Recombinant biotinylated antigen K39 for *Leishmania infantum*

CATALOG NUMBER: RAG0061BIOT **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Lesihmania infantum* K39 biotinylated (Scalone *et al.*, 2002).

DESCRIPTION: the *Leishmania infantum* antigen kinesin K39 has been prepared as a recombinant antigen fused to a his-tag in the N-terminus and monobiotinylated *in vivo*.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

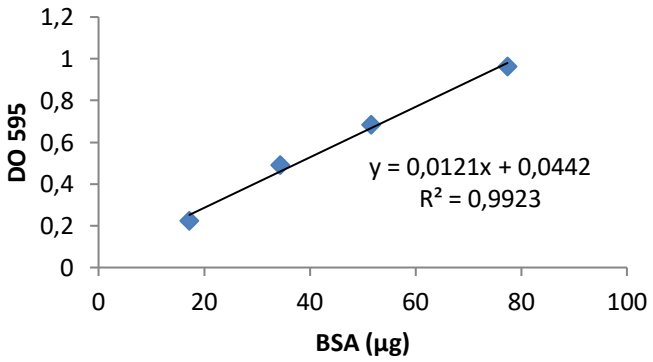
MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 45,000 and 35,000 Da, while relative molecular mass calculated from amino acid sequence is 35,241.81 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-K39	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 1 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY



This protein does not contain any Trp residues. Experience shows that this could result in more than 10% error in the computed extinction coefficient. Therefore, we have measured the protein concentration by using the colorimetric assay based on the interaction between Coomassie brilliant blue and the arginine and aromatic residues (Bradford Method) and its maximum absorption shifts from 470 nm to 595 nm. The standard curve was performed with the protein BSA. 40 µl of the protein were analysed.

DO_{595}: 0.606
CONCENTRATION: 1.16 mg/ml

2. PURITY CONTROL IN SDS-PAGE: 15%

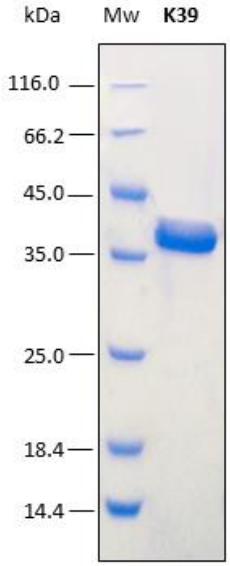


Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant K39. Purity is > 98% as determined by gel electrophoresis. Bands which slightly appear under the main one, correspond to this same protein as it is showed in a western blot performed with a his-tag monoclonal antibody.

3. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed in Rekom Biotech.

Each end user should carry out their own titration for their particular application.

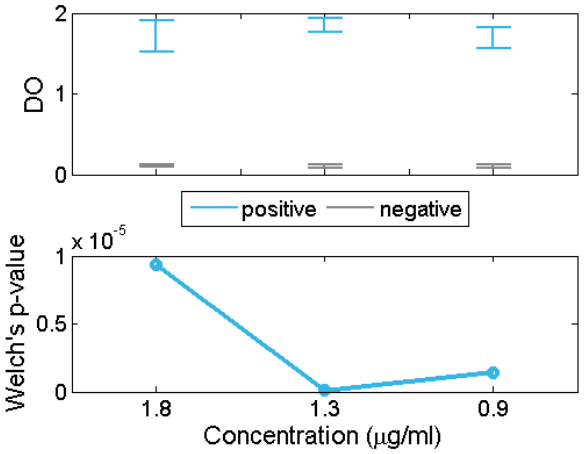


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. 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.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company

4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

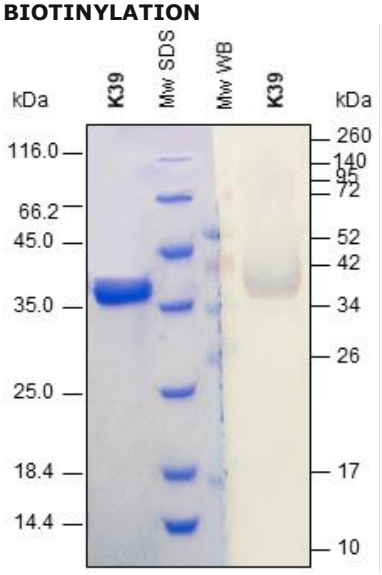


Figure 3. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.16 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.905 ml

4. SUGGESTED TITER BY ELISA: up to 1:1,275, which corresponds to 0.91 μg/ml of protein concentration in plates for IgG detection.

5. STORAGE: Protein is shipped with dry ice. Upon arrival, it should be aliquoted 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.

7. POSIBLE APPLICATIONS: WB, DB, Capture ELISA, Double Antigen Sandwich (DAS), nanoparticles binding, plate orientation. 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.

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.

RECOMMENDED MATCHED ANTIGEN PAIRS:

CAPTURE: RAG0061
DETECTION: RAG0061BIOT

RELATED PRODUCTS:

K39, KMP11.

BIBLIOGRAPHY:

Scalone, A., de Luna, R., Oliva, G., Baldi, L., Satta, G., Vesco, G., Mignone, W., Turilli, C., Mondesire, R.R., Simpson, D., Donoghue, A.R., Frank, G.R., Gradoni, L. Evaluation of the Leishmania recombinant K39 antigen as a diagnostic marker for canine leishmaniasis and validation of a standardized enzyme-linked immunosorbent assay. 2002, *Vet. Parasitol.* 104:275–285.

Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976, 131:499-503.

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 *E. coli* 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.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company