ATAGENIX LABORATORIES

Catalog Number:ATK00013

2019-nCoV Nucleocapsid ELISA Kit

Product Details

Summary

Product name	2019-nCoV Nucleocapsid ELISA Kit
description	This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for nucleocapsid protein has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any nucleocapsid protein present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labeled monoclonal antibody specific for nucleocapsid protein is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, a streptavidin- horseradish peroxidase is added to the wells and color develops in proportion to the amount of nucleocapsid protein bound in the initial step. The color development is stopped and the intensity of the color is measured. This assay recognizes natural and recombinant nucleocapsid protein in bronchoalveolar lavage fluid, nasopharyngeal swab samples or recombinant samples.
Stability &Storage	After receiving the kit, please store the standard, detection reagent A, detection reagent B, and pre-packaged plates at -20°C, and store the sealing film at room temperature, and store the remaining reagents at 4°C for later use. The kit is stored at the recommended temperature for 6 months, and the signal intensity decreases by less than 10%

Standard Operating Procedure

OTHER SUPPLIES REQUIRED

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

• Pipettes and pipette tips.

• Deionized or distilled water.

• 500 mL graduated cylinder.

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

• Test tubes for dilution of standards.

Catalog Number:ATK00013

2019-nCoV Nucleocapsid ELISA Kit

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. 20-fold Wash Buffer Concentrate: if there is crystal precipitation in the Wash Buffer Concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

2. Reconstitute the Standard with 2mL Standard/Sample Dilution Buffer, The undiluted nucleocapsid protein Standard (80 ng /mL) serves as the high standard. The multiple proportion dilution of the standard was selected. The concentration of the 7 standard sample were 80ng/mL, 40ng/mL, 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL respectively. The appropriate calibrator diluent serves as the Standard/Sample Dilution Buffer (0 pg/mL).

3. Detection A (biotin labeled) shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection A 1:100 to the working concentration with Reagent Dilution Buffer.

4. Detection B (HRP labeled) shake and mix before used, Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection B 1:100 to the working concentration with Reagent Dilution Buffer.

5. Color Reagent TMB: Protect from light, Add 100μL of Color Reagent TMB to each well. Incubate for 5-10 minutes at room temperature.

6. Stop solution: 50μL/well after color development.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 . Avoid repeated freeze-thaw cycles.

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 100μL of standard, control, or samples per well. Cover with the adhesive strip provided. Incubate for 1 hour at 37.

4. Aspirate each well and wash, repeating the process three times. 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 100μL of Detection A (biotin labeled) antibody to each well. Cover with a new adhesive strip. Incubate for 0.5 hour at 37.

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

7. Add 100μL of Detection B (HRP labeled) to each well. Cover with a new adhesive strip. Incubate for 0.5 hour at 37.

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

9. Add 100μL of Color Reagent TMB to each well. Incubate for 5-10 minutes at 37. Protect from light.

10. Add 50μL 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 10 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. 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 nucleocapsid protein 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.

LINEARITY

1.25ng/mL—80ng/mL

SENSITIVITY

The minimum detectable dose (MDD) of nucleocapsid protein 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

Intra-Assay Precision (Precision within an assay): <12%

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): <15%

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

Stability

When the kit was stored at the recommended temperature for 6 months, the signal intensity decreased by less than 10%.

Component and Instruction

MATERIALS PROVIDED & STORAGE CONDITIONS(Provided this is within the expiration date of the kit.)

ATAGENIX LABORATORIES

Catalog Number:ATK00013

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PART	Format	Description	STORAGE CONDITIONS
Capture Plate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rabbit polyclonal antibody specific for nucleocapsid protein.	Store in sealed at - 20℃.
Standard	1 bottle	160ng/bottle recombinant nucleocapsid protein in a buffered protein base with preservatives, lyophilized. Dissolute in 2ml deionized water before used. Final concentration was 80ng/mL.	Store at - 20℃.
Detection A	1 vial	120μL/vial biotin labeled anti-Nucleocapsid protein monoclonal antibody (including preservative) , 1:100 diluted by dilution buffer before used.	Store at - 20℃.
Detection B	1 vial	120μL/vial streptavidin conjugated to HRP with preservatives, 1:100 diluted by dilution buffer before used.	Store at - 20℃.
Standard/Sample dilution Buffer	1 bottle	25 ml/bottle diluent (including preservative) was used to dilute the standard or sample.	Store at 4℃.
Reagent Dilution Buffer	1 bottle	25 ml/bottle diluent (including preservative) was used to dilute the Detection A and B.	Store at 4℃.
Wash Buffer Concentrate	1 bottle	25 mL/bottle of a 20-fold concentrated solution of buffered surfactant with preservative. 1:20 diluted by deionized water before used.	Store at 4℃.
Color Reagent TMB	1 bottle	12 mL/ bottle of TMB (tetramethylbenzidine) .	Store at 4℃.
Stop Solution	1 bottle	6 mL/ bottle.	Store at 4℃.
Plate Sealer	4 strips	Adhesive strips.	RT.

SPECIFICITY

This assay recognizes natural and recombinant nucleocapsid protein in bronchoalveolar lavage fluid, nasopharyngeal swab samples or recombinant samples.

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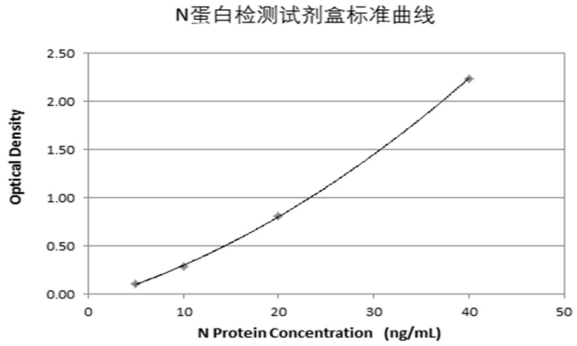
Tested Picture



Note

For research use only.