Atlas Medical

# SARS-CoV-2 TOTAL AB ELISA

IVD *For In-Vitro diagnostic and professional use only*


**Store at 2-8 °C**

**96 Tests**

## INTENDED USE

For the qualitative determination of Total antibodies to SARS-CoV-2 in human serum or plasma.

## INTRODUCTION

Coronaviruses are a group of related viruses that cause diseases in mammals and birds. In humans, coronaviruses cause respiratory tract infections that can range from mild to lethal. They are enveloped viruses with single-stranded RNA genome and a nucleocapsid of helical symmetry. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Human coronaviruses were first discovered in the late 1960s. Other members of this family have since been identified, including SARS-CoV in 2003, HCoV NL63 in 2004, HKU1 in 2005, MERS-CoV in 2012, and COVID-19 in 2019.

The COVID-19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory-syndrome, kidney failure and even death.

## TEST PRINCIPLE

Double antigen Sandwich assay. Total duration of assay: 70 minutes

Polystyrene microwell strips pre-coated with recombinant SARS-CoV-2 antigens expressed in insect cells (recombinant N-protein, S2-ECD and S1-RBD). Patient's serum or plasma sample is added, and during the first incubation step, the specific SARS-CoV-2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. A second set of recombinant antigens (recombinant N-protein, S2-ECD and S1-RBD) conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody.

The microwells are washed to remove unbound conjugate, and Chromogen solutions are added into the wells. In wells containing the antigen-antibody-antigen (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid.

The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for SARS-CoV-2 remain colorless

## MATERIALS

### Materials provided

1. Coated Microplate
   1 plate of 96 wells, 8x12 strips, Pre-coated with recombinant SARS-CoV-2 antigens (including N-protein, S2-ECD and S1-RBD).
2. Enzyme Conjugate
   1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled with recombinant SARS-CoV-2 antigens (including N-protein, S2-ECD and S1-RBD). Contains 0.1% ProClin300 preservative.
3. Sample Diluent,
   1 vial, 11 mL, ready to use
4. Substrate Solution
   1 vial, 11 mL. Ready to use, (tetramethylbenzidine) TMB.
5. Stop Solution
   1 vial,6 mL.
6. Wash Solution Concentrate
   1 vial, 30 mL (20X concentrated), PBS-Tween wash solution.
7. Positive Control:
   1 vial, 0.3 mL.
8. Negative Control:
   1 vial, 0.3 mL
9. Package insert.
10. 2 pieces of plate lid.

### Materials required but not provided

1. Microplate reader with 450nm and 620nm wave length absorbent capability.
2. Microplate washer.
3. Incubator.
4. Plate shaker.
5. Micropipettes and multichannel micropipettes delivering 50 µl with a precision of better than 1.5%.
6. Absorbent paper.
7. Distilled water

## WARNINGS AND PRECAUTIONS

1. *For in vitro diagnostic and professional use only.*
2. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
3. This package insert must be read completely before performing the test.
4. Do not use reagents beyond the labeled expiry date.
5. Do not mix or use components from kits with different batch codes.
6. Do not touch the bottom exterior 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.
7. 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.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross- contaminations.
10. Assure that the incubation temperature is 37°C inside the incubator.
11. When adding specimens, do not touch the well's bottom with the pipette tip.
12. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
13. 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.
14. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
15. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Warning: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.

17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M $H_2SO_4$ is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. 0.1% of ProClin 300 used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

**STORAGE**

1. Store all components at 2-8 °C. Do not freeze.
2. The components of the kit will remain stable through the expiration date indicated on the label and package.
3. To assure maximum performance of this ELISA kit, during storage, protect the reagents from contamination with microorganism or chemicals.

**SPECIMEN COLLECTION AND PREPARATION**

- Either serum or plasma can be used with this assay.
- Collect serum samples in accordance with correct medical practices.
- No special patient's preparation required. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
- Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric, or hemolytic specimens should not be used as they can give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
- This ELISA kit is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
- Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 7 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

**INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT**

Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one.

**PROCEDURE**

1. Bring all reagents and specimens to room temperature (18°C-25°C) before beginning the assay. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer.
2. Use only the number of wells required.
3. Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
   A1 : Blank
   B 1,C 1 : Negative Control Serum
   D 1, E 1 : Positive Control Serum
4. Transfer **100 µL of Sample Diluent** into their respective wells except the Blank.
5. Transfer **10 µL of Positive control, Negative control, and Specimen** into their respective wells except the Blank.
6. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
7. After adding Sample, the reagents in wells turns Blue color from Green.
8. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
9. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
10. Transfer **100µl of HRP-Conjugate** into each well except the Blank.
11. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
12. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
13. Transfer **100 µL of TMB Substrate** into each well induding the Blank. Incubate the plate at Room Temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in SARS-CoV- 2 Ab positive sample wells.
14. Transfer **50 µL of Stop Solution** by Using a multichannel pipette or manually into each well and mix gently. Intensive yellow color develops in Positive control and SARS-CoV-2 Ab positive sample wells.
15. Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

**INSTRUCTIONS FOR WASHING**

- A good washing procedure is essential in order to obtain correct and precise analytical data.
- It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
- To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
- Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
- In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400µl / well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
- In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
- The concentrated Wash buffer should be diluted 1 to 20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

**CALCULATION**

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 specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. The results should be calculated by subtracting the blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = NC + 0.10 (NC = the mean absorbance value for two negative controls).

Important: If the NC is lower than 0.05, take it as 0.05.

Quality control (assay validation): 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 sample being analyzed.

• The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.

• The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.

• The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining value. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality control:

Blank well A value: A1= 0.025 at 450nm.

Well No.:

Negative control A values after blanking:

Well No.:

Positive control A values after blanking:

All control values are within the stated quality control range

B1 0.022 C1 0.024

D1 2.118 E1 2.109

2. Calculation of Nc: = $\frac{0.022+0.024}{2}$ = 0.023

(Nc is lower than 0.05, so take it as 0.05)

3. Calculation of the Cut-off: (C.O.) = 0.10 + 0.05 = 0.15.

**INTERPRETATIONS OF THE RESULTS**

- Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no SARS-CoV-2 Ab has been detected with this ELISA kit. Therefore, the patient is probably not infected with SARS-CoV-2.
- Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that SARS-CoV-2 Ab has probably been detected using this ELISA kit. All initially reactive specimens should be retested in duplicates using this ELISA kit before the final assay results interpretation. Repeatedly reactive specimens could be considered positive for SARS-CoV-2 Ab and therefore there are serological indications for infection with SARS-CoV-2.
- Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.
- Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required. Clinical diagnosis should not be established based on a Single test result. It should integrate clinical and other laboratory data and findings.
- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for SARS-CoV-2 Ab. After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

**PERFORMANCE CHARACTERISTICS**

**1. Clinical specificity:**

A study of 2956 individuals was tested with 2 different ELISA kits from different manufacturers. The specificity of ATLAS SARS-CoV-2 Ab ELISA kit was 99.52%.

**2. Clinical sensitivity**:

Among 65 SARS-CoV-2 Ab confirmed were positive when tested with this ATLAS SARS-CoV-2 Ab ELISA, the sensitivity was 100%.

| Manufacturers | - | + | Confirmed positive | False Positive | Sensitiviy |
|---|---|---|---|---|---|
| ATLAS | 2927 | 29 | 15 | 14 | 99.52% |
| Other | 2928 | 28 | 15 | 13 | 99.56% |

When tested with enterprise reference, the following standards are met: the conformity rate of positive reference P1 is 1/1; the conformity rate of negative reference n1-n10 is 10/10. The test limit reference (S1-S5) requires S1-S3 to be positive, S4 to be positive or negative, and S5 to be negative. CV% not higher than 15%.

**3. Analytical Specificity**:

No cross reactivity observed with samples by RSV Ab and MP Ab positive.

No interference from rheumatoid factors up to 2000 U/ml observed. This assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

**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. Therefore, negative results obtained with SARS-CoV-2 Ab ELISA are only indication that the sample does not contain detectable level of SARS-CoV-2 Ab antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with SARS-CoV-2 or the blood unit is not infected with SARS-CoV-2.
- If, after retesting of the initially reactive samples, the assay results are negative, these samples 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.
- 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 kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

**REFERENCES**


- https://www.who.int/health-topics/coronavirus



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|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | In Vitro diagnostic medical device | | Caution |
| | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |