ATAGENIX LABORATORIES

SARS-CoV-2 (2019-nCoV) Nucleocapsid ELISA Kit

Catalog Number:ATK00013

PRODUCT SUMMARY

SARS-CoV-2 (2019-nCoV) Nucleocapsid ELISA Kit is a sandwich solid phase ELISA designed to measure Nucleocapsid protein in bronchoalveolar lavage fluid and nasopharyngeal swab samples. The microplate provided in this kit has been pre-coated with Nucleocapsid monoclonal antibody. Standards or samples are then added to the appropriate wells with a biotin-conjugated antibody specific to Nucleocapsid. Next, Streptavidin Horseradish Peroxidase (SA-HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Nucleocapsid, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The concentration of Nucleocapsid in the samples is then determined by comparing the O.D. of the samples to the standard curve.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

OTHER SUPPLIES REQUIRED

• Microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 540nm or 570nm.

• Pipettes and pipette tips.

• Deionized or distilled water.

• 500 mL graduated cylinder.

• Squirt bottle, manifold dispenser, or automated microplate washer.

• Tubes for dilution of standards.

COMPONENT

PART	CATALOG	Description	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
SARS-CoV-2 Nucleocapsid Microplate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for SARS-CoV-2 Nucleocapsid.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. Store at -20 ℃.
SARS-CoV-2 Nucleocapsid Standard	1 vial	Recombinant SARS-CoV-2 Nucleocapsid in a buffered protein base with preservatives; lyophilized. Dissoluted in 2ml Diluent Buffer before used. Final concentration was 80ng/mL	Store at -20 ℃. Avoid repeated freeze-thaw cycles.
SARS-CoV-2 Nucleocapsid Biotin-Conjugate Antibody	1 vial	120µL monoclonal antibody specific for SARS-CoV-2 Nucleocapsid conjugated to Biotin with preservatives; 1:100 diluted by Diluent Buffer before used.	Store at -20 ℃
Streptavidin -HRP	1 vial	120µL Streptavidin conjugated to HRP with preservatives; 1:100 diluted by Diluent Buffer before used.	Store at -20 ℃
Diluent Buffer	1 vial	25 mL buffer with preservatives.	Stored at 4 ℃
Wash Buffer Concentrate	1 vial	25 mL 20-fold concentrated solution of buffered surfactant with preservative. 1:20 diluted by pure water before used.	Stored at room temperature
Substrate Reagent	1 vial	12 mL/vial of TMB (tetramethylbenzidine)	Stored at 4 ℃ (Protect from light)
Stop Solution	1 vial	6 mL 2M sulfuric acid.	Stored at 4 ℃
Plate Sealers	4 strips	Adhesive strips.	N/A

OPERATING PROCEDURES

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buff er Concentrate to 380 mL of deionized or distilled water to prepare 400 mL of Wash Buff er.

Substrate Solution - Protect from light. 100 µL is required per well.

SARS-CoV-2 Nucleocapsid Standard - Refer to the vial label for reconstitution volume.

Reconstitute the SARS-CoV-2 Nucleocapsid Standard with 2ml Dilution Buffer. This reconstitution produces a stock solution of 80ng/mL. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Pipette 400µL of 80ng/mL Standard into the 80ng/mL tube. Pipette 400µL of the appropriate calibrator diluent into each remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted SARS-CoV-2 Nucleocapsid Standard (80ng/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0pg/mL). Total 8 standard points, 80ng/mL, 40ng/mL, 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0ng/ mL.

SARS-CoV-2 Nucleocapsid Biotin-Conjugate Antibody Preparation: Dilute SARS-CoV-2 Nucleocapsid Biotin-Conjugate Antibody by Dilution Buffer with a volume ratio of 1:100. For example, dilute 100 µ L of Biotin conjugated ACE2 with 9,900 µ L of Reagent Dilution Buffer to make 10mL of SARS-CoV-2 Nucleocapsid Biotin-Conjugate Antibody solution.

Streptavidin-HRP Preparation: Dilute Streptavidin-HRP by Dilution Buffer with a volume ratio 1:100. For example, dilute 100 µ L of Streptavidin-HRP with 9,900 µ L of Reagent Dilution Buffer to make 10mL of Streptavidin-HRP solution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 µL of standard, control, or samples per well. Cover with the adhesive strip provided. Incubate for 1 hours at 37℃. A plate layout is provided to record standards and samples assayed.

4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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 SARS-CoV-2 Nucleocapsid Biotin-Conjugate antibody to each well. Cover with a new adhesive strip. Incubate for 0.5 hours at 37℃.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of Streptavidin-HRP to each well. Cover with a new adhesive strip. Incubate for 0.5 hours at 37℃.

8. Repeat the aspiration/wash as in step 4.

9. Add 100 µL of Substrate Reagent to each well. Incubate for 10 minutes at 37℃. Protect from light.

10. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

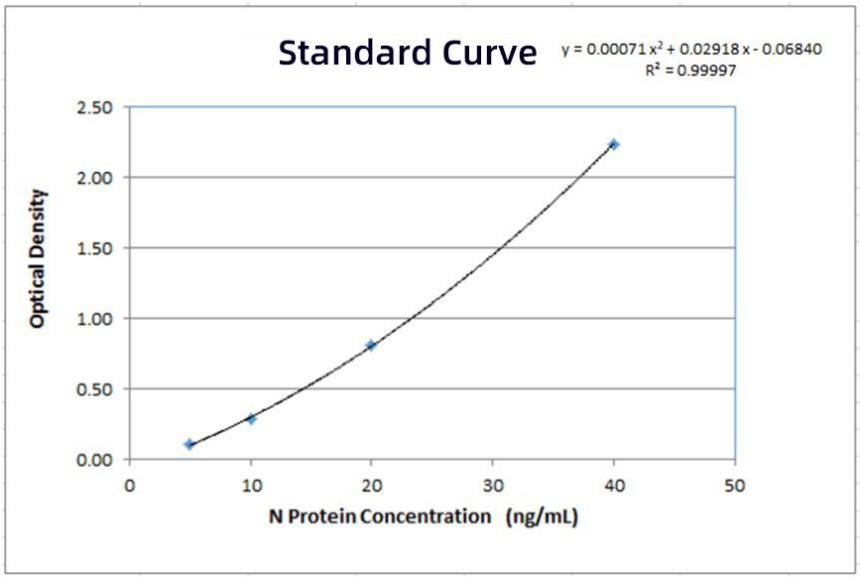
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, 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. The data may be linearized by plotting the log of the SARS-CoV-2 Nucleocapsid concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



Detection range

5ng/mL—80ng/mL

Sensitivity

The minimum detectable dose (MDD) of SARS-CoV-2 Nucleocapsid is typically less than 1ng/mL. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Intro-Assay: CV<12%

Inter-assay: CV<15%

Stability

The kit can be stored at the recommended temperature for 6 months, and the signal intensity decreases by less than 10%.

NOTE

For research use only .Not for use in clinical diagnostic procedures.