

Recombinant antigen ChimLyme for *Borrelia burgdorferi*

CATALOG NUMBER: RAG0027

LOT NUMBER: #

RECOMBINANT ANTIGEN: variable major protein-like sequence expressed (ChimLyme) of *Borrelia burgdorferi*.

DESCRIPTION: the *Borrelia burgdorferi* antigen ChimLyme has been prepared as a recombinant antigen fused to a his-tag in its N-terminus. It is produced from the complete ORF of the variable major protein-like sequence expressed of this bacteria.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

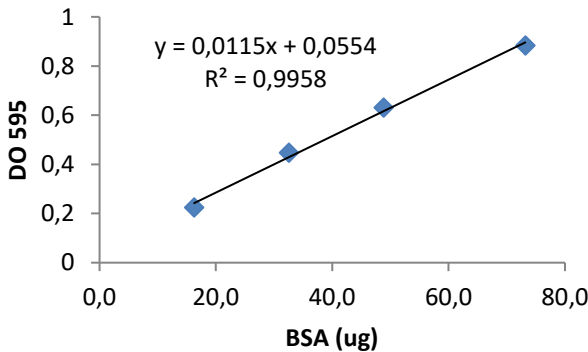
MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 66,200-45,000 Da, while relative molecular mass calculated from amino acid sequence is 39,926.9 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-ChimLyme	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 7, 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.

DO₅₉₅ = 0.578
 CONCENTRATION: 1.13 mg/ml

2. PURITY CONTROL IN SDS-PAGE: 12.5%

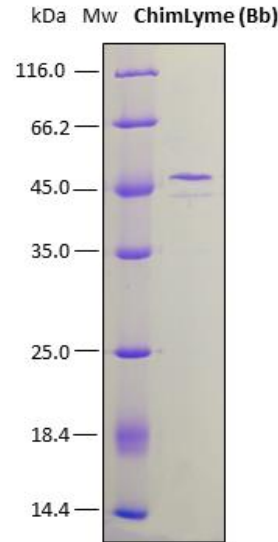


Figure 1. SDS-PAGE analysis (12.5%) of 5 µl of recombinant ChimLyme. Purity is approx. 95% as determined by gel electrophoresis.

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 their own titration for their particular application.

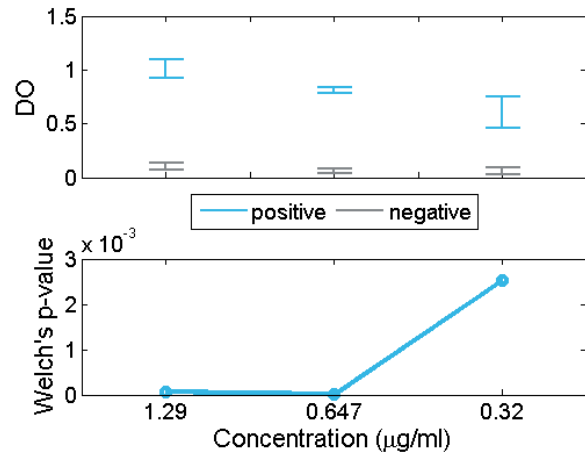


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) IgG sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. Eligible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

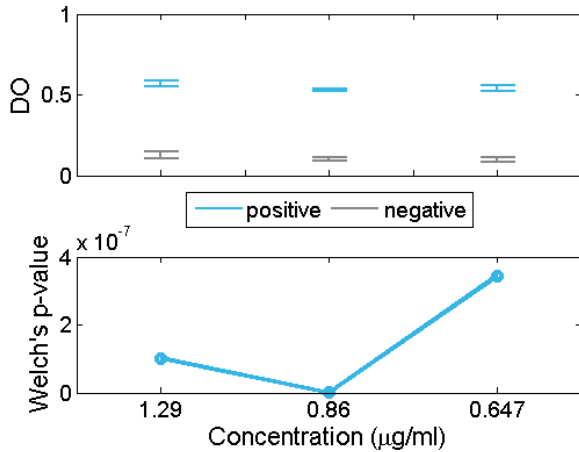


Figure 3. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. 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, the p-values corresponding concentrations of 5.8 and 2.32 µg/ml are below 0.05 and thus the intervals do not overlap. Therefore, any of these two 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:** 1.13 mg/ml
2. **TOTAL QUANTITY PER ALIQUOT:** 1 mg
3. **TOTAL VOLUME PER ALIQUOT:** 0.929 ml
4. **SUGGESTED TITER BY ELISA:** 1:3,625, which corresponds to 0.32 µg/ml of protein in plates for IgG detection and 1:1,700, which corresponds to 0.64 µg/ml of protein in plates for IgM detection.
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

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.

NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY

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 defrost 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:

ospC Ba, ospC Bb, p41 Ba, p41 Bg, p41 Bb, VlsE Bg, VlsE Ba.

BIBLIOGRAPHY:

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.