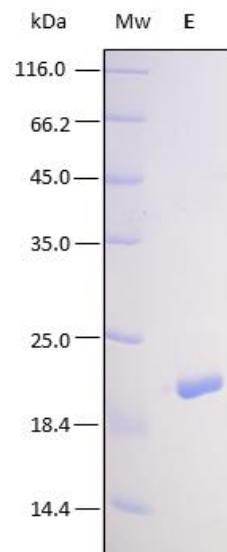


Figure 1. SDS-PAGE analysis (15%) of 2 µl of recombinant E. Purity is > 98% as determined by gel electrophoresis.

3. TITRATION CURVE 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.

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

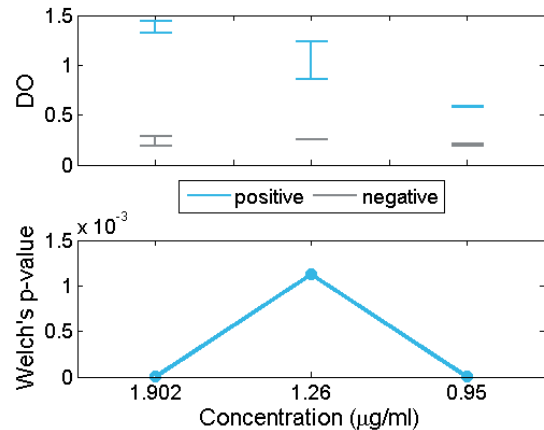


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. Eligible concentrations for the use of the antigen should present statistically significant differences between positive and negative sera. This happens when the intervals at the top do not overlap and, equivalently, when the p-value at the bottom is below 0.05. In the present figure, all p-values are below 0.05 and thus the intervals do not overlap. Therefore, any of the showed concentrations can be used to distinguish between positive and negative sera.

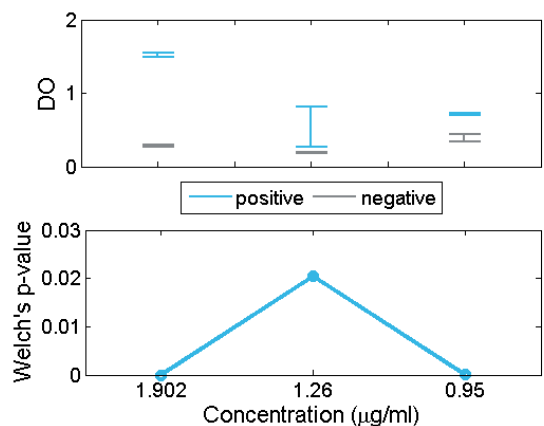


Figure 3. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. Eligible concentrations for the use of the antigen should present statistically significant differences between positive and negative sera. This happens when the intervals at the top do not overlap and, equivalently, when the p-value at the bottom is below 0.05. In the present figure, all p-values are below 0.05 and thus the intervals do not overlap. Therefore, any of the showed concentrations can be used to distinguish between positive and negative sera.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.762 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.608 ml

4. SUGGESTED TITER BY ELISA: up to 1:1,855, which corresponds to 0.95 µg/ml of protein concentration in plates for IgG and IgM 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 -20°C to -80°C. In order to defrost the protein, maintain the aliquot at 25°C without shaking to avoid aggregation.

6. TESTED APPLICATIONS: ELISA.

7. POSSIBLE APPLICATIONS: WB, DB, Indirect ELISA, positive control in direct ELISA. 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. It is recommended that the user titrates.

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 25°C 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. This protein is highly prone to micro-aggregation in absence of 6 M urea, therefore we strongly recommend using fresh protein dilutions in ELISA assay for the protein coating in the microtiter plates. Avoid using storage protein solutions at 4°C in absence of 6 M urea. As the pI of the protein is 7, we recommend using carbonate buffer for ELISA plates coating. **If you put the protein on ice and a precipitate appears, it can be urea crystals. In such a case, it would help if you put the protein at room temperature to dissolve the crystals.**

RELATED PRODUCTS:

E (RAG0065).

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 *E. coli* 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.