

## Recombinant antigen p30 (SAG1) for *Toxoplasma gondii*

**CATALOG NUMBER:** RAG0019

**LOT NUMBER:** #

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

**DESCRIPTION:** an antigenic fragment of the *T. gondii* antigen p30 has been prepared as a recombinant antigen fused to a his-tag in its N-terminal. It corresponds to the *T. gondii* major surface antigen (SAG1).

**PRESENTATION:** liquid protein solution

**SOURCE:** *Pichia pastoris*

**MOLECULAR WEIGHT:** determined by SDS-PAGE, the protein band is like a smear between molecular markers of 18,400-25,000 Da, while relative molecular mass calculated from amino acid sequence and without glycosylation is 16,886.04 Da.

**BATCH COMPOSITION:**

COMPONENTS	COMPOSITION
his-p30	recombinant antigen with a his-tag in its C-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.1 M KCl and 0.1% polyoxyethylene (10) tridecyl ether

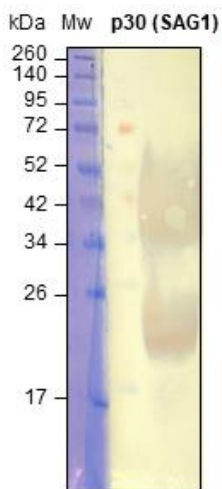
**QUALITY CONTROL:**

**1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY**

DO<sub>280</sub> = 1.38  
 A<sub>0.1%</sub> (=1 g/l) = 0.850  
 CONCENTRATION\*: 1.62 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

**2. IDENTITY CONTROL IN WESTERN BLOT**

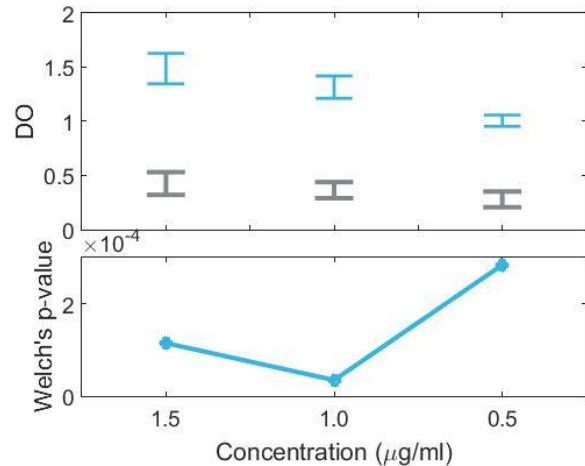


**Figure 1.** Western blot analysis of the recombinant p30 with a monoclonal antibody against His-tag. De signal which appears between markers of 52,000 and 34,000 Da corresponds to the homodimer of the protein, which is described in bibliography. The signal under de marker of 26,000 Da corresponds to the monomer.

**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 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) IgM sera for toxoplasmosis 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. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE:** ok

**LOT SPECIFICATIONS:**

- 1. CONCENTRATION:** 1.62 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.645 ml
- 4. SUGGESTED TITER BY ELISA:** up to 1:3,240, which corresponds to 0.5 µg/ml of protein concentration in plates for IgG and IgM detection. Better P/N ratios in IgG detection are obtained at protein concentrations of 0.05 µg/ml.
- 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 -20°C to -80°C
- 6. APPLICATIONS:** ELISA and Western blot assays. 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:** 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:**

p30 (SAG1) (*E. coli*), p35 (GRA8), ChimToxo1.

**BIBLIOGRAPHY:**

**Santoro, F., Charif, H. and Capron, A.** The immunodominant epitope of the major membrane tachyzoite 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.**