PROGNOSIS BIOTECH

BIO-SHIELD

Surface Ag 2019-nCoV

REF B7048

Quantitative ELISA Test | For research use only

For the quantitative detection of SARS-CoV-2 Nucleocapsid protein in working surfaces.

www.prognosis-biotech.com

This ELISA kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 9001:2015.

Use only the current version of Product Data Sheet enclosed with the kit.

Bio-Shield Surface Ag 2019-nCov, B7048, is a Quantitative ELISA test for the measurement of SARS-CoV-2 Nucleocapsid Protein (NP) in working surfaces. The ELISA kit contains all reagents required for the immunoassay method. A spectrophotometer for microtiter ELISA plate is required.

- For research use only
- Test time (incubation after sample and reagent preparation): 105min
- Standard curve range: 0 - 8.5ng/ml of N-protein
- Negative results do not rule out SARS-CoV-2 infection
- Test should only be conducted by trained personnel
- **DO NOT USE** this kit with human or animal specimen

- Test time (incubation time after specimens and reagents preparation): 105 min
- Shelf life: 12 months
- Storage: 2-8^{o}C

1. Intended Use

Bio-Shield Surface Ag 2019-nCov is a quantitative ELISA test to detect and quantify protein levels of endogenous Nucleocapsid protein of SARS-CoV-2 (COVID-19 virus) from food packaging and environmental surfaces. The kit is intended for environmental swab sampling of hard surfaces and common areas such as, working bench, tables, door handles, PIN pads, etc. Neither the kit nor the results may be used to diagnose human patients or clinical samples (i.e. nasopharyngeal samples). Prognosis Biotech does not support nor validate results for purposes apart from its intended use.

2. Principal of the assay

The principal of Bio-Shield Surface Ag 2019-nCov is based on the sandwich Enzyme Linked-immune-Sorbent Assay (Sandwich ELISA) to detect SARS-CoV-2 Nucleocapsid protein in environmental swab specimens. Each well of the supplied microtiter plate has been pre-coated with target-specific antibody. Standards and specimens are added to the wells and Nucleocapsid protein (NP), if present in the specimen, is bound to the wells coated with the immobilized antibody. Unbound substances are washed away, and HRP labeled secondary antibody against SARS-CoV-2 NP is added, producing an ''antibody -antigen-antibody'' complex. Following extensive washing, a substrate solution containing TMB is loaded and color develops in proportion to the amount of SARS-CoV-2 Nucleoprotein (NP) bound. After terminating the reaction with a STOP solution, the measurement is made at 450nm and the intensity of the produced colored complex is proportional to NP present in the specimen.

3. Introduction

A novel coronavirus (2019-nCoV) was identified in December 2019,[1] which has resulted in hundreds of thousands of confirmed human infections worldwide. Cases of severe illness and deaths have been reported. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2] As of May 28, 2020, molecular and antigen testing are the only techniques capable of detecting SARS-CoV-2 virus. Briefly, SARS-CoV-2 is an enveloped positive sense RNA virus. Molecular approaches are designed to target one or more genes (Figure 2)[3]. Common target genes include the envelope (E), nucleocapsid (N), spike protein (S), and the open reading frame (ORF) region. 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 the 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, N protein of coronavirus is chosen as a diagnostic tool. Person-to-person contact through droplets is believed to be the primary route of virus transmission. However, it has also been suggested that virus can be spread via contaminated surfaces. Surveillance, and the prevention of cross-contamination are critical in the control of foodborne illnesses. Coronaviruses have been shown to survive (remain viable) on different surfaces, for up to nine days[4], under specific conditions such as temperature, relative humidity, and light. SARS-CoV-2 can be transmitted when a healthy person touches contaminated food or surfaces (including packaging materials), and then shortly afterwards, touches their eyes, mouth, or nose. The application of sound principles of environmental sanitation, personal hygiene and established food safety practices will reduce the likelihood that harmful pathogens including SARS-CoV-2 will threaten the safety of the food supply, regardless of how the food is sourced. Environmental testing of public spaces provides a proactive alternative to human laboratory testing to monitor and prevent the spread of highly contagious and easily transmittable diseases. Clearly, at this critical time, food safety is important more than ever, and in the current pandemic, food and beverage companies, retailers, public health authorities and other businesses are seeking solutions to monitor the presence of SARS-CoV-2 in their environment.

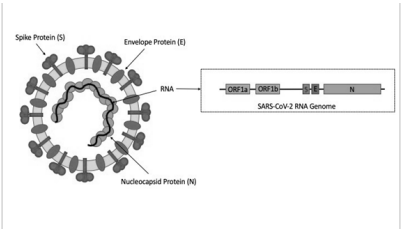


Figure 2. SARS-CoV-2 Structure and Molecular Targets. Common SARS-CoV-2 virus antigenic targets include spike, envelope, and nucleocapsid proteins.

4. Reagents Provided

Bio-Shield Surface Ag 2019-nCov ELISA kit contains sufficient reagents and materials for 48 measurements (including standard tests)

Reagents (Store at 2-8ºC)	Quantity for 48 wells	State	Vial cap color
Surface Ag 2019-nCov Single-Break Strip Plate	48 wells	Ready to use (precoated)	-
Sterile swabs	38 swab	Ready to use	-
Extraction Tubes and Caps	38 plastic tubes	Ready to use	-
Standards 1-5 (0, 1.06, 2.12, 4.25, 8.5ng/ml of N-protein)	5 glass vial (each 1ml)	Ready to use	Black
Extraction Lysis Buffer	2 plastic vials (each 10ml)	Ready to use	White
Surface Ag 2019-nCov Detection Solution	1 plastic vial (6 mL)	Ready to use	Green
Wash Buffer	1 plastic vial (50 mL)	20X Concentrate (dilute in distilled water)	White
TMB Substrate	1 plastic vial (6 mL)	Ready to use	Brown
Stop Solution	1 plastic vial (6 mL)	Ready to use	White

5. Materials required but not provided

- Vortex mixer and microtiter plate reader fitted with 450 nm filter.
- Adjustable single channel micropipettes of 10, 100 and 1000 µL with disposable tips (a repetitive pipette of 100-200 µL is preferable for the steps of Detection Solution, TMB and Stop Solution) and a multi-channel micropipette of 50 - 300 µL with disposable tips and reservoir.
- Distilled or deionized water, a graduated cylinder of 1000 mL capacity and absorbent paper.
- Protective goggles, gloves and container for biohazardous waste.
- Disposable plastic tubes

6. Storage Instructions

Store kit reagents between 2 and 8°C. Do not freeze any components provided. Reseal immediately the unused strips of the microtiter plate in the bag together **with the desiccant bag** provided and store at 2-8°C for 1 month. After use, all remaining reagents should be returned to 2-8°C where they also remain stable for 1 month. Expiry of the kit and reagents is stated on the labels respectively and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly as well as if the reagent is not contaminated by the first handling, in case of repeated use of one component. Because of the colorless TMB Substrate light sensitivity, avoid exposure to direct light.

7. Safety and Precautions for use

7.1 Health and safety precautions

- Avoid any skin contact with Standards, Stop Solution (H_3PO_4 15%) and TMB Substrate (toxic). In case of contact, wash thoroughly with water. **Use gloves, protective clothing and eye/face protection and handle appropriately with the requisite Good Laboratory Practices**. The product must only be used by trained personnel, familiar with the potential hazards.

- Dispose all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with the local, regional and national regulations. Human source material spills should be also treated as potentially infectious. Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces, with an appropriate chemical disinfectant (commonly 1:10 dilution of household bleach or 70-80% Ethanol) and should be wiped dry.

7.2 Precautions related to the procedure

- Use a clean container to prepare the wash buffer and all residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper.
- Never insert absorbent paper into the well. Read the absorbance within 60 minutes after completion of the assay.
- All reagents should be warmed in room temperature before use and covered when not in use. **Use a clean disposable plastic pipette tip for each reagent, to avoid cross-contamination. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.**
- Do not interchange individual reagents between kits of different lot numbers.

8. Indication of spoilage of kit reagents

- The bluish coloration of the TMB substrate before the ELISA test.
- Visible microbial contamination of reagents.
- DO NOT USE the kit if the packaging of components is damaged, if there is an expired reagent or if the desiccant bag is absent inside microplate bag.

9. Specimens and reagents preparation

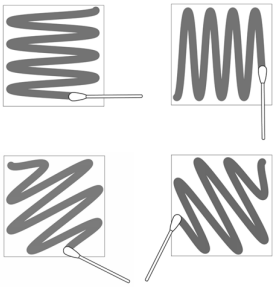
9.1 Wash Buffer 1X preparation

Dilute the 20X solution concentrate 20 fold with distilled water to give a **1X** working solution. In case of the occurrence of crystals in the Wash Buffer, the warming of the crystals by gentle dismantling (using hands) is needed. Pour the entire content of the solution concentrate (50 mL) into a clean 1000 mL graduated cylinder, rinse the vial with distilled or deionized water, pour the content again into the cylinder and fill to a final volume of 1000 mL with distilled or deionized water. Mix gently to avoid foaming, transferring the final solution from cylinder to a clean bottle and back two times. The clean bottle with 1X Wash Buffer working solution can be left out of the refrigerator during the method procedure and subsequently be stored 2-8°C for 1 month.

9.2 Environmental Swab Specimens

1. Calculate the amount of swabbing solution, tubes, caps and swabs needed, according to the number of specimens to collect.
2. Label one microcentrifuge tube for each Environmental Swab Specimen.
3. Add 350 µL of Extraction Lysis Buffer into labeled microcentrifuge tube. Define a swabbing area of 10x10 cm to 15x15cm. It is also possible to swab directly on the target surfaces in a line of 40cm instead. The area selected for analysis must be representative of the total area of interest.
4. Thoroughly swab the sampling site location by using even back and forth horizontal, vertical, and diagonal motions while rotating the swab.
5. Carefully place the swab into pre-labeled tube and while squeezing the extraction buffer tube, stir the swab more than 5 times.
6. Remove the swab while squeezing the sides of the tube to extract any residues that might be on the surface of swab.
7. The specimen is now ready to be tested.

Note: *Swabs are intended for single use only. Do not re-use any swabs that have been used for environmental swabbing.*



10. Method Procedure

10.1 *Assay Design:* Determine the number of microwell strips needed for the assay (Standards and Specimens). Considering that each specimen and standard can be tested in single, create a layout.

NOTE 1: If the number of wells is more than 32 (four strips), a repetitive pipette or multichannel pipette is necessary, for the Detection Solution, TMB Substrate and Stop Solution.

NOTE 2: The reliability of the test can be improved when duplicate values are used for each specimen.

	1	2	3	4	5	6	7	8	9	10	11	12
A	St1											
B	St2											
C	St3											
D	St4											
E	St5											
F												
G												
H												

Example plate layout

10.2 Bring all reagents to room temperature (19 - 24°C) before use. Place the appropriate number of Antibody Coated Microtiter Wells in a microwell holder. Immediately reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided. The specimens should be stored in a cool place.

10.3 Using a new pipette tip for each, add 50 µL of each Standard and surface specimen (see Chapter 9.2) to appropriate Coated Microtiter Well. Incubate at room temperature for **60 minutes**.

NOTE: Use only sufficient volume of standards and specimens as indicated in the procedure. Failure to do so may cause low sensitivity of the assay.

10.4 When the countdown is over wash the plate as follows: Aspirate the liquid from each well into the sink and tap the holder of microwells upside down strongly (four times in a row) on an absorbent paper to ensure the complete removal of liquid from the wells. Dispense **300µl** of **Wash Buffer 1X** (see 9.1) into each well with a wash bottle or multichannel micropipette using the proper reagent reservoir and shake the plate manually for a few seconds. Repeat this process for another four times **(total 5 times). CAUTION:** It is important to not allow microwells dry between working steps.

10.5 Aspirate the liquid as described above and add **50µl** of **Detection Solution** to each well. Shake the plate manually for 30 seconds and incubate at room temperature for **30 minutes.**

10.6 Wash the plate as the wash **step 10.4.**

10.7 Aspirate the liquid as described above and add **50µl** of **TMB Substrate** to each well (pour 0.5ml per 8 wells in a reservoir). Shake the plate manually for a few seconds and incubate in the dark at room temperature for **15 minutes.**

10.8 Add **50µl** of the **Stop Solution** to each well (pour 0.5ml per 8 wells in a reservoir). Mix gently by shaking again the plate manually.

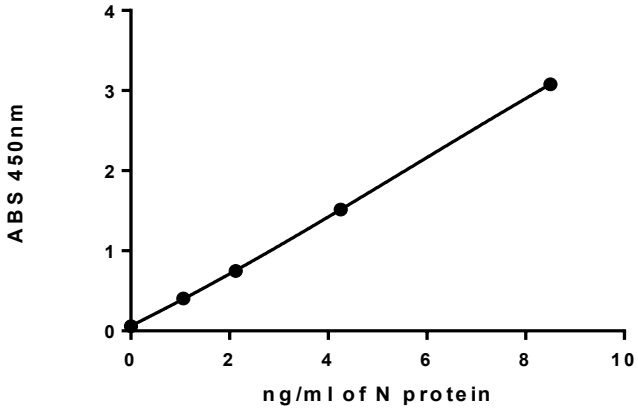
10.9 Measure the absorbance at 450nm. Read the absorbance value of each well (within 60 minutes after the step 10.8) on a spectrophotometer using 450nm as the primary wavelength and optionally 620 nm as the reference wavelength (610 nm to 650 nm is acceptable).

11. Data analysis

- **Automatically**

An assigned software, the **Prognosis-Data-Reader,** is available for free (contact:info@prognosis-biotech.com) download in order to evaluate the Bio-Shield Surface Ag 2019-nCov ELISA kit. The evaluation is carried out by a simple transfer of data values after the measurement.

12. Example of Standard Curve



13. Limitations of the immunoassay

- The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
- After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) within two hours. In this case use the cap to close the extraction tube without discarding the droplet.
- The test should be used for the detection of SARS-CoV-2 antigen in environmental swab specimens.
- Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid result.
- A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.

- Negative results indicate that the amount of SARS-CoV-2 present in the testing area is lower than the limit of detection but do not guarantee the complete absence of the virus.
- Negative environmental testing results cannot exclude the presence of SARS-CoV-2 within the setting where the investigation has been conducted.
- Positive test results do not rule out co-contamination with other pathogens.
- Results should be interpreted after verifying the validity of all controls and specimens. Interpretation must be performed by trained personnel.
- The test is not capable of distinguishing active from inactive SARS-CoV-2.

14. Immunoassay Specification

14.1 General Specification

- IC50 = 2.01 - 4.14 ng/ml.
- Each standards duplicates mean CV ≤ 6%.

14.2 LOD - LOQ - Accuracy

- The LOD of the method is 0.55ng/ml.
- The LOQ of the method is 1ng/ml.
- The lowest detectable concentration of an analyte in a method is known as LOD. In this case, we check the concentration of SARS-CoV-2 (inactivated virus) in Bio-Shield Surface Ag 2019-nCov. The LOD is the level at which 95% of the replicates is characterized as positive. The results of 48 replicates of 6 dilutions with inactivated virus are shown in table 1.

Concentration ($TCID_{50}$/ml)	Positive Replicates	Mean OD Value	CV%
1.15×10^7	48/48	Overflow	-
1.15×10^6	48/48	Overflow	-
1.15×10^5	48/48	2.844	2.99
1.15×10^4	48/48	2.032	5.22
1.15×10^3	48/48	0.450	4.12
1.15×10^2	10/48	0.131	4.67

Table 1.

14.3 Cross-reactivity

Cross reactivity of the Bio-Shield Surface Ag 2019-nCoV quantitative assay has been evaluated by testing specimens spiked with 4 different types of coronaviruses, Alpha coronavirus 229E, Alpha coronavirus NL63, Beta coronavirus OC43 and Beta coronavirus HKU1. No cross reactivity has been found with any of the specimens tested.

14.4 High Dose Hook effect

The Bio-Shield Surface Ag 2019-nCoV kit was tested with 10^7 TCID50/ml of heat-inactivated SARS-CoV -2 and no hook effect was observed.

15. Method Summary

Total procedure time (after specimens and reagents preparation): 105 min.

Add 50 µL from each standard and specimen into the Antibody Coated Microwells

⇩

Incubate 60 min at room temperature

⇩

Wash five times

⇩

Add 50 µL of Detection Solution

⇩

Incubate 30 min at room temperature

⇩

Wash five times

⇩

Add 50 µL of TMB Substrate

⇩

Let the color develop for 15 min in the dark at room temperature

⇩

Add 50 µL Stop Solution

⇩

Read Absorbance at 450 nm

16. References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html Accessed March 30, 2020.

2. BioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 30, 2020.

3. https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm.

4. Wu F, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-269.

5. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC et al. (2020). Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV -2: an observational cohort study. Lancet Infect Dis. 2020 Mar 23. pii: S1473-3099(20)30196-1.

www.prognosis-biotech.com
e: info@prognosis-biotech.com
t: +30 2410 623922 | f: +30 700 700 6262
Farsalon 153 | 41335 Larissa, Greece