Recombinant nucleoprotein (C-term) antigen for SARS-CoV (2003) (NP-CTD)

CATALOG NUMBER: RAG0080 **LOT NUMBER:** #

RECOMBINANT ANTIGEN: SARS-CoV nucleoprotein C-terminal domain (Wang *et al*. 2003). This protein shows 92.5% identity with NP COVID-19.

DESCRIPTION: a recombinant antigen has been prepared by expressing aa 212 to 396 from de nucleoprotein of SARS-CoV.

PRESENTATION: liquid protein solution

SOURCE: *Escherichia coli*

MOLECULAR WEIGHT: determined by SDS-PAGE, the protein band is between the molecular markers of 35,000 Da and 25,000 Da, while relative molecular mass calculated from amino acid sequence is 27,492.87 Da.

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
His-NP-CTD	recombinant nucleoprotein with a his-tag
Storage buffer	20 mM phosphate buffer pH 8, 0.15 M NaCl and 0.1% polyoxyethylene (10) tridecyl ether

QUALITY CONTROL:

1. PROTEIN CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{280} = 1.14
$A_{0.1\%}$ (=1 g/l) = 0.671
CONCENTRATION*: 1.69 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%

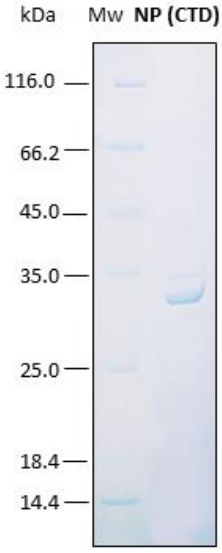

Figure 1. SDS-PAGE analysis (15%) of 1 µl of recombinant NP (CTD). Purity is > 95% as determined by gel electrophoresis.

3. POSITIVE AND NEGATIVE SERA DISCRIMINATION 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. ELISA assays were performed with a serum sample panel of pre-validated 28 SARS-CoV-2 (COVID-19) IgG-positive specimen sera obtained from Andalusian Public health System Biobank and 4 negative pre-COVID specimen sera from general population.

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

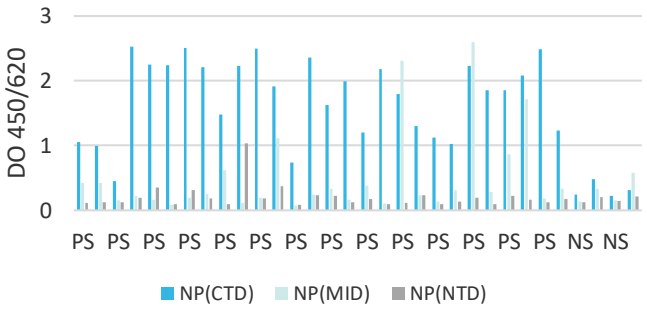

Figure 2. This plot shows an indirect IgG ELISA assay performed with the NP (CTD), NP (MID) and NP (NTD) recombinant antigens. The optical density at 450/620 nm for high-positive sera (pale blue) and negative sera (gray) are displayed in the graphic. For specimen discrimination sera, the coating was performed with 0.5 µg/ml of protein in microtiter plates.

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

LOT SPECIFICATIONS:

1. CONCENTRATION: 1.6 mg/ml

2. TOTAL QUANTITY PER ALIQUOT: 1 mg

3. TOTAL VOLUME PER ALIQUOT: 0.62 ml

4. SUGGESTED TITER BY ELISA: up to 1:3,200, which corresponds to 0.5 µg/ml of protein concentration in plates for IgG 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. 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.

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

8. 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:

S1 (RBD) SarsCoV-2, NP (CTD) SarsCoV-2.

BIBLIOGRAPHY:

Wang *et al*. The structure analysis and antigenicity study of the N protein of SARS-CoV. 2003. Genomics Proteomics Bioinformatic, 1 (2): 145-54.

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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