Recombinant antigen pp65 for *Cytomegalovirus* (CMV)

CATALOG NUMBER: RAG0016 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: CMV antigen pp65 (Lucas *et al.*, 2011).

DESCRIPTION: the CMV antigen pp65 has been prepared as a recombinant antigen fused to a his-tag in its N-terminus. It is produced from the UL83 gene, which codifies a tegument phosphoprotein located in the nucleolar matrix of lytically infected fibroblasts.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): CMV pp65 polyclonal antibody (Rekom Biotech catalog reference PAB0003)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 66,200-45,000 Da, while relative molecular mass calculated from amino acid sequence is 51,902.6 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-pp65	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.3 M NaCl, 0.1% polyoxyethylene (10) tridecyl ether and 8 M urea

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.47
$A_{0.1\,\%}$ (=1 g/l) = 1.269
CONCENTRATION*: 1.16 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 analysis.

2. PURITY CONTROL IN SDS-PAGE: 12 %

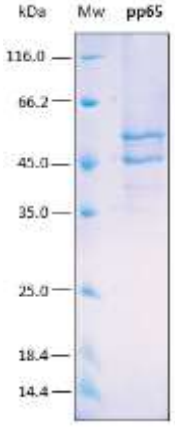

Figure 1. SDS-PAGE analysis (12%) of 5 µl of recombinant pp65. Purity is approx. 95% as determined by gel electrophoresis. The band which appears at approx. at 45 kDa, corresponds to an *in vivo* N-terminus degradation product of this same protein as it is showed in a western blot performed with a his-tag monoclonal antibody.

3. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" ELISA kit performed at Rekom Biotech.

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

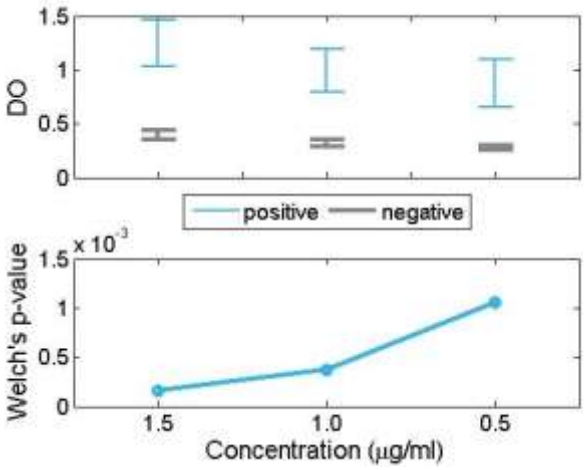

Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed.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

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.16 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.905 ml

4. SUGGESTED TITER BY ELISA: up to 1:2,320, which corresponds to 0.5 µg/ml of protein concentration in plates for IgM 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 -20ºC to -80ºC. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation.

6. TESTED APPLICATIONS: ELISA.

7. POSIBLE APPLICATIONS: WB, DB, Indirect ELISA, positive control in direct ELISA. Where this product has not been tested for use in a particular technique, this does not necessarily exclude its use in such procedures. Suggested

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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 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. **If you put the protein on ice and a precipitate appears, it can be urea crystals. In such a case, it would help if you put the protein at room temperature to dissolve the crystals.**

RELATED PRODUCTS:

pp150, pp52, pp52-Biot, ChimCMV1, ChimCMV1-Biot, ChimCMV2, ChimCMV2-Biot, pp28, ChimCMV3.

BIBLIOGRAPHY:

Kenneth G. Lucas, Lei Bao, Richard Bruggeman, Kimberly Dunham and Charles Specht. The detection of CMV pp65 and IE1 in glioblastoma multiforme. 2011, Journal of neuro-oncology, 103.

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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