Recombinant multi-epitope chimeric pepsin inhibitor precursor (ChimDiT33) for *Dirofilaria immitis*

CATALOG NUMBER: RAG0014 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: recombinant chimeric antigen DiT33 for *D. immitis* (Hong *et al.,* 1996)

DESCRIPTION: a recombinant multi-epitope chimera formed by different antigenic determinants of pepsin inhibitor precursor DiT33 for *D. immitis* has been prepared as a recombinant protein fused to a His-tag in its N-terminus.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 35,000-45,000 Da, while relative molecular mass calculated from amino acid sequence is 38,435.9 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
trxA-his-ChimDiT33	recombinant chimeric antigen with a trxA-tag and a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.95
$A_{0.1\,\%}$ (=1 g/l) = 0.781
CONCENTRATION*: 1.21 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%

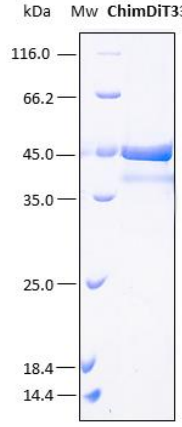

Figure 1. SDS-PAGE analysis (15%) of 4 µl of recombinant ChimDiT33. Purity is > 95% as determined by gel electrophoresis. The two fragments correspond to this same protein as it is showed in a western blot performed with a his-tag monoclonal antibody

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.21 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.867 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.

7. OBSERVATIONS: in some cases, purified proteins run at a molecular weight which is slightly different to the theoretically calculated molecular weight maybe due to the his-tag presence or sequences of hydrophobic residues, which can produce a delay in SDS-PAGE. Proteins should be maintained frozen at high concentration. The dilution to be performed for carrying out an ELISA assays, should be freshly-made with a small quantity of protein. 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:

Hong, X., Santiago M., Kumar, S., Perler, F. and Carlow, C. Cloning and expression of DiT33 from Dirofilaria immitis: a specific and early marker of heartworm infection. *Journal Parasitology*. 1996;112(3):331-338.

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 *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

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