Recombinant multi-epitope chimeric antigen for *Treponema pallidum* (Chimsyphilis1)

CATALOG NUMBER: RAG0046 **LOT NUMBER:** #

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

DESCRIPTION: a recombinant multi-epitope chimeric antigen has been prepared by expressing Tpp17 and Tpp47 proteins 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 70,590.64 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
Chimsyphilis1-his	recombinant chimeric antigen with a his-tag
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl, 0.1% polyoxyethylene (10) tridecyl ether and 5 mM EDTA

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.28
$A_{0.1\,\%}$ (=1 g/l) = 0.923
CONCENTRATION*: 1.39 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%

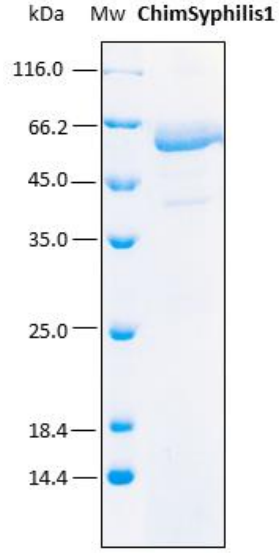

Figure 1. SDS-PAGE analysis (15%) of 5 µl of recombinant Chimsyphilis1. Purity is > 95% as determined by gel electrophoresis.

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.

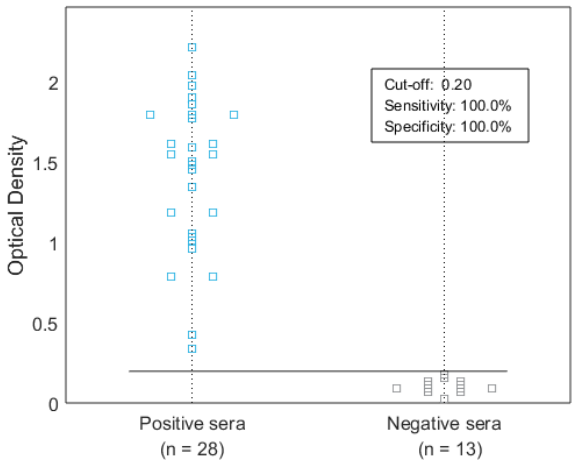

Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with a ChimSyphilis1 plate coating of 0.25 µg/ml. Pre-validated sera by Dx (IgG, Abbott-ELISA, Becton Dickinson-RPR, Spin React - TPHA) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

4. POSITIVE AND NEGATIVE SERA DISCRIMINATION 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.

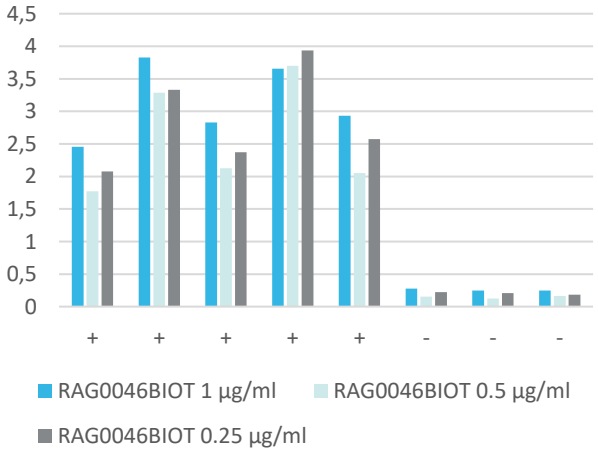

Figure 3. A DAS-ELISA assay was performed by using as detector three different concentrations of the Rekom RAG0046BIOT (1, 0.5

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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.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.39 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.756 ml

4. SUGGESTED TITER BY ELISA: up to 1:5,560, which corresponds to 0.25 μg/ml of protein concentration in plates for IgG detection in an indirect ELISA assay.

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, 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.

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: RAG0046
DETECTION: RAG0046BIOT

RELATED PRODUCTS:

TmpA, Tpp15, Tpp15-Biot, Tpp17, Tpp17-Biot, Tpp47, Tpp47-Biot, Chimsyphilis1-Biot, ChimSyphilis2, ChimSyphilis2-Biot.

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.

FOR RESEARCH AND COMMERCIAL USE *IN VITRO*: not for human *in vivo* or therapeutic use.

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