SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP ELISA Kit

Catalog Number: KIT40588

[Background] Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch.Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

Note: The use of this kit for natural samples need be validated by the end user due to the complexity of natural targets and unpredictable interference.

Materials and Reagents

1. SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with rabbit mAb antibody against
2. SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP
3. SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP Detection Antibody - 0.2 mg/ml of rabbit mAb antibody against SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP conjugated to horseradish peroxidase (HRP) with preservatives
4. SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP Standard - Recombinant SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial
5. Wash Buffer Concentrate - 25 ml of a 20-fold concentrated solution of buffered surfactant with preservatives, stored for up to 1 week at 2-8 °C
6. Dilution Buffer Concentrate - 8 ml of a 20-fold concentrated dilution buffer with preservatives, stored for up to 1 week at 2-8 °C
7. 5× Sample Lysis Buffer - 6 ml, stored for up to 1 week at 2-8 °C
8. Color Reagent A - 13 ml of stabilized hydrogen peroxide, stored for up to 1 month at 2-8 °C
9. Color Reagent B - 13 ml of stabilized chromogen (tetramethylbenzidine), stored for up to 1 month at 2-8 °C
10. Stop Solution - 8 ml of 2 N sulfuric acid, stored for up to 1 month at 2-8 °C
11. Deionized or distilled water

Other supplies required

1. pipette tips
2. Tubes for standard dilution
3. Well plate cover or seals

Equipment

1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes
3. Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
4. 500 ml graduated cylinder

Procedure

A. Sample collection and storage

Serum

1. Use a serum separator tube and allow samples to clot for 30 min before centrifugation for 15 min at 1000 *x g*.
2. Remove serum and assay immediately or aliquot and store samples at -20 °C or lower temperature. Avoid repeated freeze -thaw cycles.

Cell Culture Supernates

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 °C or lower temperature. Avoid repeated freeze-thaw cycles.

Note: If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.

Note: In order to inactive virus, add 1/5 volume of 5× Sample Lysis Buffer to sample (i.e., add 50 µl 5× Sample Lysis Buffer to 200 µl sample), vortex well, then, do 1-fold, 5-fold and 10-fold dilution in 1× dilution buffer.

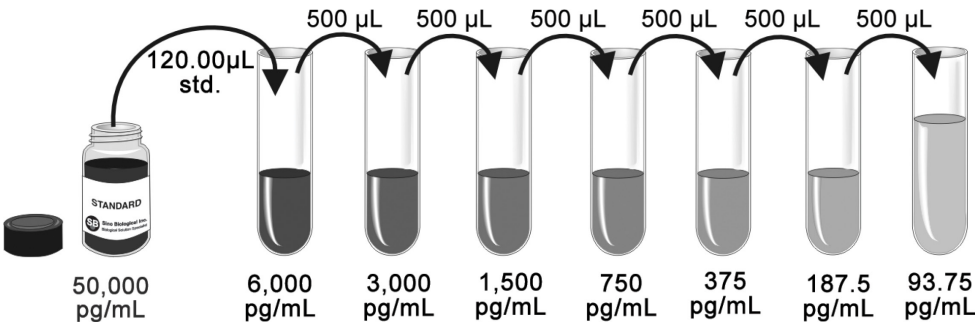
B. Reagent preparation

Note: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

1. Wash Buffer - Prepare 1× wash buffer by adding 20 ml of Wash Buffer Concentrate to deionized or distilled water to prepare 400 ml of Wash Buffer.

2. Dilution Buffer - Prepare 1× dilution buffer by adding 5 ml of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 ml of Dilution Buffer.
3. Detection Antibody - Centrifuge at 10,000 *x g* for 20 s. Dilute to work concentration of 0.5 µg/ml in Dilution Buffer before use.
4. Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 min of use. Protect from light. 200 µl of the resultant mixture is required per well. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**
5. SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP Standard - Reconstitute the SARS-CoV-2 (2019-nCoV) Nucleoprotein/NP Standard with 1 ml of Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (Do not turn the vial upside down). Mix the standard to ensure complete reconstitution prior to making dilutions.
6. Prepare serially diluted standards as described in the following step:
   a. Pipette 880 µl of Dilution Buffer into the 6000 pg/ml tube.
   b. Pipette 500 µl of Dilution Buffer into the remaining tubes.
   c. Use the stock solution to produce a dilution series as the following figure.
   d. Mix each tube thoroughly before the next transfer.
   e. The 6,000 pg/ml standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/ml). **Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.**

   The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



C. Assay procedure

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Wash each well three times with Wash Buffer (300 µl/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential

to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of each serially diluted protein standard or test sample per well including a zero standard. Ensure reagent addition is uninterrupted and completed within 15 min. Cover/seal the plate and incubate for 2 h at room temperature.
5. Repeat the aspiration/wash as in Step C3.
6. Add 100 µl of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in Step C3.
8. Add 200 µl of Substrate Solution to each well. Incubate for 20 min at room temperature. Protect from light.
9. Add 50 µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 20 min, using a microplate reader set to 450 nm.

Data analysis

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

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).
2. 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.
3. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

Notes

Recautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

Safety instructions

5. Please follow biosafety level 2 guidelines when handling virus samples before sample lysis buffer treatment.
6. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.

7. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
8. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

Techinical tips

9. Bring all reagents and samples to room temperature before use.
10. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
11. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.
12. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
13. Read the absorbance of each well within 20 min after adding the stop solution.