

Recombinant biotinylated antigen ChimToxo2 for *Toxoplasma gondii*

CATALOG NUMBER: RAG0012BIOT

LOT NUMBER: #

RECOMBINANT ANTIGEN: recombinant biotinylated chimeric antigen for *Toxoplasma gondii*.

DESCRIPTION: the *T. gondii* antigens GRA7 and GRA8 have been prepared as a recombinant antigen fused to a TrxA-his-tag in its N-terminal.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 66,200 and 116,000 Da, while relative molecular mass calculated from amino acid sequence is 66,828.43 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
trx-his-ChimToxo2	recombinant chimeric antigen with a trx and a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 6, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 0.54
 A_{0.1%} (=1 g/l) = 0.479
 CONCENTRATION*: 1.12 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: 12%

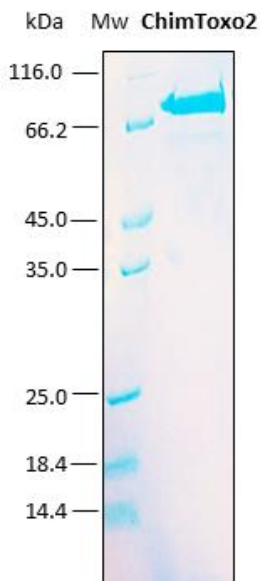


Figure 1. SDS-PAGE analysis (12%) of 4 µl of recombinant ChimToxo2. Purity is approx. 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

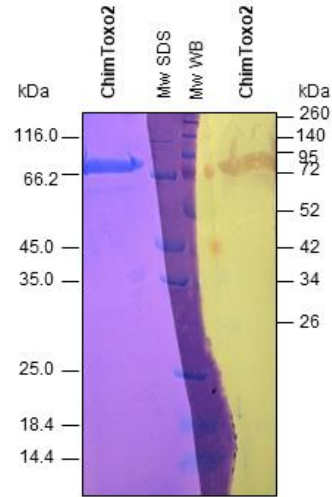


Figure 3. Western blot analysis to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

4. POSITIVE AND NEGATIVE SERA DISCRIMINATION 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 assay was performed with a serum sample panel of 13 IgG-positive specimen sera and 3 negative sera pre-validated by LIAISON® Toxoplasmosis IgG (DiaSorin).

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

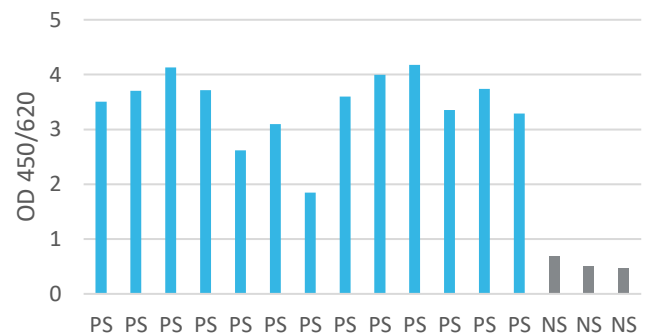


Figure 2. This plot shows an indirect IgG ELISA assay performed with the ChimToxo2 recombinant antigen. The optical density at 450/620 nm for positive sera (pale blue) and negative sera (gray) are displayed in the graphic.

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

LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 1.12 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.937 ml
- 4. SUGGESTED TITER BY ELISA:** up to 1:1,120, which corresponds to 1 µg/ml of protein 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 -20°C to -80°C. Upon defrosting, leave the solution at least 15 min homogenizing with a mild agitation at 4°C.

6. TESTED APPLICATIONS: ELISA.

7. POSSIBLE APPLICATIONS: WB, DB, Capture ELISA, nanoparticles binding, plate orientation. 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: 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 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:

p29 (GRA7), p30 (SAG1), p35 (GRA8), ChimToxo1, ChimToxo2.

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.