

Recombinant chimeric antigen ChimBc for *Babesia canis*

CATALOG NUMBER: RAG0040

LOT NUMBER: #

RECOMBINANT ANTIGEN: recombinant multi-epitope antigen ChimBc for *B. canis*.

DESCRIPTION: the recombinant chimeric antigen of *Babesia canis* contains several antigenic determinants from some proteins of this parasite 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 at the molecular marker of 66,200, while relative molecular mass calculated from amino acid sequence is 73,168.40.

BATCH COMPOSITION:

| COMPONENTS | COMPOSITION |
|----------------|---|
| his-ChimBc | recombinant chimeric antigen with a his-tag in its N-terminus |
| Storage buffer | 20 mM phosphate buffer pH 6, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether |

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 2.03
 A_{0.1%} (=1 g/l) = 1.513
 CONCENTRATION*: 1.34 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%

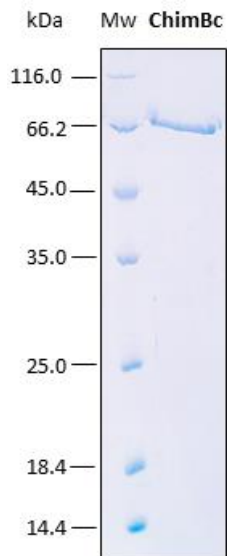


Figure 1. SDS-PAGE analysis (15%) of 1 µl of recombinant ChimBc. Purity is > 95% as determined by gel electrophoresis.

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.

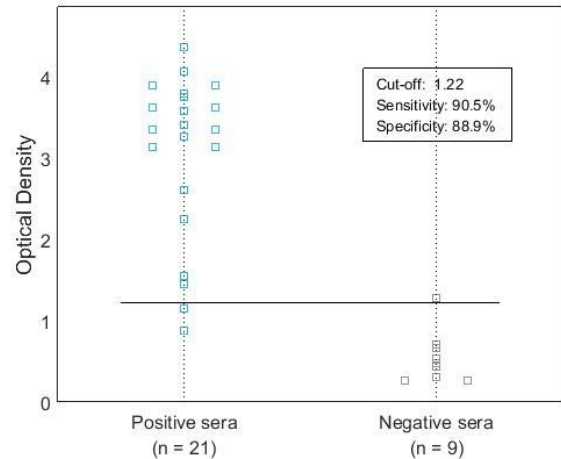


Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect ELISA with a ChimBc plate coating of 1.3 µg/ml (20 nM). Pre-validated sera to babesiosis (IFAT) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

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

LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 1.34 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.784 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, BcSA1.

BIBLIOGRAPHY:

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.