Recombinant biotinylated multi-epitope chimeric antigen ChimCMV2 for CMV

CATALOG NUMBER: RAG0110BIOT **LOT NUMBER:** #

RECOMBINANT ANTIGEN: recombinant chimeric antigen for CMV.

DESCRIPTION: the recombinant multi-epitope chimeric antigen of CMV has been prepared as a chimeric protein formed by several immunological regions from some antigens of this virus and monobiotinylated *in vivo*.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

SPECIFIC ANTIBODY (CALIBRATOR): CMV pp52 and pp150 polyclonal antibodies (Rekom Biotech catalog references PAB0001 and PAB0002)

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 116,000 and 66,200 Da, while relative molecular mass calculated from amino acid sequence is 61,935.98 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-ChimCMV2	Recombinant chimeric antigen with 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} =0.68
$A_{0.1\,\%}$ (=1 g/l) = 0.489
CONCENTRATION*: 1.39 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%

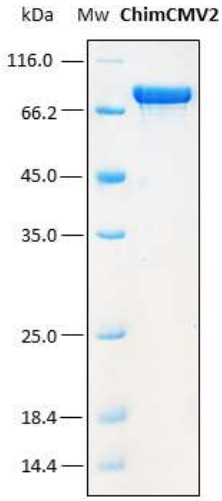

Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant chimera ChimCMV2. Purity is > 90% as determined by gel electrophoresis.

3. POSITIVE AND NEGATIVE SERA DISCRIMINATION BY AN ELISA ASSAY

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

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

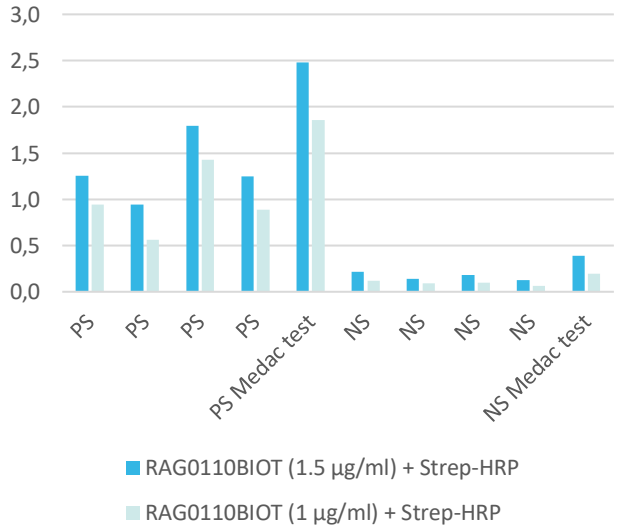

Figure 2. A capture ELISA assay was performed by using two different dilutions of the Rekom RAG0110BIOT (1.5 and 1 µg/ml) in combination with one HRP-strep dilution (1:10,000) as detector. Both, positive and negative controls from the commercial test CMV-IgM-eLA test PKS Medac were used for this assay and also specimen sera validated by using the IgM capture ELISA test from Vidas. The complex for detection formed by the biotinylated protein and the conjugated streptavidin were previously incubated 1 hour at room temperature.

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4. WESTERN BLOT WITH STREPTAVIDIN TO DETECT BIOTINYLATION

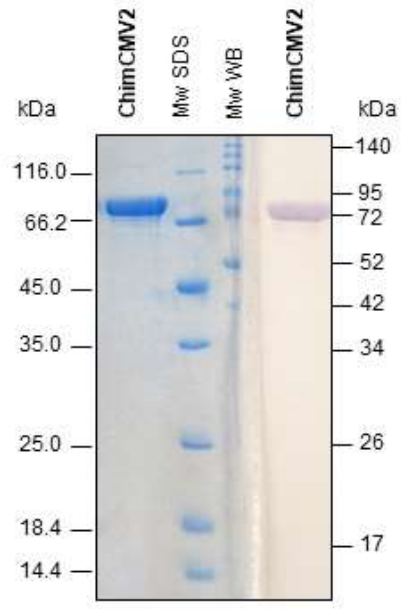

Figure 3. Western blot analysis in order to detect streptavidin /biotin reaction. The incubation was performed with HRP conjugated streptavidin (1:2500)

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.39 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.755 ml

4. SUGGESTED TITER BY ELISA: 1 to 1.5 µg/ml of biotinylated protein for IgM detection in a capture format.

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, 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.

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 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, pp52, pp52-Biot, pp65, ChimCMV1, ChimCMV1-Biot, ChimCMV2, 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, andM. 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.

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