Recombinant antigen Tpp17-biotin for *Treponema pallidum*

CATALOG NUMBER: RAG0012 **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*.

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 57024.5 Da.

BATCH COMPOSITION:

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

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 3.91
$A_{0.1\,\%}$ (=1 g/l) = 1.016
CONCENTRATION*: 3.85 mg/ml

* The meassurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and vonHippel, 1989

2. ESPECTROPHOTOMETRIC DETECTION OF BIOTIN

DO_{500} HABA/Avidin = 0.994
DO_{500} HABA/Avidin/Tpp17-biotin = 0.840
Dilution factor = 4
nmol biotin per nmol of protein = 0.95 ≈ 1

This measure has been performed with the Thermo Scientific™ Pierce™ Biotin Quantitation Kit. HABA (4´-hydroxyazobenzene-2-carboxylic acid) is a reagent that enables a quick estimation of the mole-to-mole ratio of biotin to protein

3. PURITY CONTROL IN SDS-PAGE: 12.5%

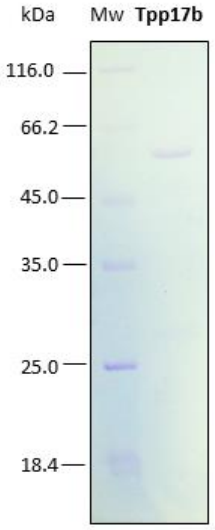

Figure 1. SDS-PAGE analysis (12.5%) of 2 µl of recombinant Tpp17. Purity is > 95% as determined by gel electrophoresis.

4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

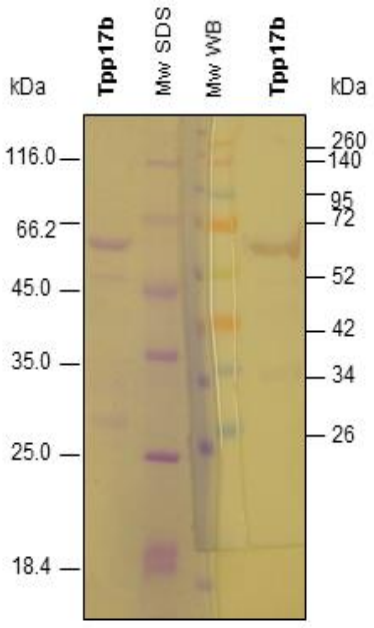

Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

5. TITRATION CURVE BY A DOUBLE ANTIGEN SANDWICH ELISA

A double antigen sandwich ELISA assay (DAS) was performed by The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech over the first lot obtained. Assays were performed by using positive and negative syphilis specimen sera pre-validated with ELISA (Abbott: Architeck); TPHA (Spin React) and RPR (BectonDickinson).

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

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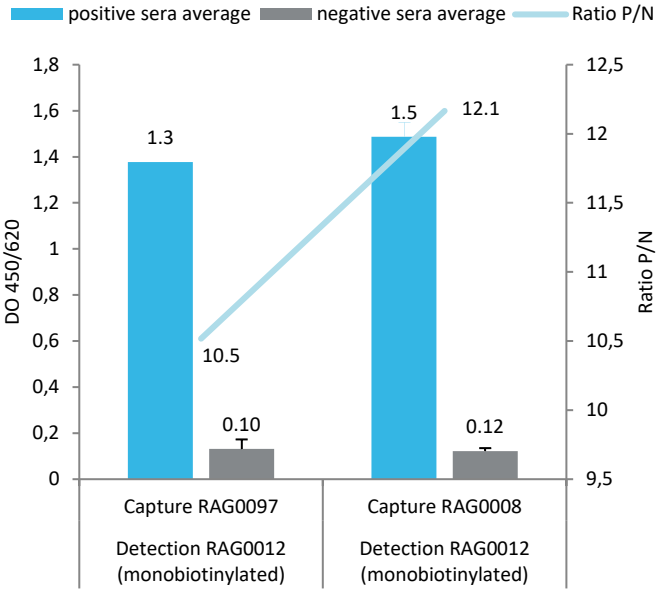

Figure 3. Double antigen sandwich ELISA assay (DAS). The plates were coating with Rekom Tpp17 RAG0097 and RAG0008 and the detection was performed with Rekom monobiotinylated Tpp17 RAG0012. In this plot, the optical density at 450/620 nm obtained in a DAS ELISA assay for several positive (blue) and negative (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 capture antigen, the detection was performed with 0.5 μg/m of biotinylated antigen, 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: 3.85 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.272 ml

4. SUGGESTED TITER FOR CAPTURE ELISA: more than a 1:7,700 dilution in PBS 1x which corresponds to 0.5 μg/ml of protein for 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: not tested. 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 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:

TmpA, Tpp15, Tpp15-monobiot, Tpp17, Tpp47, Tpp47-monobiot.

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.

NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY

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