

Recombinant biotinylated antigen pp52 for CMV

CATALOG NUMBER: RAG0090BIOT

LOT NUMBER: #

RECOMBINANT ANTIGEN: CMV phosphoprotein of 52 kDa (Maine *et al.*, 2000).

DESCRIPTION: the CMV antigen pp52 has been prepared as a truncated recombinant antigen fused to a his-tag in its N-terminal and monobiotinylated *in vivo*. It is produced from the immunological regions of the UL44 gene, which codifies the DNA polymerase processivity subunit of CMV.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): CMV pp52 polyclonal antibody (Rekom Biotech catalog reference PAB0001)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is at the molecular marker of 25,000, while relative molecular mass calculated from amino acid sequence is 21,968.5 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-pp52	recombinant antigen with a his-tag in its N-terminal
Storage buffer	20 mM phosphate buffer pH 6 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

$DO_{280} = 0.45$

$A_{0.1\%} (=1 \text{ g/l}) = 0.209$

CONCENTRATION*: 2.14 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: 15%

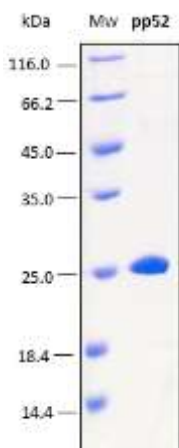


Figure 1. SDS-PAGE analysis (15%) of 2 µl of recombinant chimera pp52-Biot. Purity is > 95% as determined by gel electrophoresis.

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.

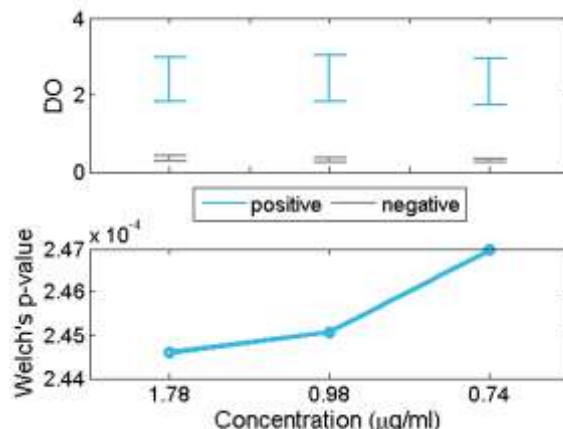


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed.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

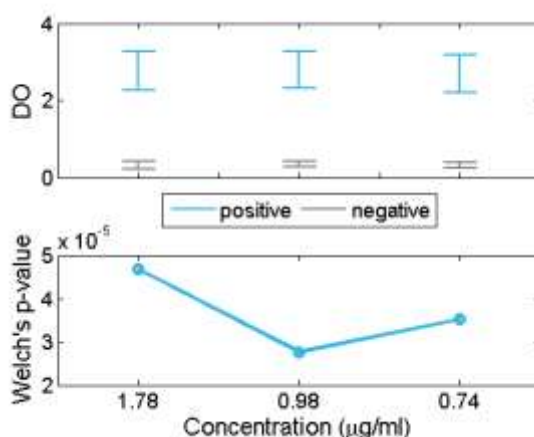


Figure 3. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed. 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. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

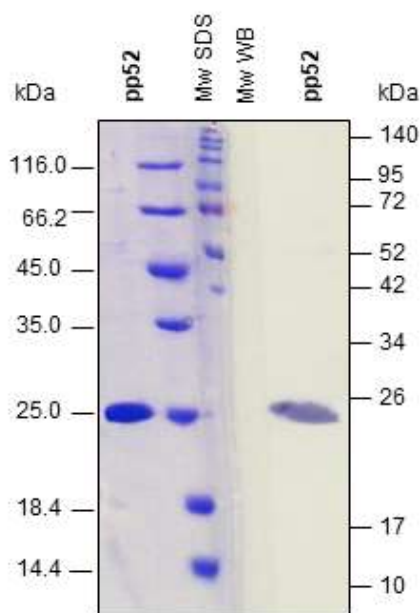


Figure 3. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 2.14 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.490 ml
- 4. SUGGESTED TITER BY ELISA:** up to 1:2,891, which corresponds to 0.74 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, Capture ELISA, nanoparticles binding, plate orientation. 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, pp52, pp65, ChimCMV1, ChimCMV1-Biot, ChimCMV2, ChimCMV2-Biot, pp28, ChimCMV3.

BIBLIOGRAPHY:

Maine, G.T., R. Stricker, M. Schuler, J. Spesard, S. Brojanac, B. Iriarte, K. Herwig, T. Gramins, B. Combs, J. Wise, H. Simmons, T. Gram, J. Lonze, D. Ruzicki, B. Byrne, J. D. Clifton, L. E. Chovan, D. Wachta, C. Holas, D. Wang, T. Wilson, S. Tomazic-Allen, M. A. Clements, G. L. Wright, Jr., T. Lazzarotto, A. Ripalti, and M. P. Landini. Development and Clinical Evaluation of a Recombinant-Antigen-Based Cytomegalovirus Immunoglobulin M Automated Immunoassay Using the Abbott AxSYM Analyzer. 2000, J Clin Microbiol., 38(4):1476-1481.

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