Recombinant antigen B13 for *Trypanosoma cruzi*

CATALOG NUMBER: RAG0103 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Trypanosoma cruzi* antigen B13 (Umezawa *et al.*, 2003).

DESCRIPTION: the *T. cruzi* antigen B13 has been prepared as a recombinant antigen fused to a his-tag in its N-terminal. It corresponds to the truncated *T. cruzi* surface antigen 2 (CA-2). Also known as Ag2, PEP2, TcR34.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): Polyclonal antibody for *Trypanosoma cruzi* (Rekom Biotech catalog reference PAB0007)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 35,000-25,000 Da, while relative molecular mass calculated from amino acid sequence is 22.532,8 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-B13	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl, 5 mM EDTA and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.33
$A_{0.1\%}$ (=1 g/l) = 0.31
CONCENTRATION*: 1.07 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%

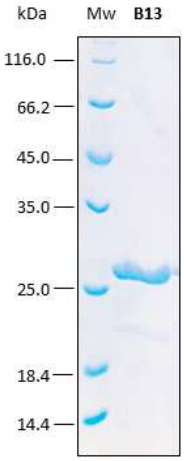

Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant B13. Purity is > 98% as determined by gel electrophoresis. Bands which slightly appear at approx. 20 and 16 kDa correspond to this same protein as it is showed in a western blot performed with a his-tag monoclonal antibody

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. ELISA assays were performed with a serum sample panel of 25 positive-specimen sera and 5 negative specimen sera, validated by chimioluminiscence (Architect, Abbot) and obtained from the Andalusian Public health System Biobank.

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

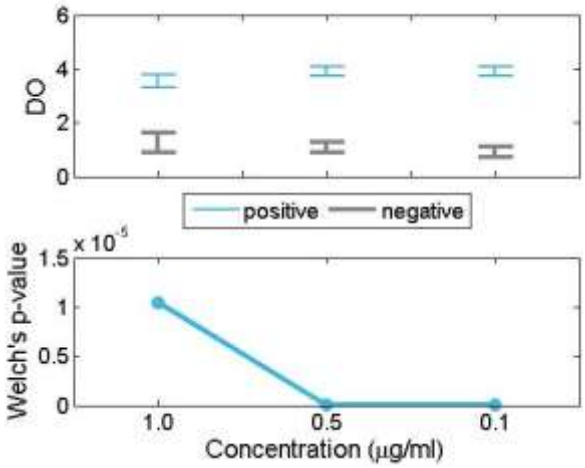

Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgG** sera for Chagas disease are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's 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: 1.07 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.981 ml

4. SUGGESTED TITER BY ELISA: up to 1:10,700, this corresponds to 0.1 µg/ml of antigen 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.

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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. It is recommended that the user titrates.

8. 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:

1F8, FRA, ChimChagas1, ChimChagas2, ChimChagas3, ChimChagas3-Biot.

BIBLIOGRAPHY:

Umezawa, E.S., Bastos, S.F., Coura, J.R., Levin, M.J., González A., Rangel-Aldao, R., Zingales, B., Luquetti, A.O., and da Silveira, J.F. An improved serodiagnostic test for Chagas´disease employing a mixture of Trypanosoma cruzi recombinant antigens. 2003, *Transfusion* 43:91-97.

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.

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