Recombinant antigen Tpp17 for *Treponema pallidum*

**CATALOG NUMBER:** RAG0008  
**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 at the molecular markers of 45,000 Da, while relative molecular mass calculated from amino acid sequence is 46,649.6 Da.

**BATCH COMPOSITION:**

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-his-Tpp17</td>
<td>recombinant antigen with a GST-tag and a his-tag in its N-terminus</td>
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<tr>
<td>Storage buffer</td>
<td>50 mM Tris-HCl pH 8 and 10 mM glutathion</td>
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**QUALITY CONTROL:**

1. **PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY**

\[
\text{DO}_{280} = 1.39  \\
A_0.1\% = 1.175  \\
\text{CONCENTRATION}^* = 1.182 \text{ mg/ml} \\
\]

* The measurement of the protein concentration has been performed with the theoretical extinction coefficient of the recombinant protein obtained from Gill and von Hippe1, 1989

2. **PURITY CONTROL IN SDS-PAGE: 12%**

**3. 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: Architect), TPHA (Spin React) and RPR (BectonDickinson).

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

![Titer determination](image)

**Figure 1.** SDS-PAGE analysis (12%) of 2 µl of recombinant Tpp17. Purity is > 95% as determined by gel electrophoresis.

**Figure 2.** 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.

![Titer determination](image)

**Figure 3.** Double antigen sandwich ELISA (DAS) for 3rd generation ELISA. The plates were coating with Rekom Tpp17 RAG0008 and the detection was performed with Rekom biotinylated Tpp17 (RAG0009BIOT 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.

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Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85  
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 Certified Company
4. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.182 mg/ml
2. TOTAL QUANTITY PER ALIQUOT: 1 mg
3. TOTAL VOLUME PER ALIQUOT: 0.888 ml
4. SUGGESTED TITER BY ELISA: 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.
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: ELISA and Western blot assays. 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 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 ANTIGEN PAIRS:

CAPTURE: RAG0008
DETECTION: RAG0008BIOT

RELATED PRODUCTS:

BIBLIOGRAPHY:


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.