SARS-CoV-2 Nucleocapsid protein (NP) High-sensitivity Quantitative ELISA Kit (CAT NO: 41A228R)

For the quantitative determination of SARS-CoV-2 Nucleocapsid protein (NP) ELISA in serum, plasma or other specimen

This package insert must be read in its entirety before using this product

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SARS-CoV-2 Nucleocapsid protein (NP) High-sensitivity Quantitative ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of SARS-CoV-2 Nucleocapsid protein

Catalog Number: 41A228R

(Please read this instruction manual carefully before use.)

WARNING! Wear appropriate protective eyewear, clothing and gloves.

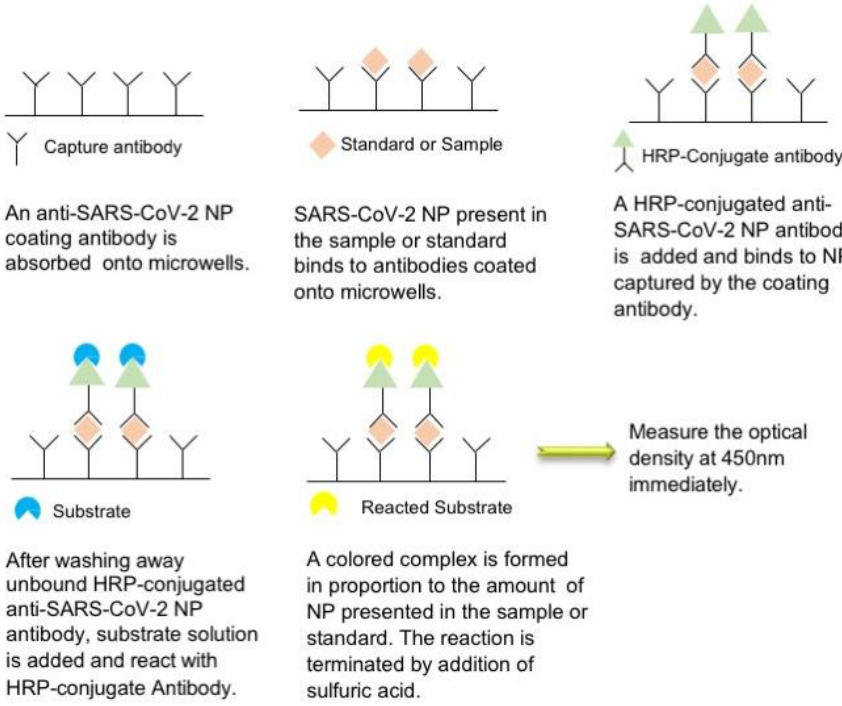
BACKGROUND

SARS-CoV-2 is an enveloped virus with a positive-sense RNA genome and a nucleocapsid of helical symmetry. During the assembly of virions, Nucleocapsid protein (NP) binds to viral RNA and leads to formation of the nucleocapsid. Because NP is the most abundant protein of coronavirus and has strong immunogenicity, NP can serve as a diagnostic marker for SARS-CoV-2 carriers. SARS-CoV-2 (2019-nCoV) Nucleocapsid protein (NP) ELISA Kit is a highly sensitive and specific immunoassay developed by ImmunoDiagnostics for the precision detection and quantitative measurement of SARS-CoV-2 NP in biological specimen.

INTENDED USE

This kit has been verified by high purity SARS-CoV-2 Nucleocapsid protein/NP(Cat#41A220,https://www.immunodiagnostics.com.hk/product-page/covid-19-nucleoprotein-np) and serum from healthy people and COVID-19 patients. It can recognize recombinant SARS-CoV-2 Nucleocapsid protein/NP or NP in serum or other samples. This kit can be used for research purpose.

ASSAY PRINCIPLE



REAGENTS SUPPLIED

Each kit is sufficient for 96 tests and contains the following components:

1. One aluminum pouch with a Microwell plate (12 strips of 8 wells each) coated with an antibody against SARS-CoV-2 NP, sealed.
2. 10×Wash buffer-40 ml.
3. 1×Assay buffer-30 ml.
4. 100×Detection antibody solution: HRP-labelled anti-NP antibody, 0.12 ml.
5. SARS-CoV-2 NP standard-2000 pg of SARS-CoV-2 NP in a buffered protein base, lyophilized.
6. Substrate solution, 12 ml, ready for use.
7. Stop solution, 12 ml, ready for use.
8. Plate cover, 1

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. Beakers, flasks, cylinders necessary for preparation of reagents.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Statistical calculator with program to perform regression analysis.
8. A horizontal micro-plate shaker

STORAGE

- The kit should be stored at 2-8℃, and all reagents should be equilibrated to room temperature before use. Immediately after use remaining reagents should be returned to cold storage (2-8℃).
- Expiry of the kit and reagents is stand on labels.
- Once opened, the strips may be stored at 2-8℃ for up to one month.

SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

- Serum and plasma were tested with this assay. Other body fluids or cell culture medium might also be suitable for use in the assay. Dilution factor needs to be determined according to the abundance of NP in the samples. 2-100fold dilution is recommended for serum/plasma samples from COVID-19 infected patients.
- Do not use grossly hemolyzed or lipemic samples.
- Samples should be aliquoted and must be stored frozen at -20℃, and avoid repeated freeze-thaw cycles.

PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. Avoid contact with skin and eyes. In the case of contact with skin or eyes wash with water.
- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light.
- Do not pipet by mouth.
- Do not eat or smoke in area where kit reagents or samples are handled.

- Avoid contact of substrate solution with oxidizing agents and metal.
- Use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Substrate solution must be at room temperature prior to use.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before use

1. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37℃ water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8℃ for up to one month.

2. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 μL of 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8℃ immediately after the necessary volume is pipetted.

PREPARATION OF STANDRADS AND SAMPLES

SARS-CoV-2 NP standard:

Reconstitute the lyophilized standard with 1 ml of 1 × assay buffer to generate the first standard solution of 2000 pg/ml. Allow the standard to sit for 10 minutes with gentle agitating prior to making dilutions. Prepare serially diluted standards using 1 × assay buffer as follow:

Standard Volume	Volume of 1 × assay buffer	Concentration
2000 pg/ml	-	2000 pg/ml
250 µL of 2000 pg/ml	250 µL	1000 pg/ml
250 µL of 1000 pg/ml	250 µL	500 pg/ml
250 µL of 500 pg/ml	250 µL	250 pg/ml
250 µL of 250 pg/ml	250 µL	125 pg/ml
250 µL of 125 pg/ml	250 µL	62.5pg/ml
250 µL of 62.5 pg/ml	250 µL	31.25 pg/ml

1x Assay buffer serves as the zero standard (0 ng/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -20℃ for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation:

Serum, plasma or the other specimen can be measured. A 2 to 100-fold dilution in 1×Assay buffer is recommended, depending on the NP concentrations in the samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µl of standard or sample per well, seal the plate with a plate cover. Incubate at room temperature for 1 hour shaking at 600 rpm on a horizontal micro-plate shaker. (Preferably shaking at incubation step which can enhance the signal)
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total of 4 times.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Determine the optical density of each well at 450 nm immediately.

CALCULATION OF RESULTS

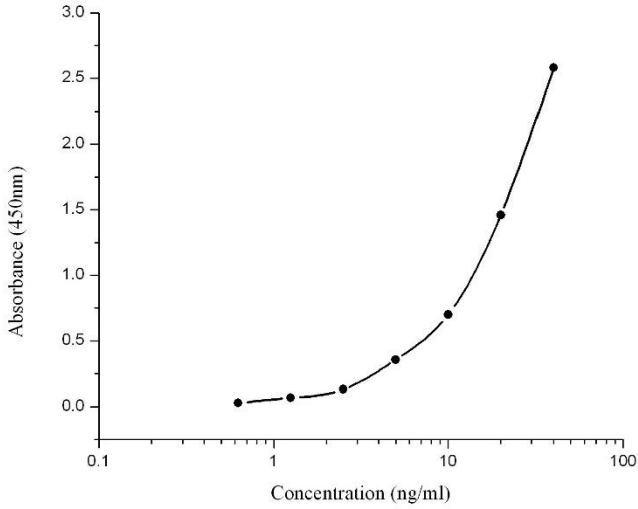
If a sample has a NP level greater than the highest standard, the sample should be diluted further, and the assay should be repeated.

- Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four-parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate.

TYPICAL RESULTS

SARS CoV-2 NP standard curve (4-parameters)



Sample	Absorbance	Concentration (pg/ml)	Calculate NP concentration (pg/ml)
Undiluted serum from healthy control	0.112	——	——
1:10 dilution of serum from healthy control	0.126	——	——
1:10 diluted serum with 2000 pg/ml NP	2.974	1831	1831
1:4 dilution of serum from severe COVID-19 patient A	0.732	418.267	1673.067
1:4 dilution of serum from severe COVID-19 patient B	0.478	248.933	995.733
1:4 dilution of serum from severe COVID-19 patient C	0.432	218.267	873.067
1:4 dilution of serum from mild COVID-19 patient D	0.694	392.933	1571.733
1:4 dilution of serum from mild COVID-19 patient E	0.513	272.267	1089.067

SPIKING AND RECOVERY

Recovery was determined by spiking different concentration of SARS-CoV-2 NP into samples listed below. Mean recoveries are as follows:

Sample Type	Average of Recovery (%)	Range (%)
Serum	91.5	80-103
Plasma	92	83-106
Cell culture medium	85.4	80-90.8

REPRODUCIBILITY

Intra-assay CV%: <5%;
Inter-assay CV%: <6%.

SENSITIVITY

The minimum detectable dose (MDD) of SARS-CoV-2 Nucleoprotein / NP is typically less than 5 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant SARS-CoV-2 Nucleoprotein/NP produced at ImmunoDiagnostics Inc. (Cat# 41A220).

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of standard or sample to each well.

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Incubate at room temperature for 1 hour shaking at 600rpm).

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Aspirate and wash each well four times.

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Add 100 µl of 1×Detection antibody solution to each well.

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Incubate at room temperature for 1 hour.

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Aspirate and wash each well four times.

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Add 100 µl of substrate solution to each well.

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Incubate at room temperature for 15 minutes

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Add 100 µl of Stop solution to each well.

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Measure absorbance of each well at 450 nm.