

# Recombinant chimeric antigen ChimChagas1 for *Trypanosoma cruzi*

**CATALOG NUMBER:** RAG0093

**LOT NUMBER:** #

**RECOMBINANT ANTIGEN:** recombinant chimeric antigen for Chagas.

**DESCRIPTION:** the recombinant chimeric antigen for Chagas has been prepared as a chimeric protein formed by several antigenic regions from some antigens of this parasite.

**PRESENTATION:** liquid protein solution

**SOURCE:** *Escherichia coli*

**MOLECULAR WEIGHT:** determined by SDS-PAGE, the protein band is between molecular markers of 66,200-45,000 Da, while relative molecular mass calculated from amino acid sequence is 58,334.2 Da.

**BATCH COMPOSITION:**

COMPONENTS	COMPOSITION
his-ChimChagas1	recombinant chimeric 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

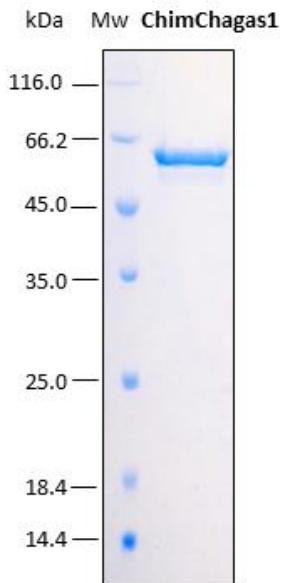
**QUALITY CONTROL:**

**1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY**

DO<sub>280</sub> = 1.03  
 A<sub>0.1%</sub> (=1 g/l) = 0.458  
 CONCENTRATION\*: 2.24 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%**

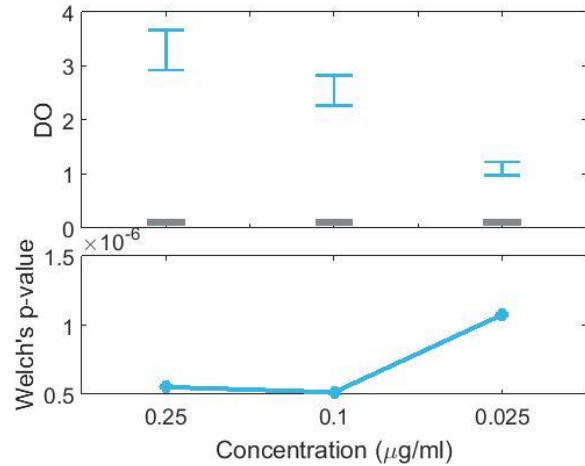


**Figure 1.** SDS-PAGE analysis (15%) of 3 µl of recombinant ChimChagas1. Purity is > 98% as determined by gel electrophoresis.

**3. TITRATION CURVE BY AN ELISA ASSAY**

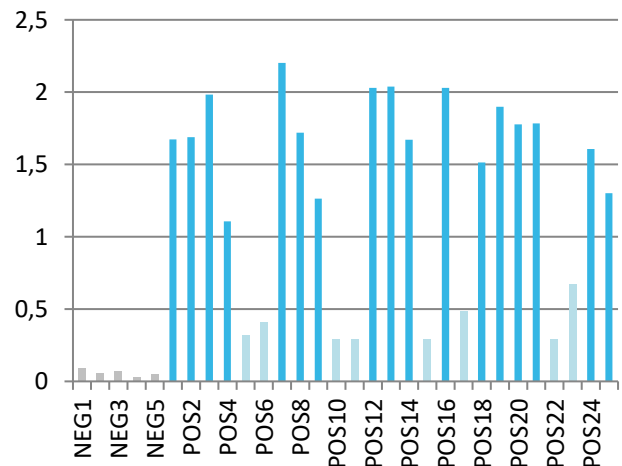
The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech over the first lot obtained. ELISA assays were performed with a serum sample panel of 25 positive- specimen sera and 5 negative specimen sera, pre-validated by a chemiluminescence test (Architect, Abbot) and 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. POSITIVE AND NEGATIVE SERA DISCRIMINATION BY AN ELISA ASSAY**



**Figure 3.** This plot shows an indirect IgG ELISA assay performed with the ChimChagas1 recombinant antigen. The optical density at 450/620 nm for high-positive sera (dark blue), low-positive sera (light blue) and negative sera (gray) are displayed in the graphic.

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

**LOT SPECIFICATIONS:**

**1. CONCENTRATION:** 2.24 mg/ml

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

**3. TOTAL VOLUME PER ALIQUOT:** 0.446 ml

**4. SUGGESTED TITER BY ELISA:** up to 1:89,600, which corresponds to 0.025 µg/ml of protein concentration in plates for IgG detection in an indirect ELISA assay.

**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, 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 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.

**RELATED PRODUCTS:**

1F8, FRA, B13, 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.**