

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*.

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

DO₂₈₀ = 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%

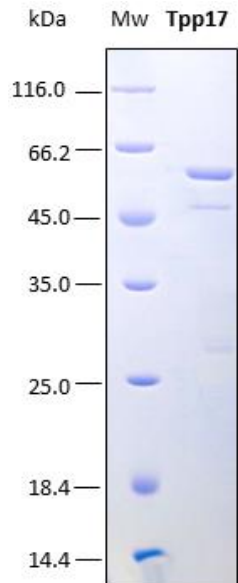


Figure 1. SDS-PAGE analysis (12%) of 1 µl of recombinant Tpp17-Biot. Purity is > 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

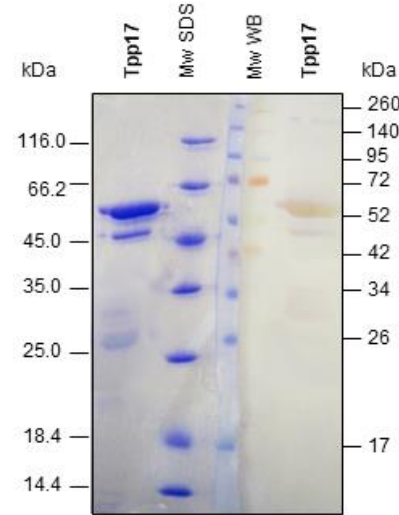


Figure 2. Western blot analysis in order to detect streptavidin/biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

4. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech on the first lot produced. Assays were performed by using positive and negative syphilis specimen sera pre-validated with ELISA (Abbott: Architekt); TPHA (Spin React) and RPR (Becton Dickinson).

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

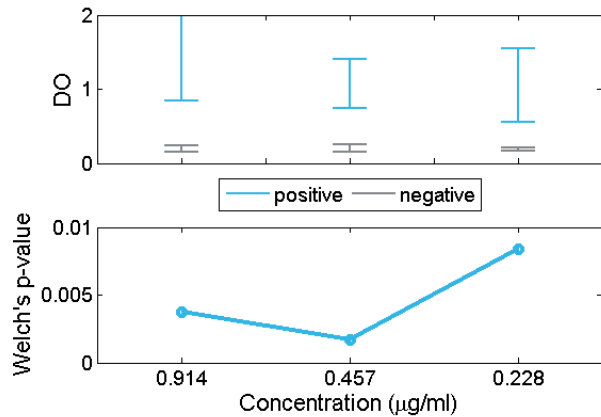


Figure 3. Indirect IgG ELISA. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, 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.

5. POSITIVE AND NEGATIVE SERA DISCRIMINATION BY A DOUBLE ANTIGEN SANDWICH ELISA

A double antigen sandwich ELISA assay (DAS) was performed by using the working pair RAG0008/RAG0008BIOT. These assays were performed with 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.

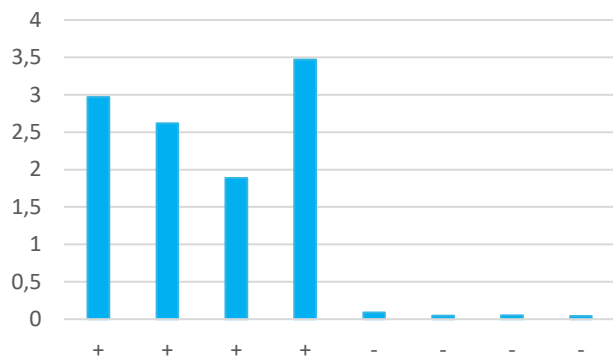


Figure 4. Double antigen sandwich ELISA assay (DAS). The plates were coated with Rekom Tpp17 RAG0008 at a final concentration of 0.25 µg/ml. The detection was performed with Rekom biotinylated Tpp17 RAG0008BIOT at a final concentration of 0.25 µg/ml also. The detection complex was formed by pre-incubation for 1 h at RT, the biotinylated antigen with 1:10,000 of HRP-streptavidin. Sera were used at 1:20 dilution.

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 1:2,940 dilution in PBS 1x which corresponds to 0.5 µg/ml of protein for detection. Up to 1:4,728, which corresponds to 0.25 µg/ml of protein concentration in plates for IgG detection and for IgG+IgM detection.

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.

5. STORAGE: Protein is shipped with dry ice. Upon arrival, it should be aliquoted to avoid repeated freezing and thawing cycles and stored at -20°C to -80°C. Upon defrosting, leave the solution at least 15 min homogenizing with a mild agitation at 4°C.

6. TESTED APPLICATIONS: ELISA.

7. POSSIBLE 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 25°C 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.