

Recombinant antigen p35 (GRA8) for *Toxoplasma gondii*

CATALOG NUMBER: RAG0084

LOT NUMBER: #

RECOMBINANT ANTIGEN: *T. gondii* antigen p35 (Lu et al., 2006).

DESCRIPTION: the *T. gondii* antigen p35 has been prepared as a recombinant antigen fused to a his-tag in its N-terminal. It corresponds to the *T. gondii* GRA8 gene.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): IgG polyclonal antibody against GRA7 and GRA8 (Rekom Biotech catalog reference PAB0005)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 35,000 and 25,000 Da, while relative molecular mass calculated from amino acid sequence is 21,757.5 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-p35	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 6, 0.15 M NaCl and 5 mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 1.528
 $A_{0.1\%} (=1 \text{ g/l}) = 1.053$
 CONCENTRATION*: 1.45 mg/ml

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and von Hippel, 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%

kDa Mw p35 (GRA8)

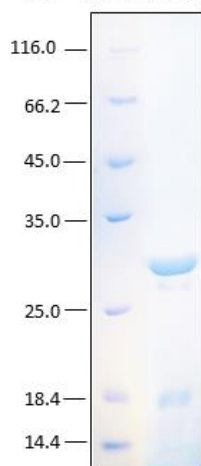


Figure 1. SDS-PAGE analysis (12%) of 2 µl of recombinant p35. Purity is approx. 95% as determined by gel electrophoresis. Band which slightly appear at approx. 16 kDa correspond to this same protein as it is showed in a western blot performed with a his-tag monoclonal antibody.

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.

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

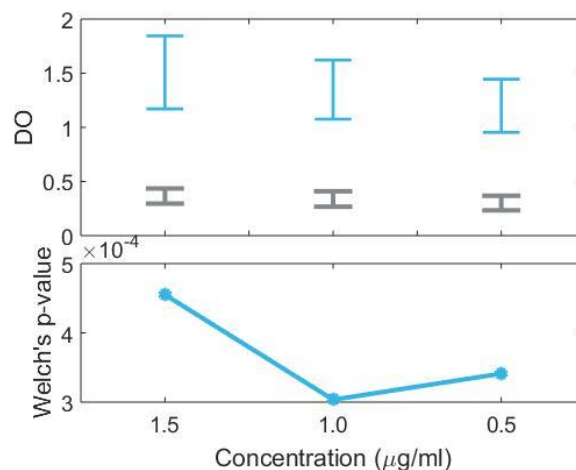


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) Toxo IgM sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. 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. 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.

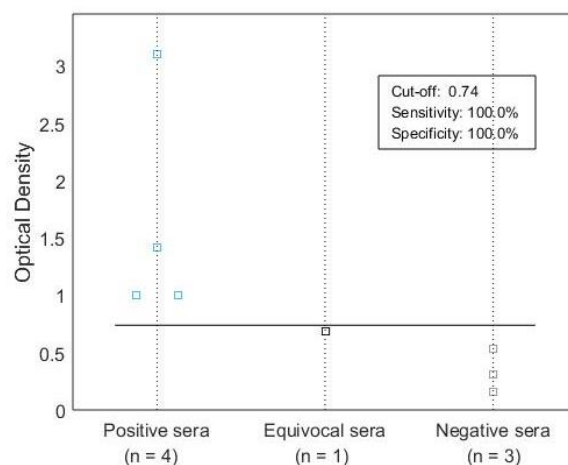


Figure 3. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with a p35 (GRA8) plate coating of 0.66 µg/ml. Pre-validated sera by Dx (IgG, LIAISON, Diasorin, CLIA) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.45 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.724 ml

4. SUGGESTED TITER BY ELISA: up to 1:2,900, which corresponds to 0.5 µg/ml of protein concentration in plates for IgM detection. Up to 1:2,197, which corresponds to 0.66 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. 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: 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), ChimToxo1

BIBLIOGRAPHY:

Lu, B., Wu, S., Shi, Y., Zhang R., Zou, L., Gao, S., Lin M. and Zhou Y. *Toxoplasma gondii*: expression pattern and detection of infection using full-length recombinant p35. 2006, *Exp. Parasitol.* 113:83-90.

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.