Recombinant biotinylated multi-epitope chimeric antigen for *Treponema pallidum* (ChimSyphilis)

CATALOG NUMBER: RAG0046BIOT **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Treponema pallidum* multi-epitope chimeric antigen.

DESCRIPTION: a recombinant multi-epitope chimeric antigen has been prepared by expressing several antigenic determinants from lipoproteins of the spirochete *Treponema pallidum*.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 116,000 Da and 66,200 Da, while relative molecular mass calculated from amino acid sequence is 79,621.21 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
ChimSyphilis-his	recombinant chimeric antigen with a his-tag
Storage buffer	20 mM phosphate buffer pH 8, 0.1 M KCl, 0.1% polyoxyethylene (10) tridecyl ether and 5 mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.11
$A_{0.1\,\%}$ (=1 g/l) = 0.838
CONCENTRATION*: 1.32 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%

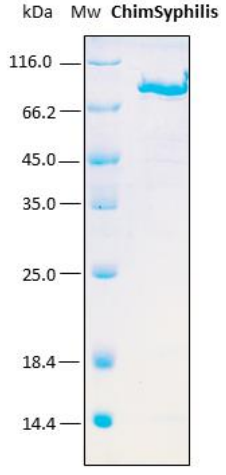

Figure 1. SDS-PAGE analysis (15%) of 2 µl of recombinant biotinylated ChimSyphilis. Purity is > 95% as determined by gel electrophoresis.

3. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" double-antigen sandwich format ELISA assay performed at Rekom Biotech over the first lot obtained, by coating the anti-IgM in the plates.

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

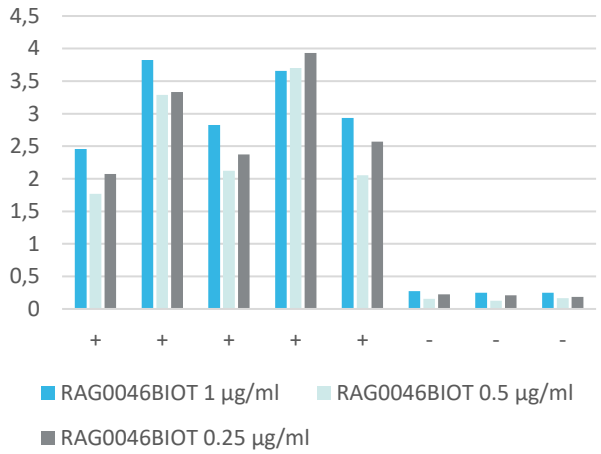

Figure 2. A DAS-ELISA assay was performed by using as detector three different concentrations of the Rekom RAG0046BIOT (1, 0.5 and 0.25 µg/ml). The plates were coated with 0.5 µg/ml by the non-biotinylated RAG0046 protein. The complex for detection formed by the biotinylated protein and the conjugated streptavidin (1/10,000) were previously incubated 1 hour at room temperature.

4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

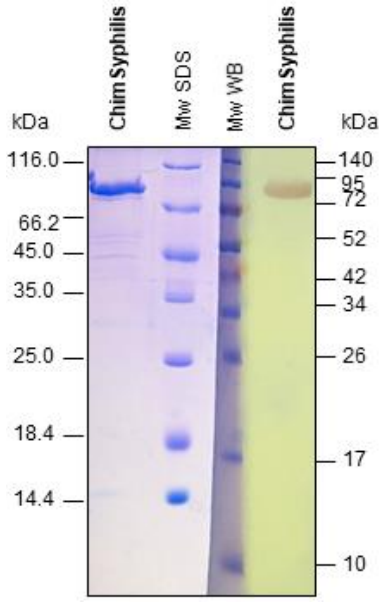

Figure 3. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.32 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.795 ml

4. SUGGESTED TITER BY ELISA: up to 1:13,000, which corresponds to 0.1 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: capture ELISA, double-antigen sandwich 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 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:

TmpA, Tpp15, Tpp15-Biot, Tpp17, Tpp17-Biot, Tpp47, Tpp47-Biot, ChimSyphilis.

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 *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

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