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User's Manual

COVID-19 / SARS-COV-2 Nucleocapsid Protein ELISA Kit

REF **DEIA-SY2335**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The COVID-19 / SARS-COV-2 Nucleocapsid Protein ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of COVID 19 N Protein in serum (plasma is not recommended in this assay) and cell culture supernatants.

General Description

The spike protein (S-protein) and nucleocapsid protein (N-protein) are encoded by all coronaviruses, including the coronavirus (COVID-19). Nucleocapsid protein is a most abundant protein of coronavirus. The nucleocapsid protein is a structural protein that binds to the coronavirus RNA genome, thus creating a shell (or capsid) around the enclosed nucleic acid. The N protein of coronavirus is chosen as a diagnostic tool since its strong immunogenicity.

Principles of Testing

This assay employs an antibody specific for COVID-19 N Protein coated on a 96-well plate. Standards and samples are pipetted into the wells and COVID-19 N Protein present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-COVID-19 N Protein antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of COVID-19 N Protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. **COVID-19 N Protein Microplate:** 96 wells (12 strips × 8 wells) coated with anti-COVID-19 N Protein. Store at 4°C for 1 month.

   Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
2. **COVID-19 N Protein Standard Protein:** 2 vials of COVID-19 N Protein. 1 vial is enough to run each standard in duplicate. Store at -80°C for 1 week.
3. **COVID-19 N Protein Detection Antibody:** 2 vials of biotinylated anti- COVID-19 N Protein. Each vial is enough to assay half the microplate. Store at 4°C for 5 days.
4. **Wash Buffer:** 25 ml of 20× concentrated solution. Store at 4°C for 1 month.
5. **HRP-Streptavidin:** 200 µl 100× concentrated HRP-conjugated streptavidin. Do not store and reuse.
6. **TMB One-Step Substrate Reagent:** 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
7. **Stop Solution:** 8 ml of 0.2 M sulfuric acid.
8. **Assay Diluent B:** 15 ml of 5× concentrated buffer. Store at 4°C for 1 month.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

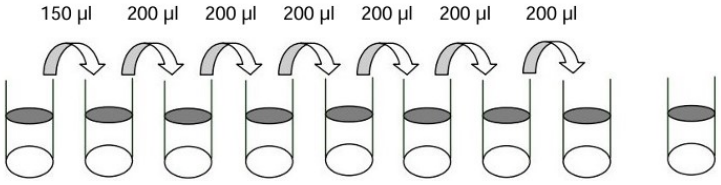
Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see 'Reagents And Materials Provided'.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1× Assay Diluent B should be used for dilution of serum, and cell culture supernatant samples. Suggested dilution for serum sample: at least 4 fold.

   Note: Levels of COVID-19 N Protein may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of standard protein. Add 600 µl 1× Assay Diluent B (should be diluted 5-fold with deionized or distilled water before use) into the Standard Protein vial to prepare a 200 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 150 µl of the COVID-19 N Protein standard solution from the vial of Standard Protein, into a tube with 450 µl 1× Assay Diluent to prepare a 50 ng/ml standard solution. Pipette 400 µl 1× Assay Diluent B 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. 1× Assay Diluent B serves as the zero standard (0 ng/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Standard + 600 µl	450 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	200 ng/ml	50 ng/ml	16.67 ng/ml	5.556 ng/ml	1.852 ng/ml	0.617 ng/ml	0.206 ng/ml	0.069 ng/ml	0 ng/ml

5. If the Wash Buffer (20×) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer into deionized or distilled water to yield 400 ml of 1× Wash Buffer.

6. Briefly spin the Detection Antibody vial before use. Add 100 µl of 1× Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1× Assay Diluent B and used in step 5 of Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 100-fold with 1× Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 100 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1× Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1× prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

Summary

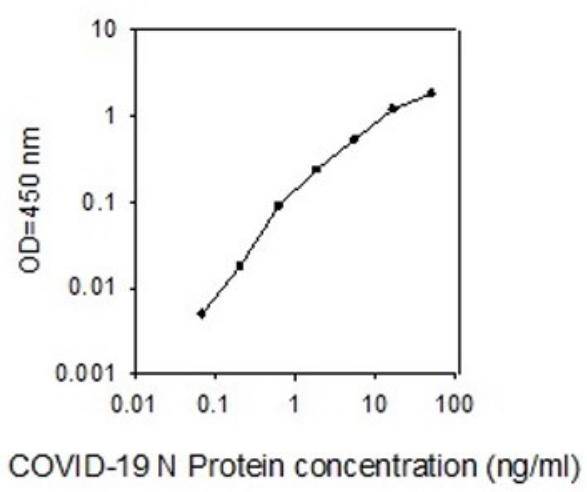
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the

average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

These standard curves are for demonstration only. A standard curve must be run with each assay.



Detection Range

0.07 ng/ml - 50 ng/ml

Sensitivity

The minimum detectable dose of COVID-19 N Protein was determined to be 0.07 ng/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Linearity

Sample Type		Serum	Cell Culture Media
1:2	Average % of Expected	111.8	100.1
	Range (%)	103-120	96-104
1:4	Average % of Expected	121.4	82.61
	Range (%)	106-137	76-89

Recovery

Recovery was determined by spiking various levels of COVID-19 N Protein into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	97.90	83-111
Cell culture media	87.26	83-92

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

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