Recombinant antigen pp150 (aa 595 to 636) for *Cytomegalovirus* (CMV)

CATALOG NUMBER: RAG0059 **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 with the first epitope formed by aa 595 to 636 and fused to a GST and 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 45,000-35,000 Da, while relative molecular mass calculated from amino acid sequence is 36,244.38 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
GST-his-pp150	recombinant antigen with a GST and a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl and 0.1 % polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 4.04
$A_{0.1\ \%}$ (=1 g/l) = 1.382
CONCENTRATION*: 2.92 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

2. PURITY CONTROL IN SDS-PAGE: 15%

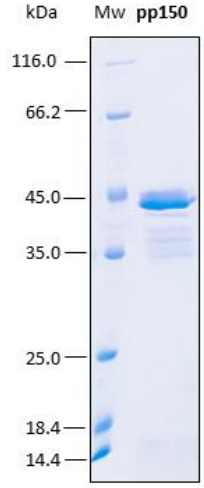

Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant pp150. Purity is approx. 95% as determined by gel electrophoresis.

3. DISCRIMINATION OF PRE-VALIDATED SERA BY AN INDIRECT ELISA ASSAY

The cut-off has been suggested about an "in-house" ELISA kit performed in Rekom Biotech.

Each end-user should carry out an analysis for their particular application.

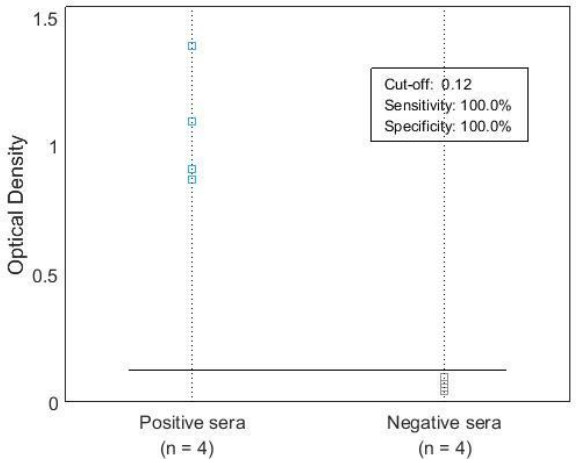

Figure 2. The dot plot graph illustrates the distribution of positive and negative sera by an indirect IgM ELISA with a protein coating of 0.5 µg/ml. Pre-validated sera by chemiluminescence (VIDAS® CMV IGM, bioMerieux) with confirmatory test by immunofluorescence, were used in this analysis. The chart shows the optical density at 450/620 nm for positive (blue) and negative (grey) IgM sera.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 2.92 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.673 ml

4. SUGGESTED TITER BY ELISA: up to 1:5,840, which corresponds to 0.5 µg/ml of protein concentration in plates for IgM detection in an indirect ELISA assay.

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. 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.

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 and 13485 Certified Company

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.

RELATED PRODUCTS:

pp150 (RAG0091), pp52, pp52-Biot, pp65, ChimCMV1, ChimCMV1-Biot, ChimCMV2, ChimCMV2-Biot.

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.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
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