Recombinant antigen pp150 for Cytomegalovirus (CMV)

CATALOG NUMBER: RAG0091
LOT NUMBER: #

RECOMBINANT ANTIGEN: CMV major structural phosphoprotein of 150 kDa (Jahn et al., 1987).

DESCRIPTION: the CMV antigen pp150 has been prepared as a truncated recombinant antigen fused to a his-tag in its N-terminal. It is produced from the UL32 gene which codifies the large structural phosphoprotein of CMV.

PRESENTATION: liquid protein solution

SOURCE: Escherichia coli

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 25,000-18,400 Da, while relative molecular mass calculated from amino acid sequence is 15,995.6 Da.

BATCH COMPOSITION:

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>his-pp150</td>
<td>recombinant antigen with a his-tag in its N-terminus</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>20 mM phosphate buffer pH 7.5, 1 M NaCl and 0.1 % polyoxyethylene (10) tridecyl ether</td>
</tr>
</tbody>
</table>

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

\[ \text{DO}_{280} = 0.549 \]
\[ \text{A} 0.1 \% \ (=1 \ g/l) = 0.437 \]

CONCENTRATION*: 1.256 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

2. PURITY CONTROL IN SDS-PAGE: 15%

3. TITRATION CURVE BY AN INDIRECT ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech over the first lot obtained.

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

![Figure 1. SDS-PAGE analysis (15%) of 2 μl of recombinant pp150. Purity is approx. 95% as determined by gel electrophoresis.](image1)

![Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV IgM 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.](image2)

![Figure 3. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV IgG 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.](image3)
4. TITRATION CURVE BY A CAPTURE ELISA ASSAY

4.A A double antigen sandwich ELISA assay (DAS) was performed by using positive and negative CMV IgM specimen sera pre-validated with the ELISA capture IgM Vidas Test. The plates were coating with Rekom pp150 and developed with Rekom pp150-HRP.

Conjugated antigen dilution

<table>
<thead>
<tr>
<th>P/N ratio</th>
<th>1:5000</th>
<th>1:10.000</th>
<th>1:15.000</th>
<th>1:20.000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average OD 450/620 negative sera</td>
<td>0.048</td>
<td>0.042</td>
<td>0.04</td>
<td>0.035</td>
</tr>
<tr>
<td>Average OD 450/620 positive sera</td>
<td>3.315</td>
<td>3.2425</td>
<td>2.9685</td>
<td>2.7275</td>
</tr>
</tbody>
</table>

In this plot, the optical density at 450/620 nm obtained in a DAS ELISA assay for positive (blue) and negative (gray) CMV IgM sera were compared. Four different conjugated dilutions were tested. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera (P/N ratio means positive/negative signal ratio).

4.B A capture ELISA assay was performed by using two different dilutions of the Rekom pp150-HRP in order to develop a reference commercial test (CMV-IgM-eLA test PKS medac).

Conjugated antigen dilution

<table>
<thead>
<tr>
<th>Reference commercial kit</th>
<th>1:10.000 Rekom pp150-HRP</th>
<th>1:20.000 Rekom pp150-HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average OD 450/620 of negative sera</td>
<td>0.081</td>
<td>0.0365</td>
</tr>
<tr>
<td>Average OD 450/620 of positive sera</td>
<td>1,288</td>
<td>2,211</td>
</tr>
</tbody>
</table>

In this plot, the optical density at 450/620 nm obtained in an ELISA capture assay for a positive (blue) and a negative (gray) CMV IgM sera were compared. Two different conjugated dilutions and the reference conjugated antigen were tested in the assay. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera. In the present assay, any of the showed dilutions can be used to distinguish between positive and negative sera in a capture ELISA assay.

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

LOT SPECIFICATIONS:
1. CONCENTRATION: 1.256 mg/ml
2. TOTAL QUANTITY PER ALIQUOT: 1 mg
3. TOTAL VOLUME PER ALIQUOT: 0.834 ml
4. SUGGESTED TITER BY ELISA: up to 1:5,024, which corresponds to 0.25 μg/ml of protein concentration in plates for IgM and IgG 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.

RELATED PRODUCTS:
pp52, pp52-Biot, ChimCMV1, ChimCMV1-Biot.

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 container’s 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**