Recombinant antigen p30 (SAG1) for *Toxoplasma gondii*

CATALOG NUMBER: RAG0030 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *T. gondii* antigen p30 (Santoro *et al.*, 1986).

DESCRIPTION: the mature anchor-less antigen p30 of the *T. gondii* (SAG1) has been prepared as a recombinant antigen fused to a his-tag in its C-terminal.

PRESENTATION: liquid protein solution

SOURCE: *Pichia pastoris*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein appears in three different positions, with one band like a smear between molecular markers of 35,000-25,400 Da, another above the marker of 45,000 Da and a higher band around marker of 66,200 Da. The relative molecular mass calculated from amino acid sequence and without glycosylation is 30,361.23 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-p30	recombinant antigen with a his-tag in its C-terminus
Storage buffer	20 mM phosphate buffer pH 7, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.90
$A_{0.1\,\%}$ (=1 g/l) = 0.715
CONCENTRATION*: 1.26 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%

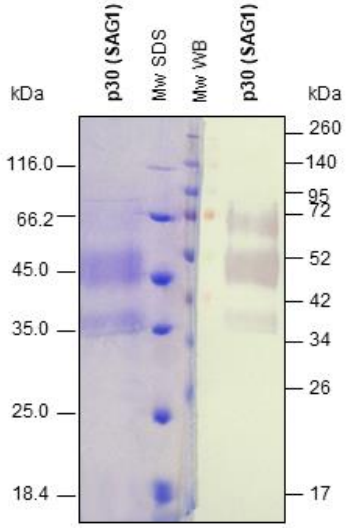

Figure 1. SDS-PAGE analysis (15%) of 5 µl of recombinant p30. Purity is > 95%, as determined by gel electrophoresis. Different bands are due to the heterogeneous glycosylation plus the dimerization capacity of this protein.

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.

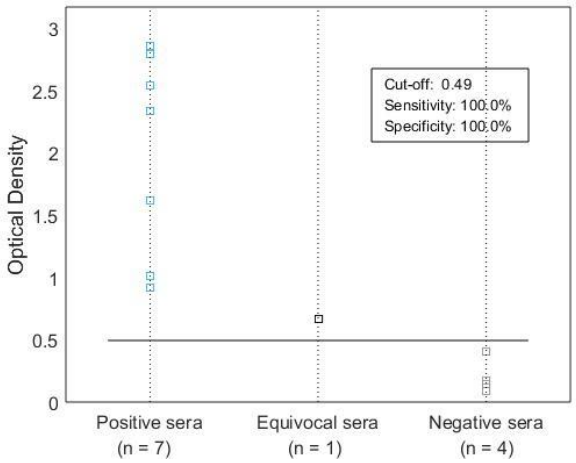

Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with a ChimToxo1 plate coating of 1 µg/ml. Pre-validated sera by Dx (IgG, LIAISON, Diasorin, CLIA) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera. **For specimen discrimination sera, 8 µg of anti-CCD blocker (Ref. Rekom SOR0001, see COA) was added to the sera 10 min before incubation with the coated antigen.**

4. WESTERN BLOT ANALYSIS

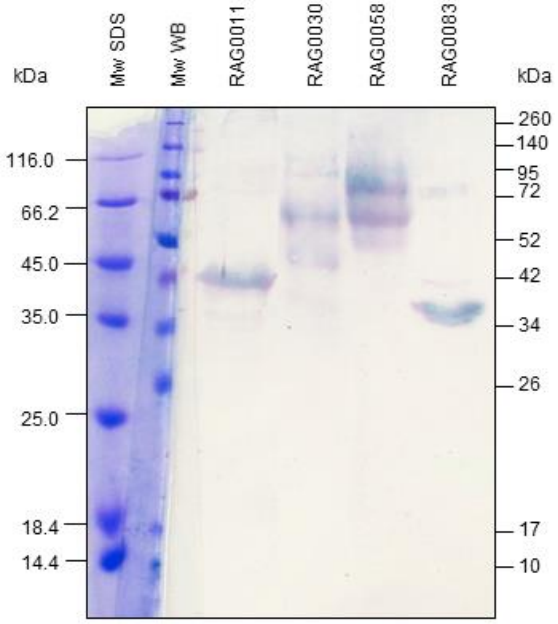

Figure 3. When a purified polyclonal Ab obtained by using as immunogen the clarified native extract of sporozoite oocysts of *Toxoplasma gondii* was used in a western blot analysis, clear signals were obtained in our recombinant antigens for *T. gondii* RAG0030 (SAG1), RAG0011 (SAG1), RAG0083 (GRA7) and RAG0058 (ChimToxo1).

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.26 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.833 ml

4. SUGGESTED TITER BY ELISA: up to 1:1,260, which corresponds to 1 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 in order 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 and western blot.

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. **Due to the nonspecific interaction with anti-CCDs present in normal human or animal sera, we strongly recommend using our blocker SOR0001 on the analyzed sera in any immunoassay.** 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 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:

p29 (GRA7), p30 (GRA7) (RAG0019, RAG0011), p35 (GRA8), ChimToxo1.

BIBLIOGRAPHY:

Santoro, F., Charif, H. and Capron, A. The immunodominant epitope of the major membrane techyzoite protein (p30) of *Toxoplasma gondii*. 1986, *Parasite Immunol.* 8:631-9.

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 *P. pastoris* 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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