

Recombinant biotinylated chimeric antigen ChimCMV1 for CMV

CATALOG NUMBER: RAG0109BIOT

LOT NUMBER: #

RECOMBINANT ANTIGEN: recombinant chimeric antigen for CMV.

DESCRIPTION: the recombinant chimeric antigen of CMV has been prepared as a chimeric protein formed by several antigenic determinants from some proteins of this virus. This recombinant antigen is fused to a his-tag in the N-terminus.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 45,000 and 35,000 Da, while relative molecular mass calculated from amino acid sequence is 34,634.4 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-ChimCMV1	recombinant antigen with a his-tag in its N-terminal
Storage buffer	20 mM phosphate buffer pH 8 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO₂₈₀ = 0.745
 A_{0.1%} (=1 g/l) = 0.652
 CONCENTRATION*: 1.14 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

2. PURITY CONTROL IN SDS-PAGE: 15%

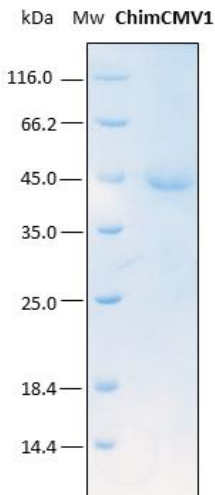


Figure 1. SDS-PAGE analysis (15%) of 2 µl of recombinant chimera ChimCMV1. Purity is > 98% as determined by gel electrophoresis.

3. TITRATION CURVE BY AN ELISA ASSAY

The titer has been suggested in reference to an "in-house" capture format ELISA assay performed at Rekom Biotech over the first lot obtained, by coating the anti-IgM in the plates.

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

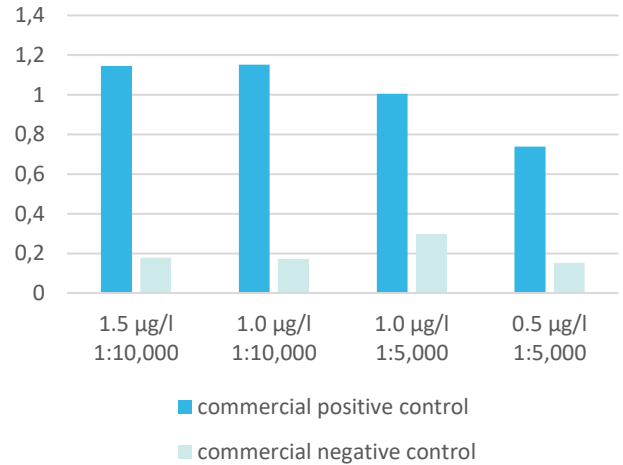


Figure 2. A capture ELISA assay was performed by using two different dilutions of the Rekom RAG0109BIOT (1.5, 1 and 0.5 µg/ml) in combination with two different HRP-strep dilutions (1:10,000 and 1:5,000) as detector in the commercial test CMV-IgM-eLA test PKS medac. Both, positive and negative controls from the commercial test were used for the assay. The complex for detection formed by the biotinylated protein and the conjugated streptavidin were previously incubated 1 hour at room temperature.

4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

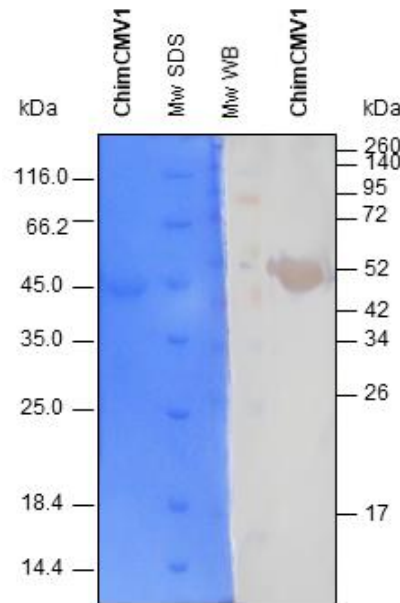


Figure 3. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 1.14 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.92 ml
- 4. SUGGESTED TITER BY ELISA:** up to 1/1,140, which corresponds to 1.0 µg/ml of protein conjugated for IgM detection in a capture format.
- 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 -20°C to -80°C.
- 6. APPLICATIONS:** immunocapture and immunometric ELISA assays, indirect ELISA on streptavidin-plates and streptavidin-nanoparticles binding procedures. 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 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, pp52-Biot, ChimCMV1.

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.

NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY