Recombinant 14-kDa internal flagellin fragment for *Borrelia afzelii*

CATALOG NUMBER: RAG0025 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: internal 14-kDa flagellin fragment of *Borrelia afzelii* (Gassmann *et al.*, 1991).

DESCRIPTION: the *Borrelia burgdorferi* antigen p41 has been prepared as a recombinant antigen fused to a his-tag in its N-terminus. It is produced from the internal fragment of the 41 kDa-flagellin of this bacteria.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 25,000 and 18,400 Da, while relative molecular mass calculated from amino acid sequence is 16,483.9 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-p41	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 6.5, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.182
$A_{0.1\,\%}$ (=1 g/l) = 0.090
CONCENTRATION*: 2.022 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

2. PURITY CONTROL IN SDS-PAGE: 15%

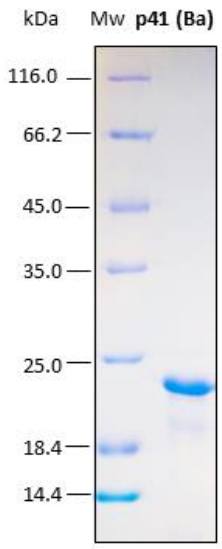

Figure 1. SDS-PAGE analysis (15%) of 2 µl of recombinant p41 Ba. Purity is approx. 95% as determined by gel electrophoresis.

3. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed in Rekom Biotech over the first lot obtained.

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

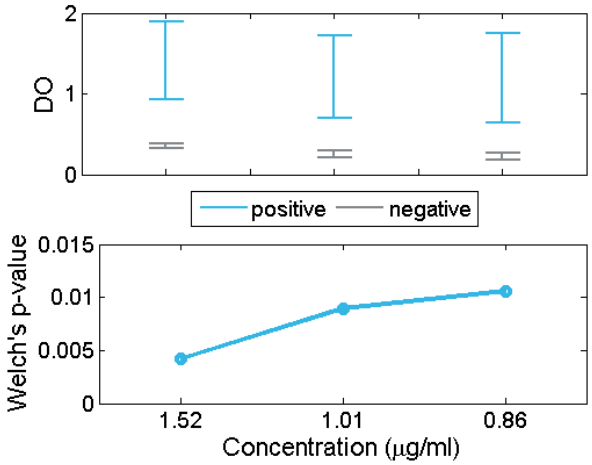

Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed.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

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 2.022 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.519 ml

4. SUGGESTED TITER BY ELISA: approx. 1/2,351 which corresponds to 0.86 µg/ml of protein concentration in plates for IgG 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 -20ºC to -80ºC.

6. APPLICATIONS: ELISA, lateral flow 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.

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7. OBSERVATIONS: in some cases, purified proteins run at a molecular weight which is slightly different to the theoretically calculated molecular weight maybe due to the his-tag presence, which can produce a delay in SDS-PAGE. 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.

RELATED PRODUCTS:

OspC Ba, OspC Bb, OspC Bg, p41 Bb, p41 Bg, VlsE Bg, VlsE Ba, ChimLyme Bb.

BIBLIOGRAPHY:

Gassmann, G. S., E. Jacobs, R. Deutzmann and U. B. Göbel. Analysis of the Borrelia burgdorferi GeHo fla gene and antigenic characterization of its gene product. 1991, *Journal of Bacteriology,* 173: 1452-1459.

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 *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.

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