Technical Reports

SAG1 (p30) from Toxoplasma gondii requires maintain its native conformation to detect IgM antibodies.

THE AIM OF THE RESEARCH PRESENTED IN THIS TECHNICAL REPORT IS THE COMPARISON OF SAG1 (p30) FROM TOXOPLASMA GONDII OVERPRODUCED IN E. COLI AND P. PASTORIS REGARDING THEIR CAPABILITY IN IgM DETECTION. WITH THIS AIM, BOTH PROTEINS WERE PRODUCED, PURIFY TO HOMOGENEITY AND USED FOR COATING PLATES IN AN INDIRECT IgM ELISA ASSAY.

SAG1 (p30) is a major surface antigen of *Toxoplasma gondii*. It is a multiepitope antigen and one of the most promising antigens for the serodiagnosis of toxoplasmosis. Although it only accounts for 3-5% of total *T. gondii* protein, most of the antibodies are reactive against SAG1 during infection. SAG1 features excellent antigenicity and immunogenicity and is valuable for both diagnosis and immunization. SAG1 is anchored at the surface of tachyzoites, and it constitutes an important immune target that results in a strong immune response against the invasive tachyzoite.

The recombinant antigen of *T. gondii* SAG1 over-produced in *E. coli*, unfortunately, produce insoluble forms and lose antigenicity due to misfolding. Although the material can be refolded to partially recover the antigenicity, the procedure is extremely inefficient mainly for Toxo IgM detection, where the identification of conformational/discontinuous epitopes formed by the secondary or tertiary structure of the protein are more frequent. It has been described in bibliography that SAG1 antigenicity is very dependent of the conformation of the protein.

Therefore, Rekom Biotech has produced SAG1, as a secreted protein, in *Pichia pastoris*, obtaining a complete folded protein thanks to the capability of this heterologous system to produce post-translational modifications such as disulfide bonds, with a high impact on the conformation. SAG1 contains a high number of cysteines. Cys disulfides are often key points in stabilizing protein structure and conformation. They frequently occur between polypeptide subunits, creating a covalent linkage to hold two chains together. Cysteine and cystine groups are relatively hydrophobic and usually can be found within the core of a protein.

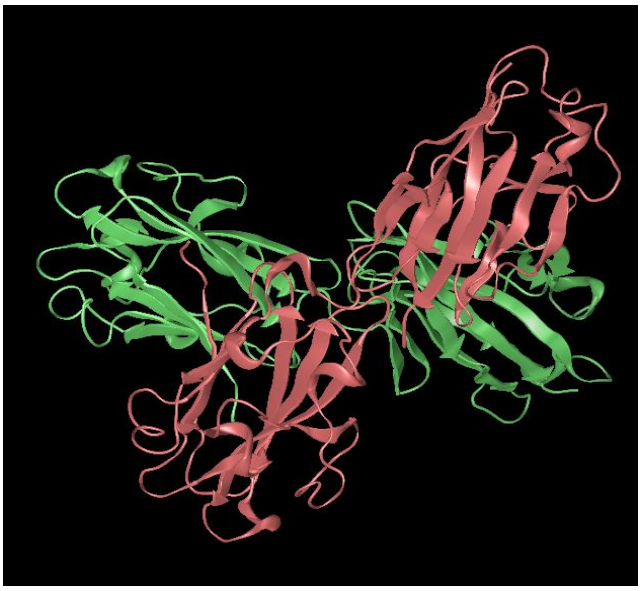

Figure 1. SAG1 3D structure (PDB code: 1KZQ)

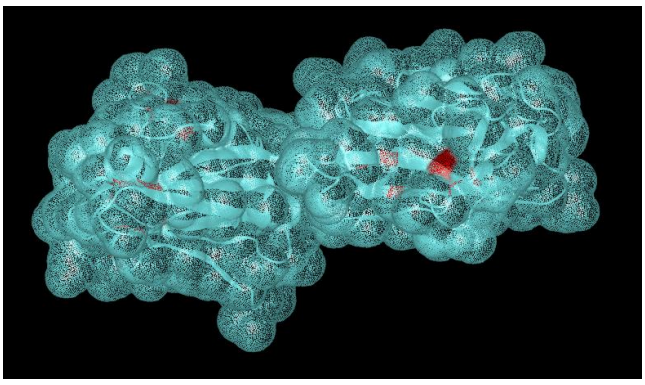

Figure 2. Sorbent accessible surface of a monomer of SAG1. All the disulfide bonds are within the core of the protein (red), maintaining a globular conformation.

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Different ELISA assays were performed to analyze our recombinant antigens:

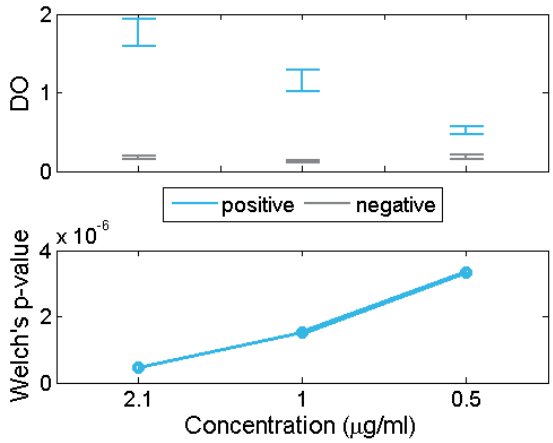

Figure 2. Indirect IgG ELISA assay by coating p30 produced in *E. coli* (RAG0011). In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) Toxo IgG sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for 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.

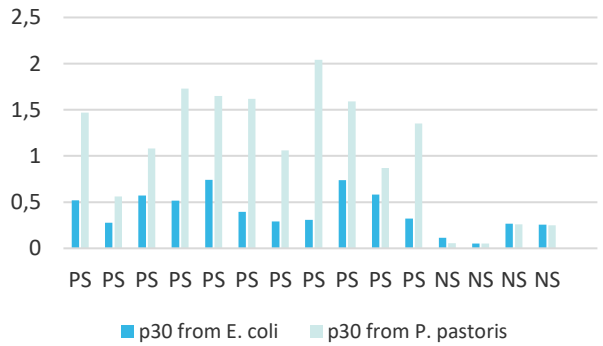

Figure 3. Indirect IgM ELISA assay by coating p30 produced in *E. coli* or p30 produced in *P. pastoris* (RAG0011 *vs* RAG0019). Proteins were coating the plates at a final concentration of 1 µg/ml. All these experiments used anti-IgG as sorbent and anti-IgM-HRP. Sera were used in a 1:100 dilution. PS means positive pre-validated sera by the Vidas test; NS means negative pre-validated sera by the Vidas test.

CONCLUSION

SAG1 (p30) produced in *E. coli* (RAG0011), aggregates as insoluble and unfolded "inclusion bodies". This microorganism, as a prokaryotic, does not possess the intracellular machinery for post-translational modifications such as disulfide bridges. Thus, SAG1 was solubilised under harsh denaturing conditions, purified to homogeneity and successfully refolded. In this way, the protein presented good properties for IgG detection, as can be seen in the indirect ELISA assay, being able to efficiently discriminate between positive and negative specimen sera. However, it exhibited low sensitivity in IgM detection, as can be seen in the indirect IgM ELISA.

SAG1 (p30) produced in *P. pastoris* (RAG0019) produces a secreted folded protein which improves the detection of IgM antibodies in the positive specimen sera, due to the presence of the conformational/discontinuous epitopes at the protein surface.

Please, find more interesting information regarding Rekom Biotech products, by visiting our web site in www.rekombiotech.com.

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