Recombinant biotinylated antigen Tpp17 for *Treponema pallidum*

CATALOG NUMBER: RAG0008BIOT **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Treponema pallidum* lipoprotein 17 kDa (Akins *et al.*, 1993).

DESCRIPTION: the Tpp17 recombinant lipoprotein has been prepared by expressing the gene which codifies the mature lipoprotein of 17 kDa of the spirochete *Treponema pallidum* 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 45,000-66,200 Da, while relative molecular mass calculated from amino acid sequence is 57,024.5 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
GST-his-Tpp17	recombinant antigen with a GST-tag and a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 7, 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.49
$A_{0.1\,\%}$ (=1 g/l) = 1.016
CONCENTRATION*: 1.47 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: 12%

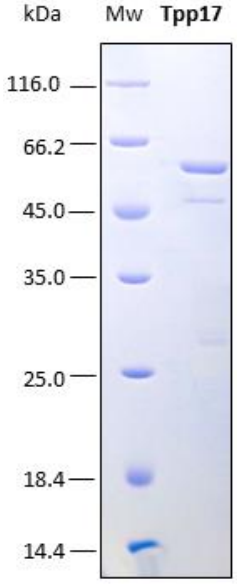

Figure 1. SDS-PAGE analysis (12%) of 1 µl of recombinant Tpp17-Biot. Purity is > 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

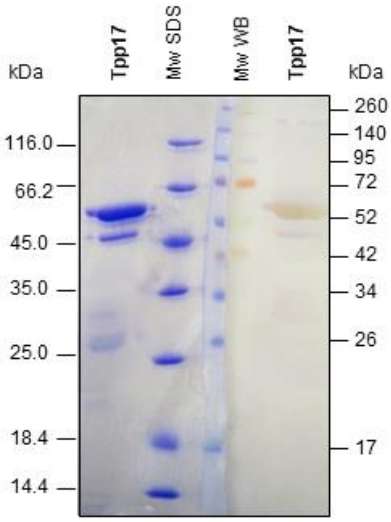

Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

4. 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.

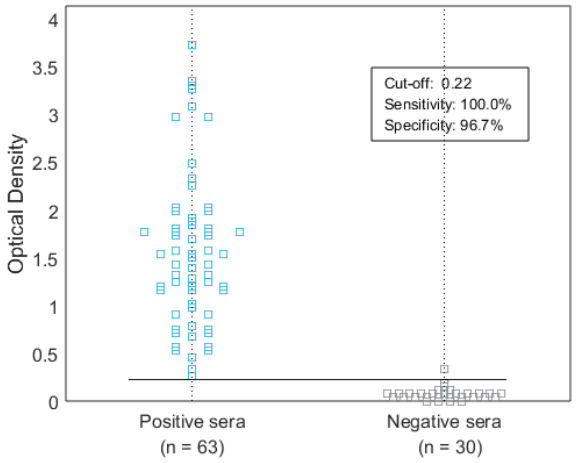

Figure 3. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgG ELISA with a Tpp17 plate coating of 0.25 µg/ml. Pre-validated sera by Dx (IgG, Abbott-ELISA, Becton Dickinson-RPR, Spin React - TPHA) were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgG sera.

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5. TITRATION CURVE BY A CAPTURE ELISA ASSAY

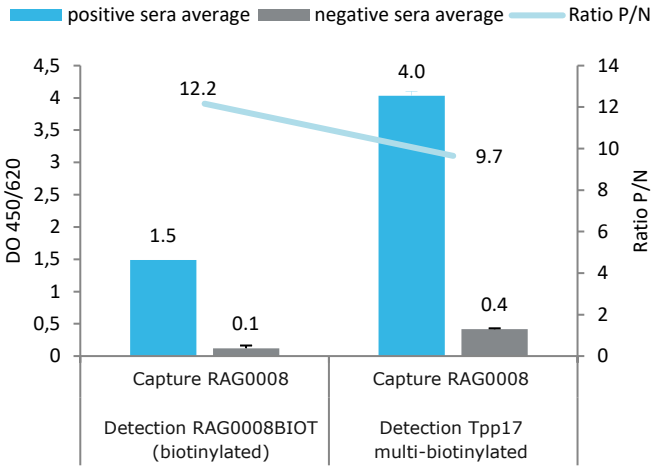

Figure 4. Double antigen sandwich ELISA assay (DAS) for 3rd generation ELISA. The plates were coating with Rekom Tpp17 RAG0008 and the detection was performed with Rekom biotinylated Tpp17 (RAG0008BIOT and Tpp17 multi-biotinylated). In this plot, the optical density at 450/620 nm obtained in a DAS ELISA assay for several positive (blue) and negative (gray) sera were compared. Also the positive and negative signal ratio was calculated for every pair matched sera for DAS. The plates were coating with 0.25 μg/ml of RAG0008, the detection was performed with 0.5 μg/ml, and the development was carried out with a 1:5000 dilution of strep-HRP.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.47 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.716 ml

4. SUGGESTED TITER FOR ELISA: Up to 1:5,880, 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, 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: RAG0008
DETECTION: RAG0008BIOT

RELATED PRODUCTS:

TmpA, Tpp15, Tpp15-Biot, Tpp17, Tpp47, Tpp47-Biot, ChimSyphilis1, ChimSyphilis1-Biot, ChimSyphilis2, ChimSyphilis2-Biot.

BIBLIOGRAPHY:

Akins, D. R., Purcell, B. K., Mitra, M. M., Norgard, M. V., and Radolf, J. D. Lipid modification of the 17-kilodalton membrane immunogen of *Treponema pallidum* determines macrophage activation as well as amphiphilicity. 1993, *Infection and Immunity*, 61: 1202-1210.

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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