Recombinant multi-epitope early antigen ChimEBV-EA for *Epstein-Barr Virus* (EBV)

CATALOG NUMBER: RAG0082 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: chimeric EBV early antigen (Neuhierl *et al.*, 2006; Mumtsidu *et al.*, 2008).

DESCRIPTION: several antigenic determinants from EBV early antigen have been prepared as a recombinant multi-epitope antigen fused to a his-tag and trxA at the N-terminal.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between molecular markers of 66,200 and 45,000 Da, while relative molecular mass calculated from amino acid sequence is 54,319.48 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
trxA-his-ChimEBV-EA	recombinant antigen with a trxA and his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 7, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} =1.27
$A_{0.1\,\%}$ (=1 g/l) = 0.832
CONCENTRATION*: 1.53 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%

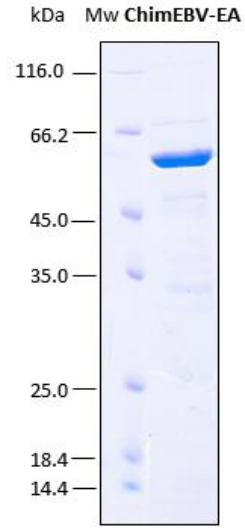

Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant chimeric EBV-EA. Purity is > 95% as determined by gel electrophoresis.

3. ANALYSIS BY AN ELISA ASSAY

To analyze the reactivity of the multi-epitope chimera, an indirect IgG ELISA was performed. The proteins were coated at 18 nM.

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

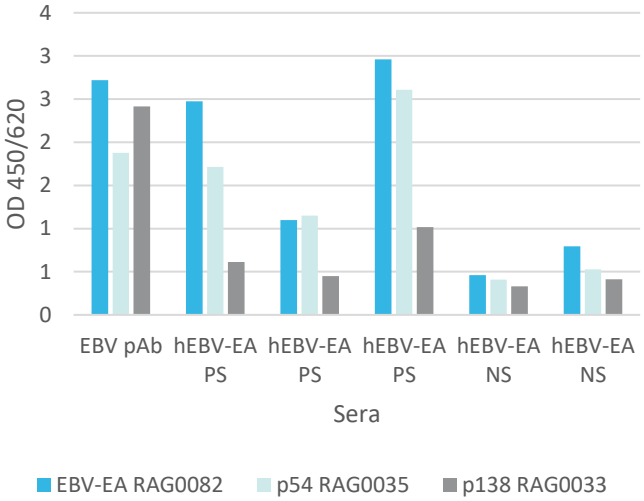

Figure 2. The plot illustrates the analysis of different sera with a coating concentration of 18 nM of EBV-EA chimera (intense blue), p54 (light blue) or p138 (grey). Human sera (hEBV-EA) were EBV IgG pre-validated as positive serum (PS) or negative serum (NS), and also a polyclonal Ab (pAb) against EBV was used. As the graphic shows, positive sera remain positive, sometimes with more reactivity, and negative sera remain negative. Furthermore, the chimera can rescue the positivity when one of the two EA antigens is negative or has lower reactivity. These limited and preliminary results suggest that each epitope of the chimera can recognize and react with its paratope with no steric hindrance with the annexed epitopes.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.53 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.686 ml

4. 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.

5. TESTED APPLICATIONS: ELISA.

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

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
E-mail: info@rekombiotech.com – Web: www.rekombiotech.com – An ISO 9001 and 13485 Certified Company

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:

EBNA1, p138, p18, p18-Biot, p23, p54, ZEBRA, ChimEBV-VCA.

BIBLIOGRAPHY:

Neuhierl, B. and Henri-Jacques Delecluse, H.J. The Epstein-Barr Virus BMRF1 Gene Is Essential for Lytic Virus Replication. *Journal of Virology*, 2006, Vol. 80, No. 10: 5078–5081.

Mumtsidu E, Makhov AM, Konarev PV, Svergun DI, Griffith JD, Tucker PA. Structural features of the single-stranded DNA-binding protein of Epstein-Barr virus. 2008 *J. Struct. Biol.* 161(2):172-87.

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.

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) – Tel: +34 958 63 70 85
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