Recombinant antigen domain III from envelope protein (EDENV4) for dengue virus serotype 4.

CATALOG NUMBER: RAG0070 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: envelope protein domain III or B domain for dengue virus serotype 4 (Ludolfs *et al*., 2002).

DESCRIPTION: the recombinant antigen EDENV4 is formed by a highly dengue virus type-specific antigenic side, the B domain or domain III, from glycoprotein E of dengue virus serotype 4.

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 14,000 Da due to the glycosylation pattern, while relative molecular mass, calculated from amino acid sequence and without glycosylation, is 14,614.41 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-EDENV4	recombinant antigen with a his-tag in its C-terminus
Storage buffer	20 mM phosphate buffer pH 7, 0.15 M NaCl, 0.25 M trehalose and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.976
$A_{0.1\,\%}$ (=1 g/l) = 0.793
CONCENTRATION*: 1.23 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%

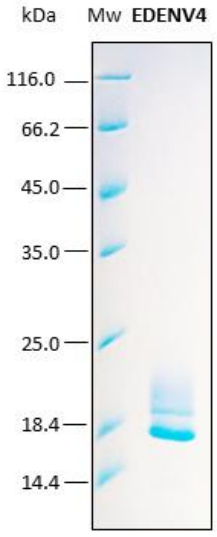

Figure 1. SDS-PAGE analysis (15%) of 10 µl of recombinant EDENV4. Purity is > 95% as determined by gel electrophoresis.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.23 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.853 ml

4. 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.

5. TESTED APPLICATIONS: none.

6. 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. It is recommended that the user titrates. **Due to the nonspecific interaction with anti-CCDs present in normal human or animal sera, we strongly recommend using of our blocker SOR0001 on the analyzed sera in any immunoassay.**

7. 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:

None.

BIBLIOGRAPHY:

Ludolfs, D., Schilling, S., Altenschmidt, J., and H. Schmitz. Serological Differentiation of Infections with Dengue Virus Serotypes 1 to 4 by Using Recombinant Antigens. 2002. Journal of Clinical Microbiology, p. 4317–4320

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

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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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