Recombinant antigen glycoprotein G for HSV-2

CATALOG NUMBER: RAG0087

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:

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>his-gG2</td>
<td>recombinant antigen with a his-tag in its N-terminus</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>20 mM phosphate buffer pH 7 and 1 M NaCl</td>
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</tbody>
</table>

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

\[ \text{DO}_{280} = 0.231 \]
\[ \text{A} 0.1\% = 0.195 \]
\[ \text{CONCENTRATION}^* = 1.18 \text{ mg/ml} \]

* 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: 12.5%

![SDS-PAGE analysis](image)

Figure 1. SDS-PAGE analysis (12.5%) of 1 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 \(-10 \times \log(P)\), where P is the probability that the observed match is a random event. Protein scores greater than 13 are significant (p<0.05).

![Mascot Score Histogram](image)

gG_HHV-2:

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

The MS was performed with a 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 his particular application.

![Titration Curve](image)

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.18 mg/ml
2. TOTAL QUANTITY PER ALIQUOT: 1 mg
3. TOTAL VOLUME PER ALIQUOT: 0.889 ml
4. SUGGESTED TITER BY ELISA: 1:7,900, 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 in order to avoid repeated freezing and thawing cycles and stored at -20ºC to -80ºC.

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: 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 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.

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.

NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY

RELATED PRODUCTS:
None.

BIBLIOGRAPHY:
