The test is time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure steps and do not modify them.

1. This test has not been FDA cleared or approved, this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.
2. This test has been authorized only for the presence of antibodies against SARS-CoV-2, not for any other virus or pathogens.
3. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revised sooner.
4. Do not change reagents from different lots or use reagents from other commercial available kits. The components of the kit are precisely matched for optimal performance of the tests.
5. Make sure that 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.

Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.

CAUTION - CRITICAL: Do not allow the reagents and specimens to reach room temperature (18-20°C) before use. Shake reagent gently before use. Return to 2-8°C immediately after use.

Use the sufficient volume of each reagent as specified in the protocol to ensure the test can be done, may cause sensitivity of the assay.

Do not use the selected bottle of the wells, fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate is bottom and there are no air bubbles inside the wells.

Never allow the microwells to dry after the wash step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.

After bottoming of the plate, proceed to the next assay step immediately. Assure same working conditions for all wells.

Calibrate the plate frequently to ensure the accuracy of specimens/makers performing. Use different disposable pipette tips for each specimen and makers in order to avoid cross-contamination.

Ensure that the incubation temperature is 37°C inside the incubator.

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 or 450/600-650nm.

The reagents of the kit are not chemically and reactive and chemical substances like sodium hypochlorite, acids, etc. Do not perform the assay in the presence of these substances.

All specimens from human origin should be considered as potentially infectious. Strict adhesion to GLP (Good Laboratory Practices) regulations can ensure that the assay is well performed.

WARNING: Materials from human origin may have been used in the preparation of the Negative Calibrator of the test. These materials may contain infectious agents in the accepted performance and found negation. The reagents and controls of the kit are inactivated and are not infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens from human origin with caution.

Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local environmental regulations.

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 step of disposal. Solution and reagents should be autoclaved by steam or other methods as determined by the local Safety Data Sheet (MSDS) available upon request.

Some reagents like the Stop solution, the Chromogens, and the Wash buffer may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided.

The Stop solution (0.5 M H2SO4) is irritant. Use with appropriate personal protective equipment.

Some reagents, like the wash buffer, can cause interaction with the skin and mucosa due to allergic skin reaction. Wipe spills immediately and wash with water if it comes into contact with the skin or eyes.

ProClin® 300® 0.1% or other compatible disinfectant solution designed for use on skin and mucous membranes, should be used. Recommended for percutaneous penetration or mucous membrane injuries. Wipe spills immediately and wash with water if it comes into contact with the skin or eyes.

INDICATIONS OF DETERIORATION OF THE REAGENT: Values of the Positive or Negative calibrator, which are out of control range, are indicators of possible deterioration of the reagents and/or operation of equipment errors. In such cases, the results should be considered as invalid and the specimens should be retested. In cases of consistent negative results, reprocess and revalidation of the reagents, immediately substitute the reagents with new one and contactWantai technical support for further advice.

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer with a pH meter. Adjust the pH of the Wash buffer with 0.1N NaOH or 0.1N HCl to 7.2±0.2 until crystal clear. Dilute the Wash buffer (100x) as indicated in the instructions. Use diluted or undiluted Wash buffer and only clean vessels to dilute the buffer. All other reagents are REQUIRED TO BE USED AS SUPPLIED.

Step 1 Preparation: Mark three wells as Negative calibrator (e.g. B1, C1, D1), two wells as Positive calibrator (e.g. B1, C1) and one well as a Blank (e.g. A1), rather specific reagent or 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.

Step 2 Adding calibrators and specimen: Add 50 μl of Positive calibrator, Negative calibrator, and 100μl in the corresponding wells, respectively.
Step 3: Incubating: Cover the plate with the cover and incubate at 37°C for 30 minutes.

Step 4: Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with 200 μl of Washing Buffer. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining samples.

Step 5: Adding HRP-Conjugate: Add 100 μl of HRP-Conjugate into each well except the Blank. Incubate at 37°C for 30 minutes.

Step 6: Incubating: Cover the plate with the cover and incubate at 37°C for 30 minutes.

Step 7: Washing: At the end of the incubation, remove the plate cover. Wash each well 5 times with 200 μl of Washing Buffer. After the final washing cycle, turn down the plate onto blotting paper or clean towel, and tap it to remove any remaining samples.

Step 8: Coloring: Add 50 μl of Chromogen Solution A and then 50 μl of Chromogen Solution B into each well including the Blank, mix gently. Incubate at the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction (the chromogen produces blue color in Positive calibrator and SARS-CoV-2 antibody positive wells).

Step 9: Stopping the reaction: Mix by tapping the plate gently. After stopping the reaction, add 100 μl of Stop Solution into each well and mix gently. Interact yellow color development in Positive calibrator and SARS-CoV-2 antibody positive wells.

Step 10: Measuring the Absorbance: 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 600–650nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. 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 microwasher machine at the best level of washing performances. In general, no less than 5 automatic washing cycles of 360°/400°/well are sufficient to avoid false positive readings and high background.

2. 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 to aspirate it automatically.

3. After the final automatic washing, the microplate wash liquid dispensing channels are not blocked or contaminated and sufficient volume of wash buffer is dispensed into each time to the wells.

4. In case of manual washing, try to suggest to cool 5 washing cycles, dispensed 360°/400°/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles and aspirating the liquid for 10 times.

5. 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 disposed of in an appropriate way.

6. The conditions of washing may be disturbed if used 21°C or below. If less than a whole plate is used, prepare the proportional volume of solution.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

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 value to the Cut-off value (C.O.) of the plate. If the Cut-off is based on the mean absorbance value of the Blank well A value from the print report values of specimens and calibrators.

Calculation of the Cut-off value (C.O.) = No. 1 + No. 16

(No. = the mean absorbance value for three negative calibrators). If No. < 0.03, take it as 0.03.

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. Negative and Positive control values should be included in every plate, and the ACO values must fall within expected ranges for the test results to be valid.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450nm.
- The A value of the ACO value is > 0.190 at 450nm.
- The A value of the Negative control must be ≥ 0.5 at 450/680≥650 or at 450nm after 45 minutes at 37°C.
- ACO value is < 0.5 at 450/680≥650 or at 450nm after 45 minutes at 37°C.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated using the remaining two values. 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

   - A value: A1 = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)
   - Well No.: B1
   - Negative A value after blanking: 0.020 ± 0.021

2. Calculation

   - Positive A value after blanking: 1.06 ± 1.082
   - At calibrator without buffer, a sample without analyte activity should be used without delayed usage. The A value is then calculated by the formula:

   \[ \text{A value} = \frac{\text{A elisa value} \times \text{Calibrator A} \times \text{Blank A}}{\text{Blank C.O. \times \text{Calibrator B}} \times \text{Blank C.O.}} \]

3. Calculation of the Cut-off (C.O.) = 0.03 ± 0.160

INTERPRETATIONS OF THE RESULTS

Negative Results (A/A/C/O: 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that SARS-CoV-2 antibodies have been detected using WANTAI SARS-CoV-2 Ab ELISA.

Positive Results (A/A/C/O: 2): Specimens giving an absorbance equal to or greater than the Cut-off value are considered positive, which indicates that SARS-CoV-2 antibodies have been detected using WANTAI SARS-CoV-2 Ab ELISA.

COMPARATIVE MEASUREMENTS

For evaluation of cross-reactivity with HIV: it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, an interval score of −0.2 in different false positive rates was calculated per a score method described by Altman). Study results and summary statistics are presented in the following tables:

<table>
<thead>
<tr>
<th>WANTAI SARS-CoV-2 Ab ELISA</th>
<th>Positive (mg/mL)</th>
<th>Negative (mg/mL)</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive %</th>
<th>Negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive a</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Site 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive a</td>
<td>0.8</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
**SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:**

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

**MARKING SYMBOLS:**

- **IVD:** In Vitro Diagnostic Medical Device
- 

---

**REFERENCES**

3. Positive Calibrator
   - Code 7: 1x0.5ml
4. HRP-Conjugate
   - Code 6: 1x12ml
5. Wash Buffer
   - Code 1: 1x50ml
6. Chromogen Solution A
   - Code 2: 1x8ml
7. Chromogen Solution B
   - Code 3: 1x8ml
8. Stop Solution
   - Code 4: 1x8ml

---

**EXAMPLE SCHEME OF CALIBRATORS / SPECIMENS DISPENSING:**

- **A:** Blank
- **B:** Norm
- **C:** Pos
- **D:** Neg
- **E:** Pos
- **F:** Neg
- **G:** S1
- **H:** S2

---

**SUMMARY OF THE ASSAY PROCEDURE:**

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

1. Add calibrator
2. Incubate
3. Add HRP-Conjugate
4. Incubate
5. Wash
6. Wash
7. Coloring
8. Stop the reaction
9. Read the absorbance

---

**EXAMPLE SCHEME OF CALIBRATORS / SPECIMENS DISPENSING:**

- **A:** Blank
- **B:** Norm
- **C:** Pos
- **D:** Neg
- **E:** Pos
- **F:** Neg
- **G:** S1
- **H:** S2

---

**EXAMPLE SCHEME OF CALIBRATORS / SPECIMENS DISPENSING:**

- **A:** Blank
- **B:** Norm
- **C:** Pos
- **D:** Neg
- **E:** Pos
- **F:** Neg
- **G:** S1
- **H:** S2

---

**MARKING SYMBOLS:**

- **IVD:** In Vitro Diagnostic Medical Device
- **+2°C~+8°C Storage Conditions**