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

Catalog No. BSKV0001(96 wells)

For use with cell culture supernates and cell culture exctracts.

For Research Use Only. Not for use in diagnostic procedures.

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INTRODUCTIONS

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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for SARS-CoV-2 (2019-nCoV) Nucleoprotein has been pre-coated onto a microplate. Standards and samples are pipetted into the wells ,and then a horseradish peroxidase conjugated detection antibody specific for SARS-CoV-2 (2019-nCoV) Nucleoprotein is added to the wells , producing an antibody-antigen-antibody "sandwich complex". Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of SARS-CoV-2 (2019-nCoV) Nucleoprotein present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from six SARS-CoV-2 (2019-nCoV) Nucleoprotein standard dilutions and SARS-CoV-2 (2019-nCoV) Nucleoprotein sample concentration determined.

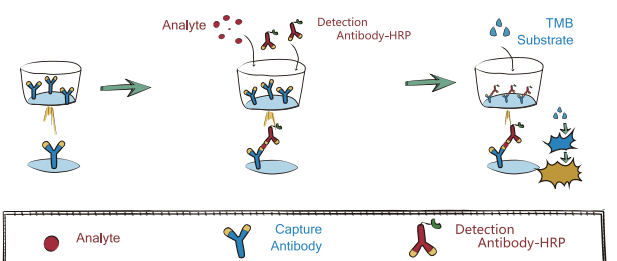

Figure 1. Schematic diagram of the assay

MATERIALS SUPPLIED

Table 1. Kit Components

Kit Components	96 wells Quantity/Size
Aluminium pouches with a Microwell Plate coated with monoclonal antibody to SARS-CoV-2 (2019-nCoV) Nucleoprotein (8×12)	1 plate
SARS-CoV-2 (2019-nCoV) Nucleoprotein standard, 25ng/ml	2 vials
Concentrated Detection antibody conjugated to horseradish peroxidase (HRP)	2 vials
Standard /sample Diluent	1 bottle
Detection antibody Diluent	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 bottle
Stop Solution	1 bottle
Adhesive Films	4 pieces
Package insert	

STORAGE

Table 2. Storage of the kit

Unopened Kit	Store at 2 - 8°C. Do NOT use past kit expiration date!	
Opened/ Reconstituted Reagents	Standard /Sample Diluent	May be stored for up to 1 month at 2 - 8°C.**
	Concentrated Detection antibody conjugated to horseradish peroxidase (HRP)	
	Detection antibody Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at -20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.**

**Provided this is within the expiration date of the kit.

MATERIALS NEEDED BUT NOT SUPPLIED

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. Microplate shaker.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit reagents between 2℃ and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).

2. Please perform simple centrifugation to collect the liquid before use.

3. To avoid cross contamination, please use disposable pipette tips.

4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.

6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.

7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.

8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.

9. Adequate mixing is very important for good result. Use a microplate shaker at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.

10. Avoid microtiter plates drying during the operation.

11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.

13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. Cell Culture Supernates - Pipette cell culture media into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4℃. Immediately aliquot supernatant and store samples at -80℃. Minimize freeze/thaw cycles.

2. Cell culture exctracts - Aspirate medium and gently wash cells once with

ice-cold PBS. Aspirate PBS and add 0.5 mL extraction buffer per 100 mm plate. Scrape cells to collect in tilted plate and remove to pre-chilled tube. Vortex briefly and incubate on ice for 15-30 min. Centrifuge at 13,000 rpm for 10 min at 4℃ to pellet insoluble contents. Aliquot supernatant (this is the soluble cell extract) to clean, chilled tubes on ice and store samples at -80℃. Minimize freeze/thaw cycles.

Cell extraction buffer recipe: 100 mM Tris(pH 7.4), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.5% Sodium deoxycholate, Phosphatase inhibitor cocktail, Protease inhibitor cocktail, PMSF.

3. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.

2. Wash Buffer - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. Standard - Reconstitute the Standard with 1mL of Standard /sample Diluent. This reconstitution produces a stock solution of 25 ng /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Prepare 5 tubes (below), Pipette 500µL of standard/sample Diluent into

the 12.5ng/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series . Mix each tube thoroughly and change pipette tips between each transfer. The 25 ng/mL standard serves as the high standard. The standard/ sample Diluent serves as the zero standard (0 ng/mL).

If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

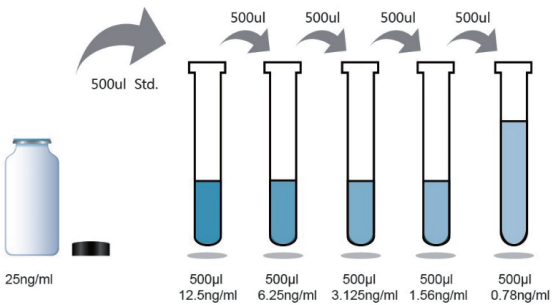

Figure 2. Preparation of SARS-CoV-2 (2019-nCoV) Nucleoprotein standard dilutions

4. Working solution of Detection antibody: Make a 1:100 dilution of the concentrated Detection antibody conjugated to horseradish peroxidase (HRP) solution with the Detection antibody Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

GENERAL ELISA PROTOCOL

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

2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.

3. Add 50µL of standard, control, or sample, per well, then add 50µL of the working solution of Detection antibody to each well. Cover with the adhesive strip provided and incubate 90 minutes at RT. Adequate mixing is very important for good result. Use a microplate shaker at the lowest frequency. Avoid placing the plate in direct light.

4. Aspirate each well and wash, repeating the process three times for a total of five washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. 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 Substrate Solution to each well. Incubate for 5-15 minutes at RT. Avoid placing the plate in direct light.

6. Add 50µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable).

ASSAY PROCEDURE SUMMARY

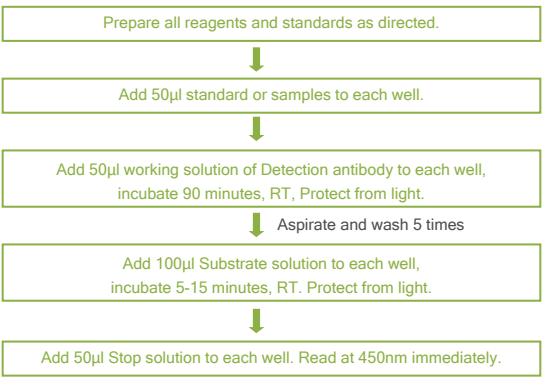

Figure 3. Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.

2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

2. 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.

3. The data may be linearized by plotting the log of the SARS-CoV-2 (2019-nCoV) Nucleoprotein 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.

4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3. Typical data using the SARS-CoV-2 (2019-nCoV) Nucleoprotein ELISA (Measuring wavelength:450nm, Reference wavelength:630nm)

Standard (ng/ml)	OD.
0	0.099
0.78	0.177
1.56	0.278
3.125	0.453
6.25	0.706
12.5	1.305
25	2.323

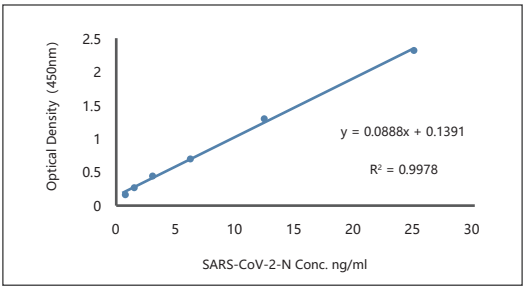

Figure 4. Representative standard curve for SARS-CoV-2 (2019-nCoV) Nucleoprotein ELISA. SARS-CoV-2 (2019-nCoV) Nucleoprotein was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

PERFORMANCE CHARACTERISTICS

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY: The minimum detectable dose was 0.4ng/mL.

SPECIFICITY: This assay can recognizes both recombinant and natural SARS-CoV-2 (2019-nCoV) Nucleoprotein， but no react with recombinant MERS-CoV Nucleoprotein .