Recombinant antigen glycoprotein G for HSV-2

CATALOG NUMBER: RAG0087 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: glycoprotein G from *herpes simplex virus* type 2 (Dolan *et al*., 1998).

DESCRIPTION: the recombinant gG-2 glycoprotein has been prepared by expressing the gene US4 from HSV-2

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is at the molecular marker of 66,200 Da, while relative molecular mass calculated from amino acid sequence is 35,870.3 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
his-gG2	recombinant antigen with a his-tag in its N-terminus
Storage buffer	20 mM phosphate buffer pH 7 and 0.15 M NaCl

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 0.243
$A_{0.1\,\%}$ (=1 g/l) = 0.195
CONCENTRATION*: 1.25 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: 12%

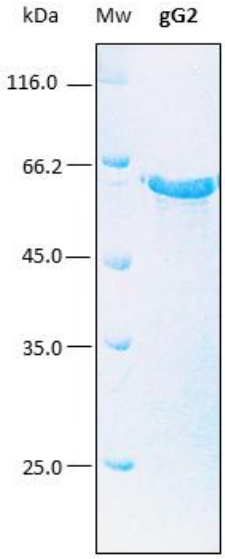


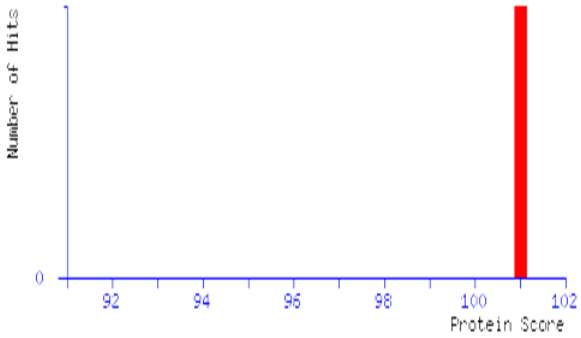
Figure 1. SDS-PAGE analysis (12%) of 2 µl of recombinant gG2. Purity is approx. 95% as determined by gel electrophoresis.

3. PROTEIN FINGERPRINT BY MASS SPECTROMETRY

Top Score: 101 for gG_HHV-2

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores greater than 13 are significant (p<0.05).



gG_HHV-2:
Mass: 35848 **Score:** 101 **Expect:** 7.9e-011 **Matches:** 10

The MS was performed with a by MALDI TOF/TOF model UltrafleXtreme (Bruker).

4. 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 their particular application.

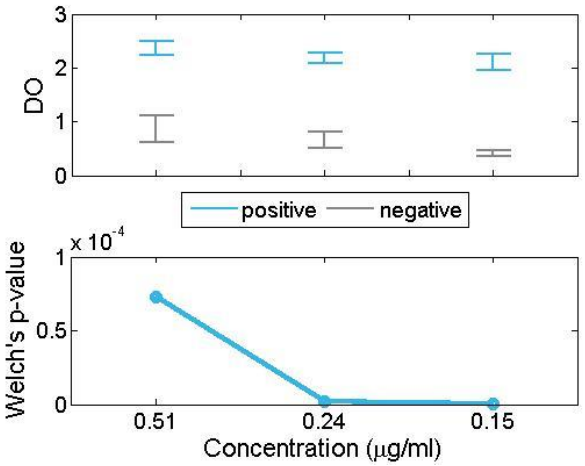


Figure 2. In this plot, the optical density at 450/620 nm for positive (blue) and negative (gray) **IgG** sera are compared for each concentration of the recombinant antigen. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, is employed.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

Rekom Biotech S.L. – BIC-Granada, Avda. Innovación, 1 – 18016, Granada (Spain) Tel: +34 958 63 70 85
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5. ABSENCE OF PRECIPITATION AFTER A FREEZING AND THAWING CYCLE: ok

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.25 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.84 ml

4. SUGGESTED TITER BY ELISA: up to 1:8,833, which corresponds to 0.15 μg/ml of protein in plates for IgG detection (24-15 ng).

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, 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, proteins with high helicity run at a molecular weight which is different to the theoretically calculated weight regarding a globular standard protein. This highly anomalous membrane protein PAGE migration behavior may be due to the hairpin conformation and therefore an altered detergent binding (Rath *et al*., 2008). 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:

gG1 (RAG0017), gG1 (RAG0105), gG1-Biot.

BIBLIOGRAPHY:

Dolan,A., Jamieson,F.E., Cunningham,C., Barnett,B.C. and McGeoch,D.J. The genome sequence of herpes simplex virus type 2.1998. J. Virol. 72 (3), 2010-2021.

Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. *Anal Biochem.* 1989 Nov 1;182(2):319-26.

Arianna Rath, Mira Glibowicka, Vincent G. Nadeau, Gong Chen, and Charles M. Deber. Detergent binding explains anomalous SDS-PAGE migration of membrane proteins. 2009. PNAS, 106:1760-1765.

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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