Recombinant antigen p23 for *Epstein-Barr Virus* (EBV)

CATALOG NUMBER: RAG0002 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: EBV antigen p23 (Färber *et al.*, 2001).

DESCRIPTION: the EBV antigen p23 has been prepared as a recombinant antigen fused to a his-tag. It is produced from the almost complete ORF of the BLRF2 gene which codifies the 23 kDa-EBV tegument protein of 23 kDa.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

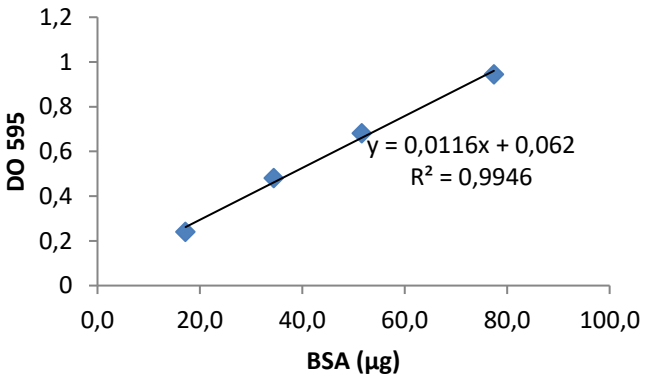
MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is at the molecular marker of 25,000 Da, while relative molecular mass calculated from amino acid sequence is 19,567.3 Da

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-p23	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 8, 0.7 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY



This protein does not contain any Trp residues. Experience shows that this could result in more than 10% error in the computed extinction coefficient. Therefore, we have measured the protein concentration by using the colorimetric assay based on the interaction between Coomassie brilliant blue and the arginine and aromatic residues (Bradford Method) and its maximum absorption shifts from 470 nm to 595 nm. The standard curve was performed with the protein BSA. 40 µl of the protein were analyzed.

DO_{595} = 0.577
CONCENTRATION: 1.11 mg/ml

2. PURITY CONTROL IN SDS-PAGE: 15%

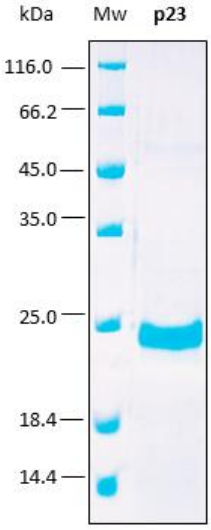


Figure 1. SDS-PAGE analysis (15%) of 3 µl of recombinant p23. Purity is > 95% as determined by gel electrophoresis.

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 his own titration for his particular application.

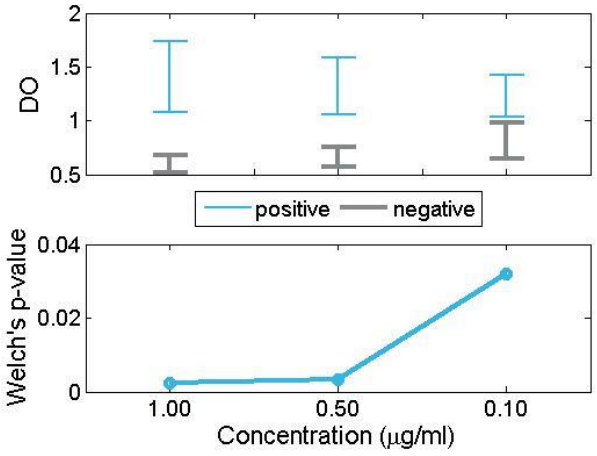


Figure 3. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgM** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed.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

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

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LOT SPECIFICATIONS:

1. CONCENTRATION: 1.11 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.9 ml

4. SUGGESTED TITER BY ELISA: between 1:2,220 and 1:11,100, this corresponds to 0.5 to 0.1 μg/ml of antigen concentration in plates for IgM 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 -20ºC to -80ºC. In order to defrost the protein, maintain the aliquot at 25ºC without shaking to avoid aggregation.

6. TESTED APPLICATIONS: ELISA.

7. POSSIBLE 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. Suggested working dilutions are given as a guide only. It is recommended that the user titrates.

8. OBSERVATIONS: in some cases, purified proteins run at a molecular weight which is slightly different to the theoretically calculated molecular weight maybe due to the his-tag existence, which can produce a delay in SDS-PAGE. 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, p54, ZEBRA, ChimEBV-EA, ChimEBV-VCA..

BIBLIOGRAPHY:

Färber, I., Hinderer, W., Rothe, M. Lang, D., Sonneborn, H.H. and Wutzler, P. Serological diagnosis of Epstein-Barr virus infection by novel ELISAs based on recombinant capsid antigens p23 and p18. 2001, J. Med. Virol, 63: 271-6.

Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. 1976, 131:499-503.

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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