

Recombinant secreted antigen 1 (BcSA1) for *Babesia canis*

CATALOG NUMBER: RAG0012

LOT NUMBER: #

RECOMBINANT ANTIGEN: recombinant antigen BcSA1 for *B. canis* (Zhou *et al.*, 2016).

DESCRIPTION: recombinant antigen formed by the mature protein BcSA1 for *B. canis* and fused to a His-tag in its N-terminus.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 66,200 and 45,000 Da, while relative molecular mass calculated from amino acid sequence is 47,711.61.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
BcSA1-His	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM MOMPS pH 7, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

$DO_{280} = 2.59$

$A_{0.1\%} (=1 \text{ g/l}) = 1.648$

CONCENTRATION*: 1.57 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: 15%

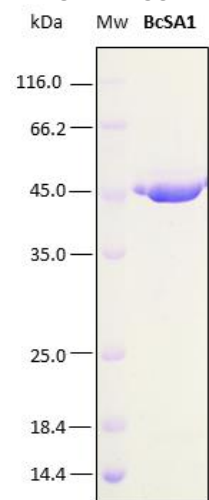


Figure 1. SDS-PAGE analysis (15%) of 1 μ l of recombinant BcSA1. Purity is > 95% as determined by gel electrophoresis.

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.

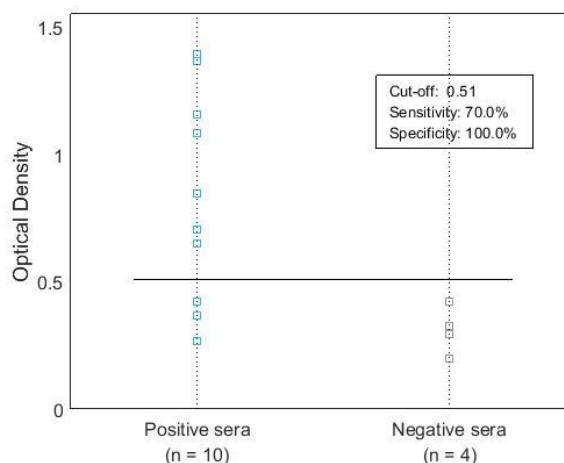


Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect ELISA with a BcSA1 plate coating of 1 μ g/ml. Pre-validated sera to babesiosis (IFAT) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.57 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.669 ml

4. 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.

5. TESTED APPLICATIONS: ELISA.

6. 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.

7. OBSERVATIONS: Proteins should be maintained frozen at high concentration. The dilution to be performed for carrying out an ELISA assays, should be freshly-made with a small quantity of protein. 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:

Bc28.1, BcMSA1, BcMSA1-Biot, ChimBc, ChimBg.

BIBLIOGRAPHY:

Zhou1, M., Cao, S., Luo, Y., Liu, M., Wang, G., Moumouni, P.F.A., Jirapattharasate, C., Iguchi, A., Vudriko, P., Terkawi, M. A., Löwenstein, M., Kern, A., Nishikawa, Y., Suzuki, H., Igarashi, I. and X. Xuan. Molecular identification and antigenic characterization of a merozoite surface antigen and a secreted antigen of *Babesia canis* (BcMSA1 and BcSA1). *Parasites & Vectors* (2016) 9:257.

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.