Recombinant antigen Tpp15-biotin for *Treponema pallidum*

CATALOG NUMBER: RAG0013 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: *Treponema pallidum* lipoprotein 15 kDa (Purcell *et al.*, 1990).

DESCRIPTION: the Tpp15 recombinant lipoprotein has been prepared by expressing the ORF of the corresponding gene which codifies the mature lipoprotein of 15 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 66,200 and 45,000 Da, while relative molecular mass calculated from amino acid sequence is 56,818.7 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
GST-his-Tpp15-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, and 1 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.897
$A_{0.1\,\%}$ (=1 g/l) = 1.068
CONCENTRATION*: 0.840 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 = 1.003
DO_{500} HABA/Avidin/Tpp15-biotin = 0.857
Dilution factor = 1
nmol biotin per nmol of protein = 0.909 ≈ 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%

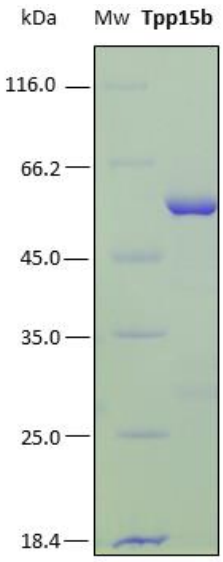

Figure 1. SDS-PAGE analysis (12.5%) of 5 µl of recombinant Tpp15. Purity is > 95% as determined by gel electrophoresis.

4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

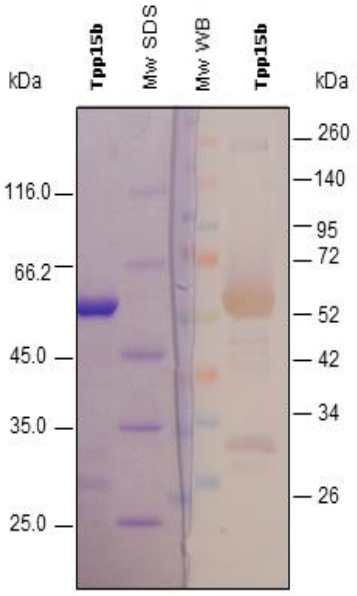

Figure 2. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 0.840 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 1.249 ml

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4. 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

5. 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.

6. 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, Tpp17, Tpp17-monobiot, Tpp47, Tpp47-monobiot.

BIBLIOGRAPHY:

Purcell BK, Swancutt MA and Radolf JD. 1990. Lipid modification of the 15 kilodalton major membrane immunogen of *Treponema pallidum*. *Mol. Micobiol.*, 4:1371-1379.

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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