Recombinant biotinylated antigen Tpp47 for *Treponema pallidum*

CATALOG NUMBER: RAG0010BIOT **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Treponema pallidum* lipoprotein 47 kDa (Félix de Miranda and Satomi Sato, 2008).

DESCRIPTION: the Tpp47 recombinant lipoprotein has been prepared by expressing the ORF of the corresponding gene which codifies the mature lipoprotein of 47 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 116,000-66,200 Da, while relative molecular mass calculated from amino acid sequence is 88,321.7 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
GST-his-Tpp47	recombinant antigen with a GST-tag and a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.3 M NaCl, 5 mM EDTA and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.39
$A_{0.1\,\%}$ (=1 g/l) = 1.11
CONCENTRATION*: 1.25 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%

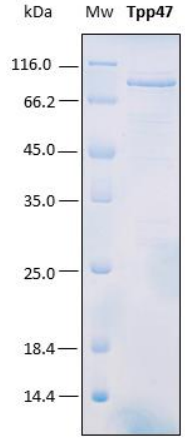

Figure 1. SDS-PAGE analysis (12%) of 1 µl of recombinant Tpp47-Biot. Purity is approx. 95% as determined by gel electrophoresis.

3. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

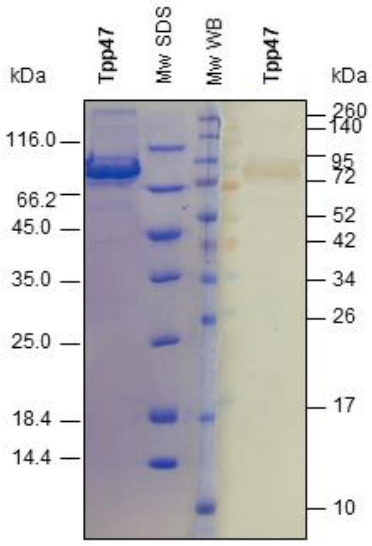

Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

4. TITRATION CURVE BY A DOUBLE ANTIGEN SANDWICH ELISA

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.

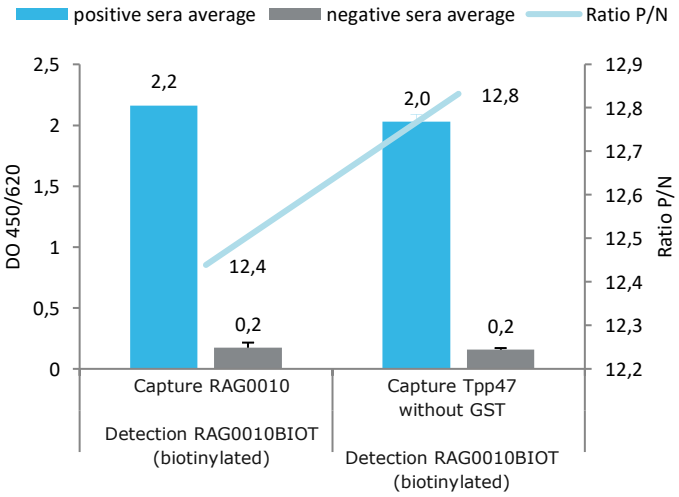

Figure 3. Double antigen sandwich ELISA assay (DAS). The plates were coating with Rekom Tpp47 without GST and RAG0010 and the detection was performed with Rekom biotinylated Tpp47 RAG0010BIOT. In this plot, the optical density at 450/620 nm obtained in a DAS ELISA assay for several positive (blue) and 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.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.25 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.84 ml

4. SUGGESTED TITER FOR CAPTURE ELISA: up to 1:2,500 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 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: RAG0010
DETECTION: RAG0010BIOT

RELATED PRODUCTS:

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

BIBLIOGRAPHY:

Ana Paula Félix de Miranda and Neuza Satomi Sato. Profile of Anti-Tp47 antibodies in patients with positive serology for syphilis analized by western blot. 2008, *The Brazilian Journal of Infectious Diseases* 12(2):139-143.

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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