# SARS-CoV-2 NP Ab ELISA kit

*For use under the Emergency Use Authorization (EUA) only*
*For in vitro diagnostic use*

Instructions for Use

(96 tests/kit)

Rx Only

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Version 01 Released on April 09, 2020

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# 1. Intended Use

SARS-CoV-2 NP Ab ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of total antibodies against the nucleocapsid protein (NP) of SARS-CoV-2 virus in serum or plasma samples from individuals with signs and symptoms of infection who are suspected of COVID-19.

Results are for the identification of antibodies against the nucleocapsid protein (NP) of SARS-CoV-2 virus. The anti-NP antibodies is generally detectable in plasma or serum samples during and even after the phase of SARS-CoV-2 virus infection.

Samples with positive results should be retested in duplicate using SARS-CoV-2 NP Ab ELISA before data interpretation. Only repeatable positive results in the same sample can be finally considered as the successful detection of total antibodies to NP of SARS-CoV-2 and can be used as serological indications of COVID-19 currently or in the past. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 NP Ab ELISA kit is intended for use by ***trained clinical laboratory personnel specifically instructed and trained in the techniques of ELISA and in vitro diagnostic procedures***. The SARS-CoV-2 NP Ab ELISA kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

# 2. Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-CoV-2), which has resulted in thousands of confirmed human infections in many countries including the United States. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

Nucleocapsid protein (NP) is the most abundant protein on the helical nucleocapsid of coronaviruses, which envelopes the entire genomic RNA. NP also interacts with other viral structural proteins to play important roles during host cell entry and virus particle assembly and release. Anti-NP antibodies have been shown to be the earliest and the most predominant antibodies detectable in patient's blood samples after coronavirus infection.

SARS-CoV-2 NP Ab ELISA kit is a two-step incubation immunoassay kit that for the detection of antibodies against the nucleocapsid protein (NP) of SARS-CoV-2 virus and aids the diagnosis COVID-19. The product contains recombinant nucleocapsid protein of SARS-CoV-2, HRP conjugated SARS-CoV-2 NP, and control material used in immunoassay for the in vitro qualitative detection of antibodies against NP protein of SARS-CoV-2 virus in human blood specimens.

## 3. Test Principles

SARS-CoV-2 NP Ab ELISA kit is a two-step incubation immunoassay kit. Recombinant nucleocapsid protein (NP) of SARS-CoV-2 pre-coated onto the polystyrene microwell strips can specifically recognize anti-NP antibodies in human serum or plasma specimens. After a 1-hour incubation, anti-NP antibodies are captured by immobilized NP protein while the unbound components are washed away. Afterwards, a detection solution containing HRP-conjugated NP is added for another 1-hour incubation, wherein HRP-conjugated NP binds to the total antibodies previously bound to NP protein on the plate. After removal of nonspecific bindings, a HRP substrate solution containing 3,3′,5,5′-Tetramethylbenzidine (TMB) is added, resulting in the formation of a blue color. Color reaction is stopped by 2M $H_2SO_4$, transforming the blue color to yellow signals, which is quantified by an absorbance microplate reader at 450nm. The color intensity is proportional to the amount of anti-NP total antibodies captured inside the wells.

## 4. Materials Provided

***SARS-CoV-2 NP Ab ELISA kit***

| Item (96 tests/kit) | Specifications and Quantity |
|---|---|
| SARS-CoV-2 NP coated ELISA plate | 12 strips of 8 wells (96 wells in total) in a white strip holder and sealed in a foil bag with desiccant. Each well contains recombinant NP of SARS-CoV-2. The microwell strips can be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C. |
| 5x Assay Buffer | 1 x 20 ml |
| 10x Wash Buffer | 1 x 40 ml of 10X concentrated Wash Buffer is used as directed in Test Procedure. |
| 100x Detection Antibody Solution | 1 x 0.12 ml |
| Substrate Solution | 1 x 12 ml |
| Stop Solution | Is used to terminate the reaction as directed in the Test Procedure. 1 x 12 ml |
| Negative Control | The negative control aids in verifying the validity of the kit .1 x 1 ml |

Table 1: Components and volumns of SARS-CoV-2 NP Ab ELISA kit

## 5. Materials and Equipment Required but not Provided*

- A microplate reader capable of reading absorbency at 450 nm.
- Vortex mixer.
- Microcentrifuge.
- 96-well plate or manual strip wash.
- Buffer and reagent reservoirs.
- Distilled water or deionized water.
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL).
- Multichannel micropipettes (5-100 µL).
- Aerosol barrier pipette tips (2 or 10 µL, 200 µL and 1000 µL).
- 1.5 mL microcentrifuge tubes (DNase/RNase free).
- Racks for 1.5 mL microcentrifuge tubes.
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
- Disposable powder-free gloves and surgical gowns.
- Paper towels or absorbent paper.

*All above equipments no specific brand requirement.

## 6. Warnings and Precautions

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

- Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
- Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
- CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (20-25°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
- Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
- Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
- Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
- Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
- Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
- When adding specimens, do not touch the well's bottom with the pipette tip.

- When measuring with a plate reader, determine the absorbance at 450nm.
- The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
- All specimens from human origin should be considered as potentially infectious for COVID 19. Strict adherence to CDC guideline regulations can ensure the personal safety.
- Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
- Chemical should be handled and disposed of only in accordance with the current CDC guideline and the local or national regulations.

# 7. Reagent Storage, Handling, and Stability

- The kit is stable until the expiry date only when stored at 2-8°C in sealed foil pouches. The expiry date is the last day of the month stated on the foil pouch and kit container (current data support 1month expiration).
- The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antigen-coated strips from the microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month. Open and reconstituted solution can be stored 2-8°C for up to one month. To assure maximum performance, it is recommended to use up all reagents and microplate strips at once, avoided unnecessary repeated temperature equilibration.

# 8. Specimen Collection, Storage, and Transfer

CAUTION: Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

- Only serum or plasma should be used for this assay, and the usual precautions for venipuncture should be observed. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25℃) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute recommendations (CLSI Approved Guideline-Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests).
- Do not use sera if any indication of microbial growth is observed.

# 9. Laboratory Procedures

## 9.1 sample preparation

- CAUTION: Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

- Serum or plasma sample requires a 100-fold dilution in the 1×Assay buffer. A suggested dilution step is to add 10 µl of sample to 990 µl of 1×Assay buffer.
- Test samples are suggested to be assayed immediately after separation of serum or plasma, or preferably stored frozen (-20℃ or below) in aliquots. Multiple freeze-thaw cycles should be avoided. Duplicate test is recommended.
- Serum or plasma specimens with EDTA, sodium citrate or heparin can be tested. Highly lipaemic, icteric, or hemolytic specimens are not recommended. Specimens with visible microbial contamination should not be used.
- When required, vortex test serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 to 15,000 rpm for 5 minutes prior to assay to remove particulates. Do not omit this centrifugation step if samples are cloudy and containing particles.

## 9.2 preparation of reagent supplied

- **1×Assay buffer:** Prepare 1x assay buffer by mixing the 5x assay buffer (30 ml) with 120 ml of distilled water or deionized water. If precipitates are observed in the 5x assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1x assay buffer may be stored at 2- 8°C for up to one month.
- **1xWash buffer:** Prepare 1xWash buffer by mixing the 10xWash buffer (40ml) with 360ml of distilled water or deionized water. If precipitates are observed in the 10xWash buffer bottle, warm the bottle in a 37°C water batch until the precipitates disappear. The 1xWash buffer may be stored at 2-8°C for up to one month.
- **1x Detection solution:** Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

## 9.3 Running a Test

- Please equilibrate all the reagents to room temperature (20-25°C) for at least 30 minutes before use.

| | |
|---|---|
| **Step 1** | **Adding Negative Control, Test Sample and Blank:**<br>Add 50µl of Negative Control, 100µl of test sample and 100µl of 1 x Assay. Buffer as Blank into their respective wells. Triplicate test is recommended for Negative Control and duplicate test is recommended for Blank and test samples.<br>Note: Use a separate disposal pipette tip for each test sample, Negative Control and Blank to avoid cross-contamination. Mix by tapping the plate gently. |
| **Step 2** | **Incubation:**<br>Cover the plate and incubate at room temperature for 1 hour. |
| **Step 3** | **Washing:** |

| | |
|---|---|
| | Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes. |
| **Step 4** | **Adding HRP-conjugated Detection Solution:**<br>Add 100 µl of 1×Detection Solution to each well. |
| **Step 5** | **Incubation:**<br>Cover the plate and incubate at room temperature (normally ranged from 20-25$^0$C) for 1 hour. |
| **Step 6** | **Washing:**<br>Wash each well 4 times as described in step 3. |
| **Step 7** | **Coloring:**<br>Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**. |
| **Step 8** | **Stopping Reaction:**<br>Add 100 µl of Stop solution (Warning: corrosive material) to each well, gently tap the plate frame for a few seconds to ensure thorough mixing. |
| **Step 9** | **Measurement:**<br>Measure absorbance of each well at 450 nm immediately.<br>Note: read the absorbance within 10 minutes after stopping the reaction. Warning: Microplate need to be discarded following GLP procedures. |

Table 2 procedures of SARS-CoV-2 NP Ab ELISA kit

# 10. Quality Control and Interpretation of Results

**Quality control:**

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's absorbance value to the Cut-off value.

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The Absorbance value of the Blank well should be < 0.100 at 450nm.

- The Absorbance value of the Negative control should be < 0.200 at 450nm.

If the Absorbance value of over one Negative controls do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

**TYPICAL RESULTS (Examples only)**

| **Samples** | **OD450** |
|---|---|
| Negative Control | 0.198 |

| | |
|---|---|
| | 0.177 |
| | 0.153 |
| | 0.162 |
| Serum from healthy subjects | 0.207 |
| | 0.175 |
| | 0.223 |
| Serum from COVID-19 patients | 3.656 |
| | 0.754 |
| | 0.362 |

Table 3 The typical test reading of SARS-CoV-2 NP Ab ELISA

**CUT-OFF VALUE**

| **Absorbance 450nm** | **Indication** |
|---|---|
| < 0.25 | Negative |
| ≥ 0.25 | Positive |

Table 4 The cut-off value of the test

This Cut-off value has been validated in, however, it is highly recommended that each laboratory should establish its own normal and pathological reference range for anti-NP Ab levels. Furthermore, it is also recommended that each laboratory should include its own control samples in the assay.

**Interpretation of results:**

- Assessment of clinical specimen test results should be performed only if the Quality Control criteria for the test kit are fulfilled. It is also recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

**Negative Result (sample's absorbance/cut-off <1.0):**

- Test result is negative when the ratio between sample's absorbance to cut-off value is less than 1.0. A Negative result indicates that the SARS-CoV-2 NP Ab ELISA test has not identified human antibodies reactive to SARS-CoV-2 NP.

**Positive Result (sample's absorbance/cut-off ≧1.0):**

- Test result is positive when the ratio between sample's absorbance to cut-off value is equal to or more than 1.0. Positive result indicates the presence of SARS-CoV-2 NP antibodies and is detected by using SARS-CoV-2 NP Ab ELISA.

- Samples with positive results should be retested in duplicate using SARS-CoV-2 NP Ab ELISA before data interpretation. Only repeatable positive results in the same sample can be finally considered as the successful detection of antibodies to SARS-CoV-2 NP, and can be used as serological indications of COVID-19 currently or in the past.

- If negative results show up during the repeated test, the previous positive results are false positive and these samples should be considered as negative. False positive results can occur in many sensitive ELISA kits due to several reasons, majority of which are related to, but not limited to, inadequate washing or contamination. For more information regarding ELISA troubleshooting, please contact iCarbonX's tech support.

**Indeterminate Result (sample's absorbance/cut-off = 0.9-1.2):**

- Test result is considered as indeterminate result when the ratio between sample's absorbance to cut-off value is between 0.9 and 1.2. The indeterminate result is considered uninterpretable at the time of testing. It is highly suggested to follow up with other clinical and laboratory diagnosis methods, as the clinical diagnosis should not be established based on a single kind of test only.

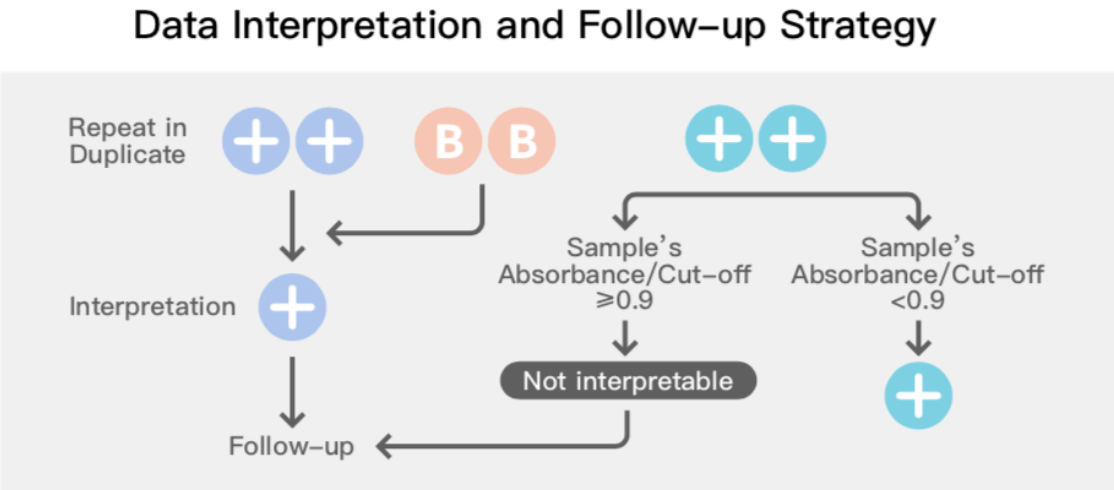


Image 1: Data interpretation and follow-up strategy

# 11. Limitations

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.

- Antibodies may be undetectable during the early stage of the disease and in some immune-suppressed individuals. Therefore, negative results obtained with iCarbonX SARS-CoV-2 NP Ab ELISA are only indication that the specimen does not contain detectable level of antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with the virus.
- If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding iCarbonX ELISA Troubleshooting, please contact iCarbonX technical support for further assistance.
- The most common assay mistakes are using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
- The prevalence of the marker will affect the assay's predictive values.
- This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.
- iCarbonX SARS-CoV-2 NP Ab ELISA is a qualitative assay and the results cannot be used to measure antibody concentration.

# 12. Performance Characteristics

### Clinical Evaluation

Clinical validation study of iCarbonX SARS-CoV-2 NP Ab ELISA was conducted in 2020 in Shenzhen, China. Samples were collected from COVID-19 confirmed cases with clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period. The kit showed higher positive detection rate in specimens from patients with delayed onset. Therefore, the interpretation of the test results should consider the specimen's collection time.

| **Sensitivity** | **94.59% (n=74)** |
|---|---|
| **Specificity** | **95.24% (n=63)** |
| **Inter Assay Precision** | |
| **Samples** | **CV** |
| **1** | **5.26%** |
| **2** | **4.95%** |
| **3** | **6.11%** |
| **Intra Assay Precision** | |
| **Samples** | **CV** |
| **1** | **5.73%** |
| **2** | **4.51%** |
| **3** | **6.68%** |

Table 5 clinical evaluation of SARS-CoV-2 NP Ab ELISA

1) Limit of Detection (LoD) - Analytical Sensitivity:

As this is a qualitative assay, we cannot provide standards or an exact Ab number/unit to indicate the limit of detection. However, a detection limit was suggested by using clinical diagnosed positive samples. Clinical positive samples were prepared with serial dilution factors until the test result at two consecutive intervals were negative and positive respectively. For example, positive samples were made to have 2x cut-off OD reading, around cut-off reading and with 1/2 of the cut-off value. Within this concentration range, the detection limit were considered to be set only when the results can be repeated for 19 out of 20 tests. The results show that the limit of detection is suggested to be around the cut-off value, which is 0.25.

2) Inclusivity (analytical sensitivity):

The sequence of the recombinant nucleocasip protein to SARS-CoV-2, which were used here in the kit to coat the plate, were provided from the supplier (ImmunoDiagnostics). If the homologies of this recombinant nucleocapsid protein sequence matches well to all published SARS-CoV-2 sequence, preferably reach 100%, it indicates a good inclusivity with this strain/isolate of SARS-CoV-2. If the homology is less than 100%, it predicts a potential false negative result from this specific strain/isolate of SARS-CoV-2.

| **Pathogen** | **Genbank** | **%Homology** |
|---|---|---|
| SARS-CoV-2 | QIK50415.1 | 100% |
| SARS-CoV-2 | 6M3M_A | 100% |
| SARS-CoV-2 | QIK02783.1 | 100% |
| SARS-CoV-2 | 6VYO_A | 100% |
| SARS-CoV-2 | QIK02784.1 | 100% |
| SARS-CoV-2 | BCA37476.1 | 100% |
| SARS-CoV-2 | QII87776.1 | 100% |

Table 6 *In silico* inclusivity analysis results record

Wet test were also conducted here to confirm the inclusivity of this the SARS-CoV-2 NP Elisa kit. All blood samples are from COVID-19 confirmed cases diagnosed based on clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period. The test sensitivity and specificity was determined by comparing the results from in total 74 patient cases and 63 healthy case.

| Sensitivity (74 cases) | Specificity (63 cases) |
|---|---|
| 94.59% | 95.24% |

Table 7 Sensitivity and specificity of SARS-CoV-2 NP Ab ELISA

3) Cross-reactivity (Analytical Specificity):

Purpose: Evaluate the specificity of SARS-CoV-2 NP Ab ELISA kit in detecting SARS-CoV-2 coronavirus and its cross-reactivity with other pathogens commonly found in respiratory specimen from patients with symptoms of fever, fatigue, and/or cough. The pathogens to be tested here including other coronavirus strain (HKU1, NL63, OC43, or 229E), FluA, FluB and Rhinovirus etc. Due to the difficulties on obtaining reference samples from patients infected by pathogens listed above, an *in silico* analysis is performed to predict the potential false positive and false negative results.

Cross-reaction evaluation using in silico analysis: *In silico* analysis is conducted to evaluate the cross-reactivity of SARS-CoV-2 NP Ab ELISA kit in detecting SARS-CoV-2 coronavirus and other pathogens that cause similar symptoms of COVID-19 but are hard to be evaluated by wet testing due to the lack of testing samples. The *in silico* analysis will be performed by blasting the sequence of the nucleocapsid protein, the detecting protein of the SARS-CoV-2 NP Ab ELISA kit, against p**u**blicly available genomic sequences of following pathogens: SARS, MERS, FluA, FluB, Rhinovirus ect.

Pass/Fail Criteria: If the homology of the nucleocapsid protein sequence between SARS-CoV-2 coronavirus and another pathogen is not higher than 50%, it indicates that a cross-reaction between this pathogen and SARS-CoV-2 nucleocapsid protein is unlikely to happen. Otherwise, if the homology is higher than 60%, it predicts that a cross-reaction between this pathogen and SARS-CoV-2 nucleocapsid protein is likely to happen.

Table8 *In silico* cross-reactivity analysis results record

| Pathogen | Strain | Taxonomy ID | % Homology Test |
|---|---|---|---|
| Human coronavirus | 229E | 11137 | <50% |
| Human coronavirus | NL63 | 277944 | <50% |
| Human coronavirus | OC43 | 31631 | <50% |
| Human coronavirus | HKU1 | 290028 | <50% |
| MERS-CoV | | 1335626 | 50% |
| Adenovirus 1 | | 10533 | <50% |
| Adenovirus 2 | | 10515 | <50% |
| Adenovirus 4 | | 28280 | <50% |
| Adenovirus 5 | | 28285 | <50% |
| Aspergillus fumigatus | | 746128 | <50% |

| | | | |
|---|---|---|---|
| Candida albicans | | 5476 | <50% |
| Candida glabrata | | 5478 | <50% |
| Chlamydia pneumoniae | | 83558 | <50% |
| Chlamydia psittaci | | 83554 | <50% |
| Corynebacterium Diphtheriae | | 1717 | <50% |
| Cryptococcus neoformans | | 5207 | <50% |
| cytomegalovirus | | 10358 | <50% |
| EB virus | | 10376 | <50% |
| Enterovirus A | | 138948 | <50% |
| Enterovirus B | | 138949 | <50% |
| Enterovirus C | | 138950 | <50% |
| Enterovirus D | | 138951 | <50% |
| Human cytomegalovirus 5 | | 10359 | <50% |
| Klebsiella pneumonia | | 573 | <50% |
| Legionella Pneumophila | | 446 | <50% |
| Leptospira interrogans | | 173 | <50% |
| Measles virus | | 11234 | <50% |
| metapneumovirus | | 162387 | <50% |
| Mumps virus | | 1979165 | <50% |
| Mycobacterium tuberculosis | | 1773 | <50% |
| Mycoplasma pneumoniae | | 2104 | <50% |

| | | | |
|---|---|---|---|
| Neisseria elongata | | 495 | <50% |
| Neisseria Meningitidis | | 487 | <50% |
| Norovirus | | 142786 | <50% |
| Human respirovirus 1 | | 12730 | <50% |
| Human rubulavirus 2 | | 1979160 | <50% |
| Human respirovirus 3 | | 11216 | <50% |
| Parechovirus A | | 1803956 | <50% |
| Parechovirus B | | 1803957 | <50% |
| Pseudomonas aeruginosa | | 287 | <50% |
| Rhinovirus A | | 147711 | <50% |
| Rhinovirus B | | 147712 | <50% |
| Rhinovirus C | | 463676 | <50% |
| rotavirus | | 10912 | <50% |
| Staphylococcus aureus | | 1280 | <50% |
| Staphylococcus epidermis | | 1282 | <50% |
| Streptococcus pneumoniae | | 1313 | <50% |
| Streptococcus pyogenes | | 1314 | <50% |
| Streptococcus salivarius | | 1304 | <50% |

4) Clinical Evaluation:

All blood samples are from COVID-19 confirmed cases diagnosed based on clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period. The test sensitivity and specificity was determined by comparing the results from in total 74 patient cases and 63 healthy case. The test kit related cut off value of 0.25 (absorbance 450nm) was determined. An absorbance reading at 450nm <0.25 is considered negative and an absorbance reading at 450nm >=0.25 is considered positive. However, it is highly recommended

that each laboratory should establish its own normal and pathological reference range for anti-NP Ab levels. Furthermore, it is also recommended that each laboratory should include its own panel of control samples in the assay. A typical test reading was displayed as below

| Samples | OD450 |
|---|---|
| Negative Control | 0.198 |
| | 0.177 |
| | 0.153 |
| | 0.162 |
| Serum from healthy subjects | 0.207 |
| | 0.175 |
| | 0.223 |
| Serum from COVID-19 patients | 3.656 |
| | 0.754 |
| | 0.362 |

# 13. Contact Information and Product Support

For technical and product support, contact iCarbonX directly:
Product support website:
https://www.icarbonx.com/en/products.html
Company Address:
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Nanshan District, Shenzhen, China
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No. 1 Taoyuan Road, Songshan Lake High-tech Park
Dongguan, Guangdong,
China.