# Recombinant biotinylated multi-epitope chimeric antigen ChimChagas2 for *Trypanosoma cruzi*

**CATALOG NUMBER:** RAG0094BIOT **LOT NUMBER:** #

**RECOMBINANT ANTIGEN:** recombinant biotinylated multi-epitope chimeric antigen for Chagas.

**DESCRIPTION:** the recombinant biotinylated multi-epitope chimeric antigen for Chagas has been prepared as a chimeric protein formed by several antigenic regions from some antigens of this parasite and monobiotinylated *in vivo*.

**PRESENTATION:** dry powder (stabilized with 5% trehalose)

**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 116,000-66,200 Da, while relative molecular mass calculated from amino acid sequence is 59,913.57 Da.

**BATCH COMPOSITION:**

| COMPONENTS | COMPOSITION |
|---|---|
| his-ChimChagas2 | recombinant biotinylated chimeric antigen with a his-tag in its N-terminus |
| Storage buffer before lyophilisation | 20 mM phosphate buffer pH 8.5, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether |

**QUALITY CONTROL:**

**1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY**

$DO_{280}$ = 0.21
$A_{0.1\,\%}$ (=1 g/l) = 0.148
CONCENTRATION*: 1.40 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%**

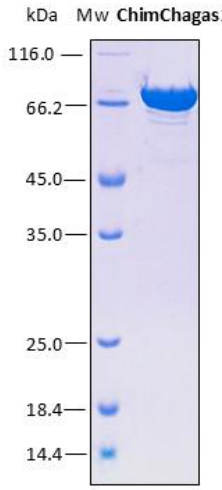


**Figure 1.** SDS-PAGE analysis (15%) of 3 µl of recombinant biotinylated ChimChagas2. Purity is > 95% as determined by gel electrophoresis.

**3. DISCRIMINATION OF PRE-VALIDATED SERA BY AN INDIRECT ELISA ASSAY**

The cut-off has been suggested about an "in-house" ELISA kit performed in Rekom Biotech.

Each end-user should carry out an analysis for their particular application.

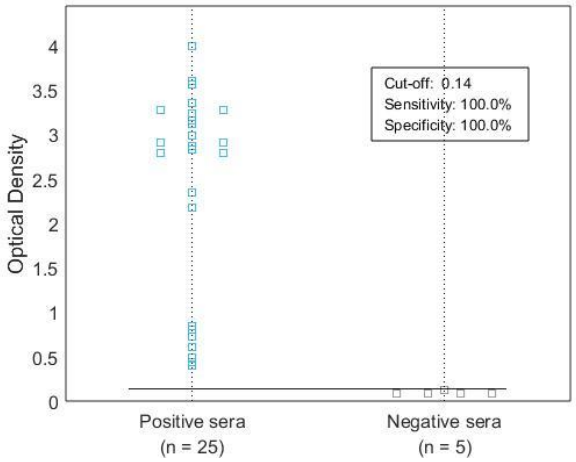


**Figure 2.** The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with a protein coating of 0.025 µg/ml. Pre-validated sera by chemiluminescence (Abbott Architect) with confirmatory test by immunofluorescence, were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.



Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company

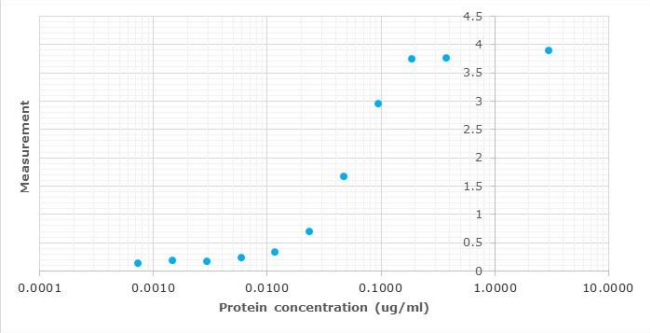


**Figure 3.** Binding capacity to a streptavidin-coated plate. The plate was coated with streptavidin at 2.5 µg/ml; increasing amounts of the monobiotinylated protein were attached, and the system was developed using the calibrator (2 µg/ml) and an anti-rabbit IgG labelled with HRP. As seen in this ELISA assay, the reactivity of the protein can be adjusted to a 4PL, reaching its maximum at a protein concentration on the plate of 0.1 µg/ml.

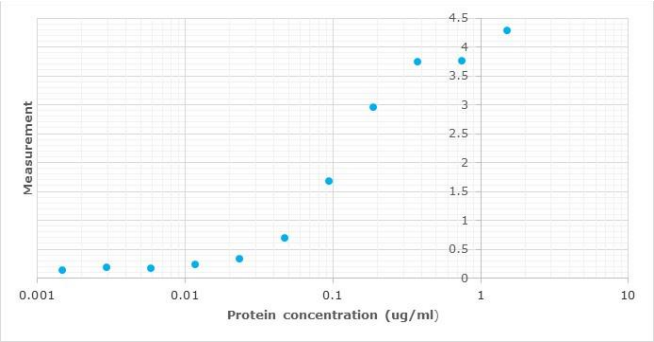


**Figure 4**. Capacity to develop the assay. For this purpose, an anti-rabbit IgG was used to coat the plate (2.5 µg/ml), capturing the calibrator (2 µg/ml). Subsequently, increasing amounts of monobiotinylated protein were used in the assay, revealing the same by using peroxidised streptavidin at a dilution of 1:1000. The reactivity of the protein can be adjusted to a 4PL, reaching its maximum at a protein concentration on the plate of 0.4 µg/ml.

**4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION**

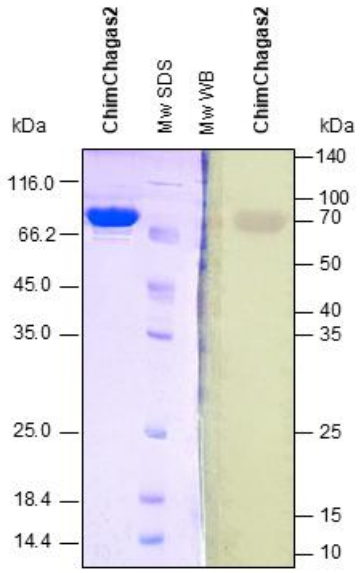


**Figure 5.** Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

**LOT SPECIFICATIONS:**

**1. RECONSTITUTION:** with 0.654 ml of sterile double-distilled water, a final concentration of 1.40 mg/ml will be obtained. The solubilisation of the cake should be developed for 15 min to allow a homogeneous protein solution, considering that part of the cake can be on the glass-walls of the container. Please keep in mind that the final volume of the reconstituted protein solution may differ from the reconstitution volume mentioned in the instructions due to the hygroscopic nature of trehalose. As the protein is reconstituted, the final volume may slightly increase to reach the specified amount mentioned in the certificate of analysis. Upon reconstitution, leave the solution at least 15 min homogenizing with a mild agitation at 4ºC. Avoid vigorous shaking that can cause foaming and protein denaturation. After those minutes, centrifuge the vial to ensure that all the product remains at the base and do not lose any of it on the walls. With this reconstitution, the protein will be maintained at pH 8.5. It is recommended that the users carry out their absorbance determinations to avoid concentration variabilities due to the equipment used, mainly in reproducibility analysis.

**2. TOTAL QUANTITY PER ALIQUOT:** 1 mg

**3. SUGGESTED TITER BY ELISA:** up to 1:56,000, which corresponds to 0.025 µg/ml of protein concentration in plates, for IgG detection in an indirect ELISA assay.

**4. STORAGE:** Protein is shipped at room temperature. Upon arrival, it should be stored at 4° to -20°C in vertical position, avoiding all possible humidity and maintaining the vials dry. Once reconstituted, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC.

**5. TESTED APPLICATIONS:** ELISA.

**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.

**RECOMMENDED MATCHED ANTIGEN PAIRS:**

CAPTURE: RAG0094
DETECTION: RAG0094BIOT



Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company

**RELATED PRODUCTS:**

1F8, B13, FRA, FRA-Biot, ChimChagas1, ChimChagas2, ChimChagas3, ChimChagas3-Biot.

**BIBLIOGRAPHY:**

**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.**



Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company