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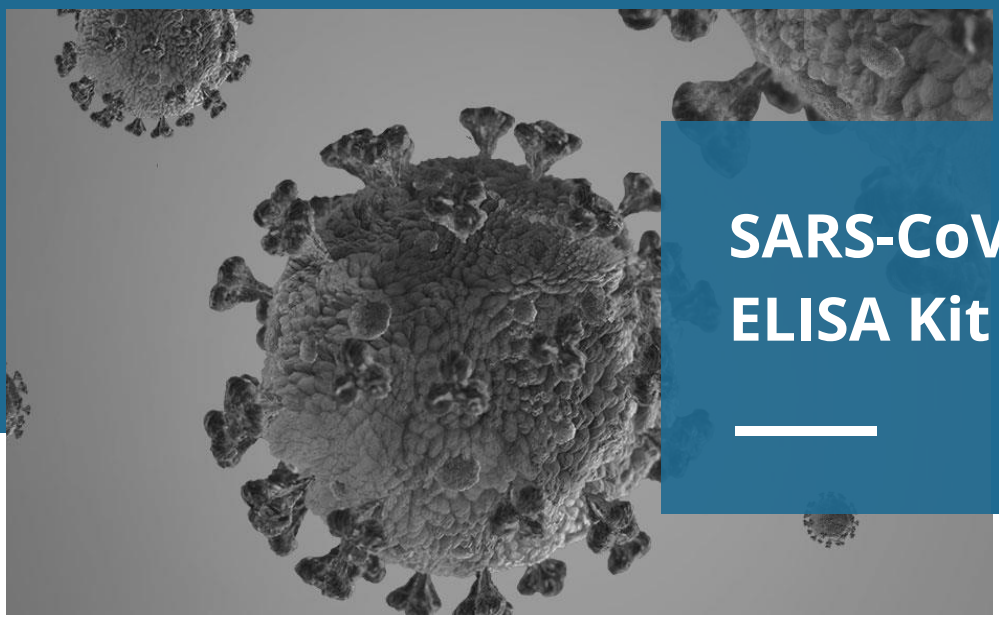


SARS-CoV-2 NP Antigen ELISA Kit (DEIA2020)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Size 96T

Intended Use

This kit is intended for the quantitative detection of the recombinant SARS-COV-2 nucleoprotein antigen in human serum. The use of this kit for natural samples need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

This assay is **not** FDA cleared or approved and is for research use only.

Principles of Testing

The SARS-COV-2 NP antigen detection kit is based on the principle of a double antibody sandwich enzyme- linked immunoassay. The microplate is pre-coated with an anti-SARS-CoV-2 N protein antibody. When the serum to be detected contain the SARS-CoV-2 NP antigen, it will bind to the specific antibody coated on the microplate. After the HRP labeled secondary antibody against the SARS-CoV-2 NP was added, an "antibody- antigen-enzyme- labeled antibody" complex is formed. After adding the chromogenic substrate TMB, HRP enzyme will catalyze the color development. After terminating the solution, the microplate reader will determine the Absorbance value to determine the presence or absence of SARS-CoV-2 NP in each test sample.

Reagents and Materials Provided

Component	Content	Quantity
The Antibody Coated Microplate	8 x 12 strips in a ziplock foil pouch with desiccant, containing 96 polystyrene microtiter wells coated with anti NP antibody in each well. Stable at 2-8°C until the expiration date.	1

SARS-CoV-2 NP Standard	One vial, 2 μg lyophilized recombinant NP. Stable at 2-8°C until the expiration date. After the standard is dissolved, store it at -80 ° C. Do not freeze or thaw repeatedly.	1
Sample Diluent	One bottle, 24 mL. Stable at 2-8°C until the expiration date.	1
HRP-Enzyme Conjugate (100 ×)	One vial, 120 μL, containing horseradish peroxidase-labeled NP monoclonal antibody. Stable at 2-8°C until the expiration date.	1
Conjugate Diluent	One bottle, 12 mL. This contains the diluent solution for the 100X Conjugate. The 100× conjugate is diluted directly into this solution. After diluting 100× Conjugate into this solution, the now ready-to-use conjugate may be stored up to 2 weeks at 2-8°C before it should be discarded. Stable at 2-8°C until the expiration date.	1
Wash Buffer (20 x)	One bottle, 50 mL. 20 x concentrated phosphate buffered saline with Tween 20 (pH 6.8-7.0). Stable at 2-8°C until the expiration date.	1
TMB Solution	One bottle, 12 mL, ready to use. Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric acid-citrate buffer (pH 3.3-3.8). Stable at 2-8°C until the expiration date.	1
Stop Solution	One bottle, 6 mL, read to use. 2M Sulfuric Acid. Used to stop the reaction. Stable at 2-8°C until the expiration date. *Warning: Strong acid. Wear protective gloves, mask and safety glasses. Dispose all materials according to all applicable safety rules and regulations*	1
Plate Cover		2

Materials Recommended but Not Provided

1. ELISA spectrophotometer capable of absorbance measurement at 450 nm
2. Biological or high-grade water
3. Automatic plate washer
4. 37°C incubator without CO2 supply
5. 1-10 μL single-channel pipettors, 50-200 μL single- and multichannel pipettors
6. Polypropylene tubes or 96 well dilution plates
7. Timer
8. Vortex

Storage

The kit should be stored at 2 ~ 8 °C, protected from light, and valid for 6 months.

Sample Collection

1. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; GP44).
2. Testing should be performed as soon as possible after collection. Do not leave serum at room temperature for prolonged periods. Separated serum should remain at 20-25°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated at 2-8 °C. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours, serum should be frozen at or below -20 °C. Do not add preservative sodium azide.
3. Avoid repeated freezing and thawing of samples more than four times as this can cause analyte deterioration. Frost-free freezers are not suitable for sample storage.
4. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.

Reagent Preparation

1. Preparation of 1x Wash Buffer

   Dilute the 20X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 50 mL 20X wash buffer with 950 mL distilled (or deionized) water. Mix thoroughly to ensure that any precipitate is dissolved and that the solution is uniform. Once diluted to 1X, the solution can be stored at room temperature for up to 6 months. Properly label the 1X wash buffer solution and carefully note the expiration date on the label. Check for contamination prior to use.

   Discard if contamination is suspected.

2. Preparation Conjugate Solution

   Add 120 µL of 100x HRP-Enzyme Conjugate directly to the 12 mL bottle of Conjugate Diluent for

   SARS-CoV-2 NP (1 part : 100 parts). Alternatively, use a clean pipette to remove the required volume of Conjugate Diluent and add the necessary volume of 100x Conjugate into a clean polypropylene test tube in order to maintain the 1:100 ratio. Mix by inverting the solution several times. *This solution may be stored for up to 2 weeks if stored at 2-8 C. After 2 weeks, this conjugate solution should be discarded and no longer used in this assay.*

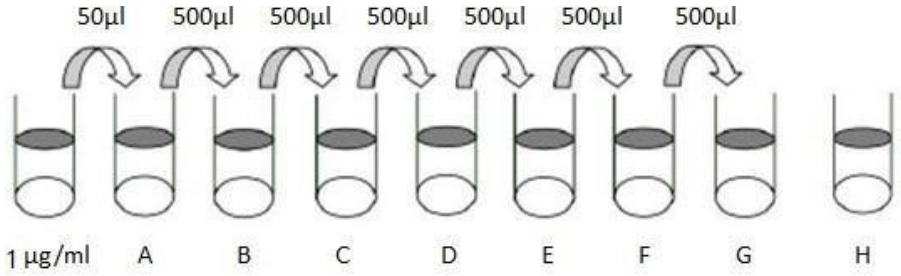
3. Preparation of Standard Solution

   Add 2 mL of the standard diluent to the lyophilized standard vial and mix thoroughly to prepare a 1 µg/mL standard. Add 50 µL of 1 µg/mL standard into a tube with 950 µL Sample Diluent to obtain a 50 ng/ml standard. Then Pipette 500 µL of Sample Diluent into each tube. Use the 50 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Sample diluent serves as the zero standard (0 ng/ml).

Notes:

1. Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.
2. After the standard is dissolved, please store it at -80 ° C. Do not freeze or thaw repeatedly.

3. To ensure the accuracy of the experiment, it is recommended that the volume of the standard gradient is not less than 500 µL each time.



		A	B	C	D	E	F	G	H
Diluent Vol.	2 mL	950 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
Conc. (ng/mL)	1 µg/mL	50	25	12.5	6.25	3.125	1.5625	0.78	0

Assay Procedure

1. Equilibrium: Recover samples, microplates and required reagents for 30 minutes at room temperature.
2. Sample incubation: Fix the required microwells on the rack. Add **100 µL** of each serially diluted protein standards (0, 0.78, 1.5625, 3.125, 6.25, 12.5, 25, 50 ng/mL) to the set wells. Add **50 µL** of the sample diluent and **50 µL** of the serum samples to the other wells. Cover the plate and incubate at **37 °C** for **60 minutes**.
3. Washing: Discard the liquid in each well, fill the microwells (300 µL/well) with 1x wash buffer, and discard the liquid in the well after standing for 30 seconds; repeat 5 times, and pat dry on the tissue paper after the last washing.
4. Prepare the Conjugate Solution (120µL of 100X Conjugate: 12mL of Conjugate Diluent) and add **100 µL** per well of this Conjugate Solution into all wells using a multi-channel pipettor. Seal the plate with a plate cover and place it in 37 °C, incubate for **30 minutes**.
5. Washing: Discard the liquid in each well, fill the microwells (300 µL/well) with 1x wash buffer, and discard the liquid in the well after standing for 30 seconds; repeat 5 times, and pat dry on the tissue paper after the last washing.
6. Color development: Add **100 µL** of TMB substrate to each well, mix by shaking slightly, and set the color at 37 °C in the dark for **30 minutes**.
7. Measurement: Add **50 µL** of stop solution to each well. Select the microplate reader with the main wavelength of 450nm and the reference wavelength of 630 nm to determine the absorbance of each well.

Quality Control

50 ng/mL standard OD ≥ 1.5, 0 ng/mL standard OD ≤ 0.10, this test is valid; otherwise, it is deemed invalid, and repeated tests should be performed.

Calculation

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

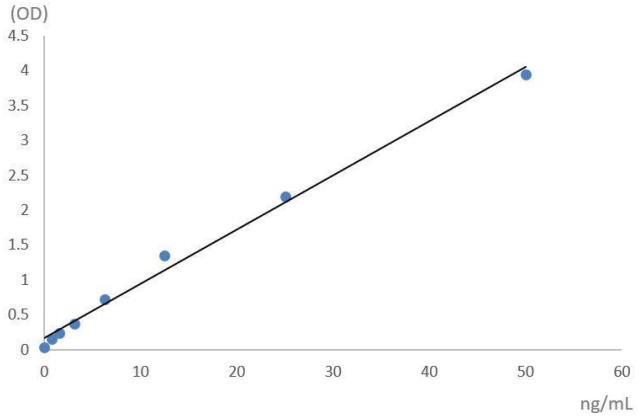
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

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. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Standard Concentration (ng/mL)	Absorbance Values
50	3.9481
25	2.2007
12.5	1.3513
6.25	0.7215
3.125	0.3775
1.5625	0.2461
0.78	0.1517
0	0.0333



Analytical Reactivity Study

The LOD of this kit is 1 ng/mL of SARS-COV-2 nucleoprotein.

The analytical reactivity was performed to determine the limits of detection (LODs) of SARS-CoV-2 NP ELISA when tested with recombinant NP diluted in an COVID-19 negative serum. The LOD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Recovery

Dilute the N protein antigen with 25% serum samples to obtain the N protein concentration in three gradients and determine the recovery efficiency of the standard. The results are as follows:

Sample (ng/mL)	Average Recovery	Recovery Range
50 ng/mL	84.4%	80.6%-86.1%
25 ng/mL	88.6%	85.8%-91.8%
12.5 ng/mL	81.5%	79.8%-84.7%

Precision

Three samples of known concentration were tested twelve times on one plate to assess intra-assay precision and were tested in five separate assays to assess inter-assay precision. to evaluate the precision in the kit batch:

Intra-assay Precision

Sample (ng/mL)	n	CV (%)
80 ng/mL	12	4.25
20 ng/mL	12	6.64
5 ng/mL	12	8.44

Inter-assay Precision

Sample (ng/mL)	n	CV (%)
80 ng/mL	6	4.13
20 ng/mL	6	7.25
5 ng/mL	6	13.44

Limitations

1. This product is only used for testing of serum or plasma samples.
2. The test results of this product are for clinical reference only, and they should not be used as the sole basis for the diagnosis of new coronavirus.
3. Due to the window effect of the virus infection and the sensitivity of the kit detection, Samples collected from patients within five (5) days after the onset of clinical symptoms should be evaluated with this assay (day 0 – day 5). A negative test result cannot exclude the possibility of virus infection.

Precautions

1. The kit should be taken out from the refrigerated environment and should be equilibrated to room temperature before being opened for use. The reagent should be thoroughly shaken before use.
2. The strips of the kit can be removed, and the unused pre-coated strips should be sealed in a desiccant bag.
3. Do not mix reagent components from different batches of the kit.
4. If crystals appear in the 20-fold concentrated washing solution, they should be placed at 37 ° C until the crystals are completely dissolved before use.
5. Read the results of the microplate reader within 30 minutes after the reaction is terminated.
6. This kit and all waste in the test are potentially contaminating and should be treated strictly in accordance with medical contamination.

Trouble Shooting

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C. The reconstituted standards should be aliquoted and avoid repeated freeze-thaw cycles.
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	Color Reagent should be clear and colorless prior to addition to wells	Color Reagent should be clear and colorless priorto addition to wells
	Use clean tubes and pipettes tips	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples