

# Recombinant chimeric antigen ChimCMV1 for Cytomegalovirus (CMV)

**CATALOG NUMBER:** RAG0109

**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.

**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 31,695.4 Da.

**BATCH COMPOSITION:**

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

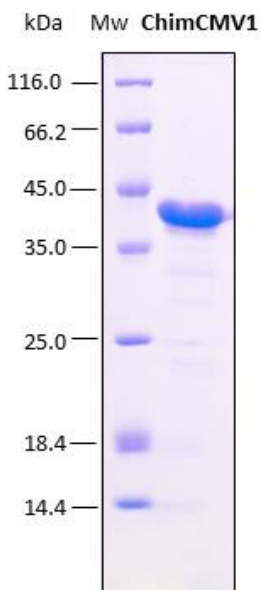
**QUALITY CONTROL:**

## 1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO<sub>280</sub> = 0.648  
 A<sub>0.1%</sub> (=1 g/l) = 0.539  
 CONCENTRATION\*: 1.2 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%

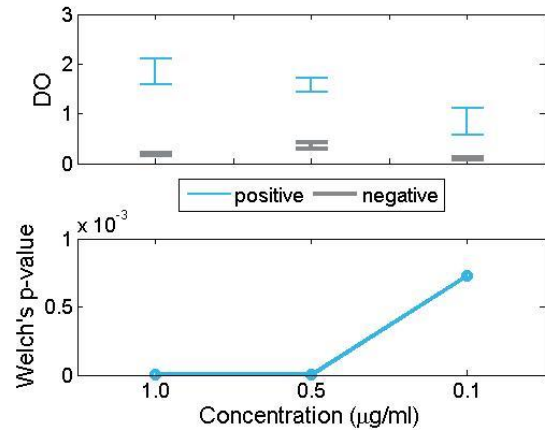


**Figure 1.** SDS-PAGE analysis (15%) of 3 µl of recombinant ChimCMV1. Purity is > 95% as determined by gel electrophoresis.

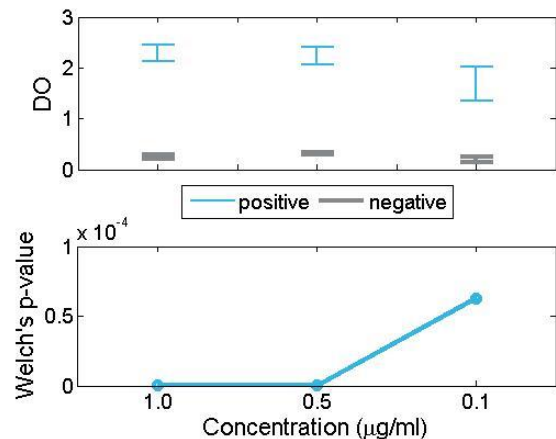
## 3. TITRATION CURVE BY AN ELISA ASSAY

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

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



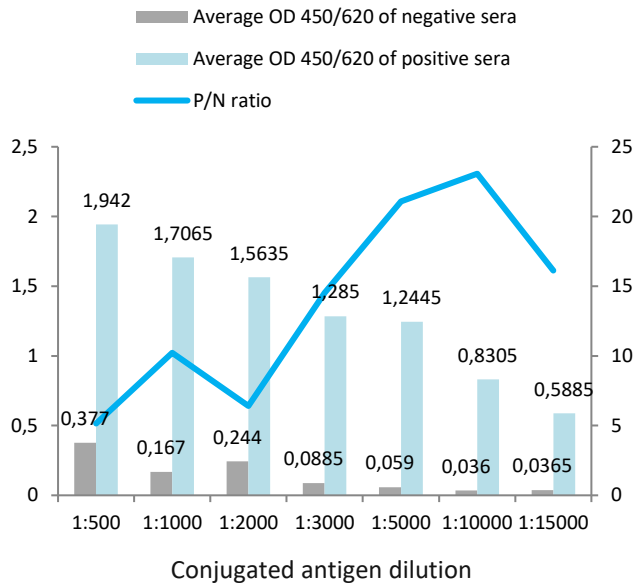
**Figure 2.** In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) CMV **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is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

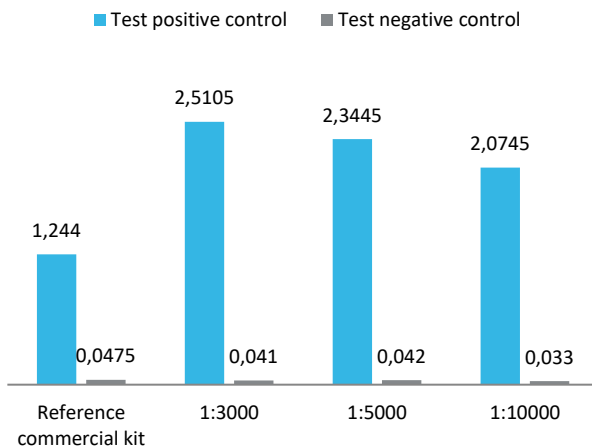
#### 4. TITRATION CURVE BY A CAPTURE ELISA ASSAY

**4.A.** A capture ELISA assay titration was performed by using different dilutions of the Rekom ChimCMV1-HRP.



**Figure 4.** In this plot, the optical density at 450/620 nm obtained in a capture ELISA assay for positive (blue) and negative (gray) CMV **IgM** sera were compared. Seven different conjugated dilutions of the conjugated ChimCMV1 to HRP were tested. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera. Therefore, in the present assay, any of the showed dilutions can be used to distinguish between positive and negative sera (P/N ratio means positive/negative signal ratio).

**4.B** A capture ELISA assay was performed by using three different dilutions of the Rekom ChimCMV1 in order to develop a reference capture commercial test (CMV-IgM-eLA test PKS medac).



**Figure 5.** In this plot, the optical density at 450/620 nm obtained in an ELISA capture assay for a positive (blue) and a negative (gray) CMV **IgM** sera were compared. Two different conjugated dilutions and the reference conjugated antigen were tested in the assay. Eligible dilution for the use of the conjugated antigen should present statistically significant differences between positive and negative sera. In the present assay, any of the showed dilutions can be used to distinguish between positive and negative sera in a capture ELISA assay.

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

#### LOT SPECIFICATIONS:

- 1. CONCENTRATION:** 1.2 mg/ml
- 2. TOTAL QUANTITY PER ALIQUOT:** 1 mg
- 3. TOTAL VOLUME PER ALIQUOT:** 0.875 ml
- 4. SUGGESTED TITER BY ELISA:** 1/12,000, which corresponds to 0.1 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 in order to avoid repeated freezing and thawing cycles and stored at -20°C to -80°C
- 6. APPLICATIONS:** ELISA and Western blot assays. 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 defrost 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, pp72.

#### 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  $\mu$ l 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**