

## 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*

**SPECIFIC ANTIBODY (CALIBRATOR):** CMV pp150 polyclonal antibody (Rekom Biotech catalog reference PAB0002)

**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:

COMPONENTS	COMPOSITION
his-pp150	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 7.5, 0.15 M NaCl and 0.1 % polyoxyethylene (10) tridecyl ether

### QUALITY CONTROL:

#### 1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

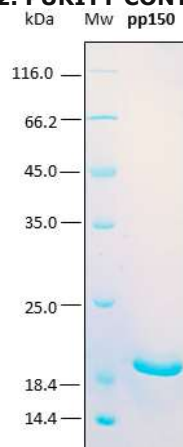
DO<sub>280</sub> = 0.598

A<sub>0.1 %</sub> (=1 g/l) = 0.437

CONCENTRATION\*: 1.37 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 Hippel, 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: 15%

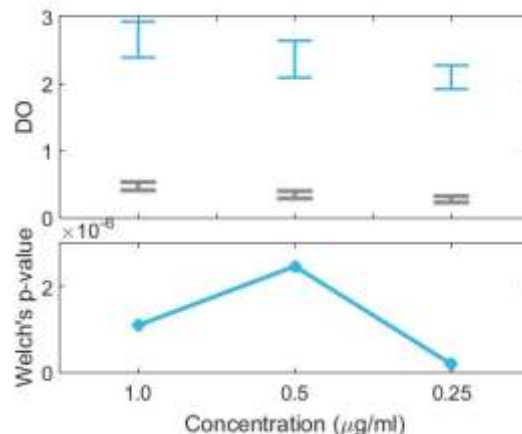


**Figure 1.** SDS-PAGE analysis (15%) of 3 µl of recombinant pp150. Purity is approx. 95% as determined by gel electrophoresis.

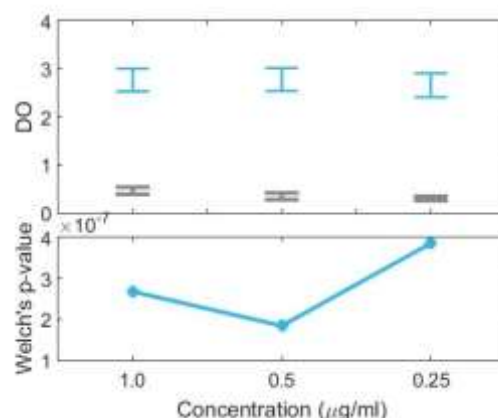
### 3. TITRATION CURVE BY AN ELISA ASSAY

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.



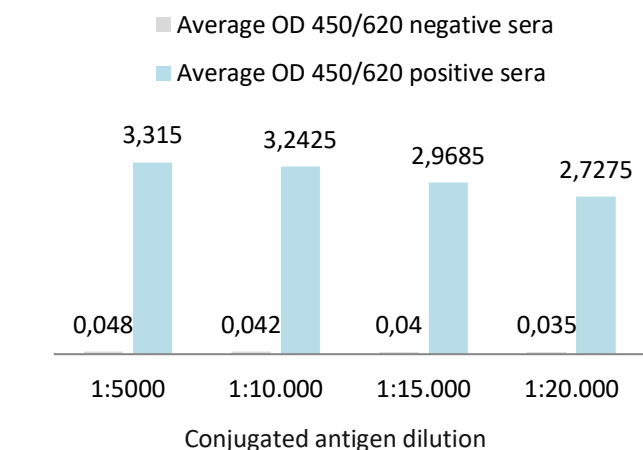
**Figure 2.** In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is 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.



**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.

#### 4. TITRATION CURVE BY A CAPTURE ELISA ASSAY

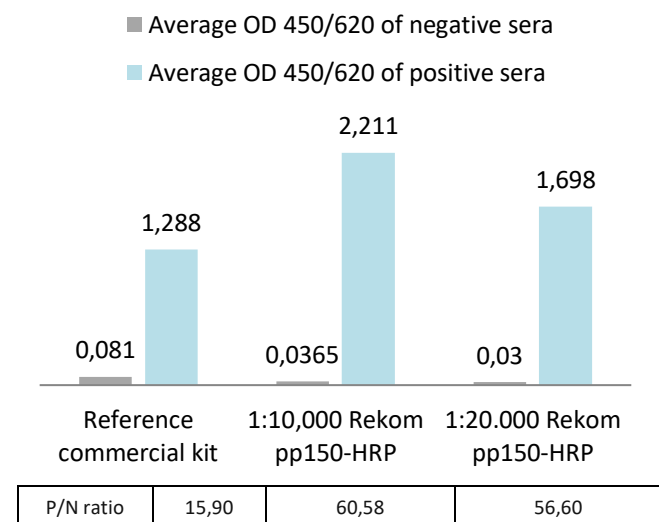
**4.A** A double antigen sandwich ELISA assay (DAS) was performed by using positive and negative CMV IgM specimen sera pre-validated with the ELISA capture IgM Vidas Test. The plates were coating with Rekom pp150 and developed with Rekom pp150-HRP.



P/N ratio	69	77	74	78
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**Figure 4A.** 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. Therefore, in the present assay, any of the showed dilutions can be used to distinguish 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).



**Figure 4B.** 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:** 1.37 mg/ml

**2. TOTAL QUANTITY PER ALIQUOT:** 1 mg

**3. TOTAL VOLUME PER ALIQUOT:** 0.729 ml

**4. SUGGESTED TITER BY ELISA:** up to 1:5,480, 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 to avoid repeated freezing and thawing cycles and stored at -20°C to -80°C. In order to defrost the protein, maintain the aliquot at 25°C without shaking to avoid aggregation.

**6. TESTED APPLICATIONS:** ELISA.

**7. POSSIBLE APPLICATIONS:** WB, DB, Indirect ELISA, positive control in direct ELISA, CLIA, lateral-flow. 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.

#### RELATED PRODUCTS:

pp150 (RAG0059), pp52, pp52-Biot, pp65, ChimCMV1, ChimCMV1-Biot, ChimCMV2, ChimCMV2-Biot, pp28, ChimCMV3

#### BIBLIOGRAPHY:

Jahn, G., Kouzarides, T., Mach, M., Scholl, B.-C., Plachter, B., Traupe, B., Preddie, E., Satchwell, S.C., Fleckenstein, B. and B. G. Barrell. Map position and nucleotide sequence of the gene for the large structural phosphoprotein of Human Cytomegalovirus. 1987. J. of Virol., 61, 1358-1367.

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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Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85  
E-mail: [info@rekombiotech.com](mailto:info@rekombiotech.com) – Web: [www.rekombiotech.com](http://www.rekombiotech.com) – An ISO 9001 and 13485 Certified Company

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