# ACCEL ELISA® COVID-19 Kit

## For Detection of Total Antibody to SARS-CoV-2 in Human Serum

CE IVD REF AE301

**Intended Use**
The Plexense ACCEL ELISA® COVID-19 test is a serological microplate-based enzyme linked immunosorbent assay (ELISA) intended for the qualitative detection of total antibodies (including IgG, IgM and/or IgA) to SARS-CoV-2 in human serum. ACCEL ELISA® COVID-19 test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection.
At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. ACCEL ELISA® COVID-19 test should not be used to diagnose acute SARS-Cov-2 infection.

**Special Conditions for Use**
For in vitro diagnostic use only.
For Professional use only

**Principle of Procedure**
This assay utilizes the microplate-based enzyme immunoassay technique. Diluted serum and diluted positive and negative controls are added to microtiter wells coated with SARS-CoV-2 recombinant nucleocapsid protein. A horseradish peroxidase (HRP)-labeled polyclonal goat anti-human Immunoglobulin detector conjugate is then added to each well and the wells incubated. During this incubation, anti-SARS-CoV-2 antibodies in the serum bind to the SARS-CoV-2 nucleocapsid antigens bound to the test wells. The HRP conjugate (detector) also attaches to the anti-SARS-CoV-2 antibodies forming the recombinant antigen/human anti-SARS-CoV-2/HRP conjugate complex. Following incubation, unbound protein is removed from the wells by a washing step. A substrate solution is then added, followed by a brief incubation. Any bound HRP conjugate will catalyze a reaction with the substrate, resulting in a color change that is measured by a spectrophotometric microplate reader. The degree of substrate color change is directly related to the amount of conjugate bound to the microtiter well and is proportional to the amount of the anti-SARS-CoV-2 total antibody level in the tested specimen.

**Summary and Explanation of the Test**
The 2019 novel coronavirus (COVID-19 or SARS-CoV-2) is a single-stranded RNA coronavirus.[1] Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV-2 and bat coronaviruses.[2] In humans, coronaviruses cause respiratory infections.[3] Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N).[4] Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry.[5] Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing.[6]

SARS-CoV-2 antibodies generally become detectable following infection in immunocompetent individuals.[7] The presence of antibodies indicates seroconversion following SARS-CoV-2 infection. Negative results do not preclude SARS-CoV-2 infection as results may vary from person to person based on immunocompetency. Seroconversion may be variable from person to person therefore the time between infection to positive antibody detection in immunocompetent individuals can vary. It is also presently unknown how long human anti-SARS-CoV-2 circulating antibodies persist following infection and the level of protection these may provide.

Results are for the detection of SARS-CoV-2 antibodies. Antibodies (IgG, IgM and/or IgA) to SARS-CoV-2 are generally detectable in blood several days after initial infection although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the Europe and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of ACCEL ELISA® COVID-19 early after infection is not known. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results with ACCEL ELISA® COVID-19 may occur due to cross-reactivity from preexisting antibodies or other possible causes.

**Reagents Provided**
1. COVID-19 antigen coated Microplate: 96 wells in a 1 x 8 strip format. Each strip is packaged within a frame enclosed in a resealable foil pouch that includes a desiccant. Each well is coated with recombinant SARS-CoV-2 nucleocapsid antigen. Ready for use as supplied. Do not use wells if the foil pouch shows evidence of damage, such as tears or holes in the foil pouch.
2. Conjugate Dilution Buffer (10 mL): Ready to use as supplied.
3. Sample Dilution Buffer (10 mL): Ready to use as supplied.
4. 10X Wash Buffer (15 mL): Dilute prior to use.
5. HRP Conjugate (30 µL): HRP-labeled goat anti human globulin. Dilute prior to use.
6. TMB Substrate (13 mL): 3,3´,5,5´-Tetramethylbenzidine. Ready for use as supplied.
7. Positive Control: Prepared from serum from patients with confirmed infection with SARS-CoV-2. Dilute prior to use.
8. Negative Control: Prepared from serum derived from donors shown to be negative for SARS-CoV-2. Dilute prior to use.

**Materials Required but not Provided**
1. Red stoppered blood collection tubes without neutral gel separators.
2. General laboratory centrifuge for serum separation
3. Vortex mixer
4. Interval timer
5. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL, etc.
6. Repeating dispenser suitable for delivering 100 µL
7. Disposable pipette tips suitable for above volume dispensing
8. Disposable microcentrifuge tube or microplate for sample dilution
9. Deionized or distilled water 135 mL
10. Multichannel pipettor capable of delivering 150-200 µL
11. Spectrophotometric microplate reader capable of reading absorbance at 650 nm

**Storage and Stability**
Store at 2–8 °C. **Do not freeze**. Return to 2–8 °C immediately after use. Do not use after expiration date indicated on the kit box and/or component and reagent labels.

**Warnings and Precautions**
1. This test kit is for in-vitro diagnostic use only.
2. The microplate wells contain dried bovine serum albumin.
3. Wear gloves while performing this assay and handle all reagents as if they were potentially infectious. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.
4. Dispose of human specimens and used tests as medical waste.
5. Do not use kits beyond the expiration date.
6. In the case of damaged packaging on arrival, contact your technical support representative (contact details listed at the end of this instructions for use).

**Sample Preparation**
Samples stored at room temperature (15-30 °C) can be used within 8 hours. Samples stored at 2-8 °C can be tested within 48 hours. Samples stored longer than 48 hours should be placed at ≤ -22 °C. Samples should not be frozen and thawed repeatedly. Severely hemolyzed samples should not be used.

**Preparation of Reagents and Sample**

**Dilution of HRP Conjugate:**
1. Vortex the HRP Conjugate thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute HRP Conjugate 1:500 with Conjugate Dilution Buffer (e.g., adding 20 µL of HRP Conjugate stock to 10 mL of the Conjugate Dilution Buffer). Diluted conjugate should be used immediately after dilution. Unused diluted conjugate should be discarded at the end of a test run.

**Dilution of Negative Control:**

1. Vortex the Negative Control thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute Negative Control 1:10 with Sample Dilution Buffer (e.g., adding 20 µL of Negative Control stock to 180 µL of Sample Dilution Buffer). Diluted Negative Control should be used immediately. Unused diluted Negative Control should be discarded at the end of a test run.

**Dilution of Positive Control:**

1. Vortex the Positive Control thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute Positive Control 1:10 with Sample Dilution Buffer (e.g. adding 10 µL of Positive Control stock to 90 µL of Sample Dilution Buffer). Diluted Positive Control should be used immediately. Unused diluted Positive Control should be discarded at the end of a test.

**Dilution of Wash Buffer:**

1. Vortex the Dilution of Wash Buffer thoroughly to homogenize before opening vial and starting the dilution.
2. Before use, dilute 10X Wash Buffer 1:10 with Deionized or Distilled Water (e.g. adding 15 mL of 10X Wash Buffer to 135 mL of Deionized or Distilled Water). Diluted Wash Buffer should be used immediately. Unused diluted Wash Buffer should be discarded at the end of a test.

**Dilution of Samples:**

1. Collect blood sample into a red stoppered collection tube by venipuncture. (Do not use tubes with neutral gel separators.)
2. Allow the blood to clot.
3. Separate the serum from the clot by centrifugation.
4. Carefully transfer the serum into a clean pre-labeled tube.
5. Dilute serum 1:10 with Sample Dilution Buffer (e.g., adding 10 µL of serum to 90 µL of Sample Dilution Buffer). Diluted sample should be used immediately.

**Procedural Notes**

1. Keep light-sensitive reagents (HRP Conjugate, TMB Substrate) in the original bottles and avoid unnecessary exposure to the light.
2. Store any unused test strips in the resealable foil pouch with desiccant to protect from moisture.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may adversely affect the results.
5. Avoid introducing air bubbles in the test wells as this could result in lower binding efficiency.
6. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

**Assay Procedure:**

1. Mix diluted HRP Conjugate and each diluted Positive Control, and serum sample for 1:1 ratio (e.g., adding 80 µL of diluted HRP Conjugate to 80 µL of diluted sample and positive control)

   Mix diluted HRP Conjugate and diluted Negative Control for 1:1 ratio

   (e.g., adding 180 µL of diluted HRP Conjugate to 180 µL of diluted negative control). Mixing should occur in a clean microcentrifuge tube or clean microwell plate intended for dilution.)

   See item 8 of the Materials Required but not Provided section of this insert.
2. Transfer 100 µL of each HRP Conjugate-Serum mixture and HRP Conjugate-Positive Control mixture to individual microplate wells, and transfer 100 µL of each HRP Conjugate-Negative Control mixture to 3 microplate wells to incubate for 20 minutes at room temperature (15-30 ℃)
3. Wash the wells with Wash Buffer.
   i. Decant the contents of the wells manually with a hard, rapid downward motion. Fluid should be captured in a receptable designed to collect medical waste. With the microwells facing downward, remove all remaining residual fluid by vigorously tapping the plate on absorbent paper until the wells appear dry. It is important to remove all residual fluid. Due to the physical shape of the microwells, it is necessary to tap vigorously.
   ii. Fill each well with 120 µL 1X Wash Buffer with a multichannel pipettor.
   iii. Decant the Wash Buffer from the wells with a hard, rapid downward motion. With the microwells facing downward, remove all remaining residual fluid by vigorously tapping the plate on absorbent paper until the wells appear dry. It is important to remove all residual fluid. Due to the physical shape of the microwells, it is necessary to tap vigorously.
4. Repeat steps ii and iii four more times (total of 5 washes).

   Do not leave any residual moisture in the wells following the last wash step.
5. Add 100 µL of TMB Substrate to each well and Incubate the wells at room temperature (15-30 °C) for 10 minutes to allow for color (blue) development.
   i. For the last 30 seconds of the incubation period, shake the wells until the color (blue) is evenly distributed throughout each well. Shaking can be performed manually or by using the shake feature of the spectrophotometer.
6. Immediately read the absorbance value of each well using a microplate reader at 650 nm. The OD readings of the plate must be read within 1 minute of test completion.

**Interpretation of Results**

Calculate positive and negative results by dividing each sample's or control's reaction value at OD 650 nm by the cutoff value determined during the assay procedure.

The cut off value is determined by + 3 SD from the average OD of triplicated negative controls.

Sample and control results should be interpreted as follows:

| Calculated Value | Result | Interpretation |
|---|---|---|
| < 1.0 | Negative | Absence of antibodies to SARS-CoV-2 or that antibody levels are below the detection limits of the assay. |
| ≥ 1.0 | Positive | Presence of antibodies (IgG, IgM or IgA or any combination) indicating exposure to SARS-CoV-2 |

Test results are qualitative for total antibodies to SARS-CoV-2. Results are reported as either positive or negative. There are no equivocal results associated with this assay. Results of this test can be used only to determine prior exposure to SARS-CoV-2. They cannot be used to make a diagnosis of acute or active infection.

**Quality Control**

Test the Positive and Negative Controls with each test run. Failure of the controls to produce the expected results indicate either a technical error (i.e., dispensing error, washing error, incubation error, etc.) or that one or more test reagents were inactive or contaminated at the time of testing. When one or both controls fail, repeat both control and sample tests. Do not report results for samples, as they are considered invalid. Contact Plexense, Inc. Product Support for assistance with troubleshooting if controls repeatedly fail on retesting. (contact details listed at the end of this instructions for use).

**Limitations of the Procedure**

1. Negative results do not preclude SARS-CoV-2 infection. If active infection is suspected, direct testing for SARS-CoV-2 is necessary.
2. The sensitivity of the test early after infection is not known.
3. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.
4. This test is only for qualitative detection of total antibody to SARS-CoV-2 in human serum. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19 or SARS-Cov-2) must be combined with the patient's clinical signs in conjunction to other tests.
5. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
6. Positive results may be due to past or present infection with nonSARS-CoV-2 coronavirus strains such as HKU1, NL63, OC43, or 229E.
7. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
8. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.
9. **Residual liquid remaining in test wells after washing can interfere with the substrate and lead to erroneous readings.**
10. **Failure to shake the contents of the wells following the TMB incubation step will lead to lowered OD readings.**
11. Not for screening of donated blood

## Specific Performance Characteristics

### Repeatability

One Lot of ACCEL ELISA COVID-19 was tested Twice a day for 20 days. %CV of all OD values were less than 15%. The data are summarized in the table below.

| Samples | %CV | | |
|---|---|---|---|
| | Results | Criteria | Pass /Fail |
| Low Positive (LP) | 9.0% | ≤ 15% | Pass |
| Moderate positive (MP) | 11.0% | | Pass |
| High Positive (HP) | 12.1% | | Pass |
| High Negative (HN) | 13.0% | | Pass |
| Low Negative (LN) | 11.4% | | Pass |

**Reproducibility**

Two Lot of ACCEL ELISA COVID-19 were tested Twice a day for five days in two different experimenters and two different Laboratories. The data are summarized in the table below.

| Reproducibility | | Samples | %CV | | |
|---|---|---|---|---|---|
| | | | Results | Criteria | Pass/Fail |
| Inter Lot | PXCOV091420 | Low Positive (LP) | 4.7% | ≤ 15% | Pass |
| | | Moderate positive (MP) | 8.2% | | Pass |
| | | High Positive (HP) | 9.3% | | Pass |
| | | High Negative (HN) | 11.4% | | Pass |
| | | Low Negative (LN) | 11.1% | | Pass |
| | PXCOV091620 | Low Positive (LP) | 5.5% | | Pass |
| | | Moderate positive (MP) | 7.9% | | Pass |
| | | High Positive (HP) | 8.9% | | Pass |
| | | High Negative (HN) | 10.0% | | Pass |
| | | Low Negative (LN) | 12.2% | | Pass |
| Inter Lot | Man 1 | Low Positive (LP) | 5.2% | | Pass |
| | | Moderate positive (MP) | 8.1% | | Pass |
| | | High Positive (HP) | 8.2% | | Pass |
| | | High Negative (HN) | 10.8% | | Pass |
| | | Low Negative (LN) | 10.8% | | Pass |
| | Man 2 | Low Positive (LP) | 5.4% | | Pass |
| | | Moderate positive (MP) | 8.5% | | Pass |
| | | High Positive (HP) | 9.9% | | Pass |
| | | High Negative (HN) | 9.7% | | Pass |
| | | Low Negative (LN) | 11.5% | | Pass |
| Inter Lot | Lab 1 | Low Positive (LP) | 5.2% | | Pass |
| | | Moderate positive (MP) | 7.5% | | Pass |
| | | High Positive (HP) | 9.0% | | Pass |
| | | High Negative (HN) | 11.6% | | Pass |
| | | Low Negative (LN) | 11.7% | | Pass |
| | Lab 2 | Low Positive (LP) | 5.4% | | Pass |
| | | Moderate positive (MP) | 9.2% | | Pass |
| | | High Positive (HP) | 9.1% | | Pass |
| | | High Negative (HN) | 9.7% | | Pass |
| | | Low Negative (LN) | 11.3% | | Pass |

**Cross-reactivity Studies:**

ACCEL ELISA COVID-19 was evaluated for potential cross-reacting antibodies. A total of 40 specimens from 16 different categories were tested. There was no cross-reactivity seen with the ACCEL ELISA COVID-19 in any specimens that were tested. The data are summarized in the table below

| No. | Microorganism | Cross-reactivity |
|---|---|---|
| 1 | COVID 229E | 0 / 3 |
| 2 | COVID NL63 | 0 / 3 |
| 3 | COVID OC43 | 0 / 3 |
| 4 | COVID HKU1 | 0 / 3 |
| 5 | Influenza A virus | 0 / 3 |
| 6 | Influenza B virus | 0 / 2 |
| 7 | Adenovirus | 0 / 2 |
| 8 | Parainfluenza virus | 0 / 3 |
| 9 | Respiratory syncytial virus | 0 / 3 |
| 10 | Enterovirus group A | 0 / 1 |
| 11 | Rhinovirus group A | 0 / 3 |
| 12 | Metapneumovirus | 0 / 3 |
| 13 | Anti-HBV | 0 / 2 |
| 14 | Anti-MERS-CoV | 0 / 3 |
| 15 | Anti-SARS-CoV | 0 / 2 |
| 16 | Antinuclear Antibodies (ANA) | 0 / 1 |

**Interference Studies:**

As a result of analyzing ACCEL ELISA COVID-19 kit after adding interfering substances with concentration like below chart on 3 LP samples and 1 negative sample, every LP samples added interfering substances was positive and every N samples added interfering substances was negative.

Therefore, No interference was observed on ACCEL ELISA COVID-19 kit with 9 interfering substances.

| No. | Microorganism | Minimum targeted final concentration in serum |
|---|---|---|
| 1 | Acetaminophen | 30 µg/mL |
| 2 | Acetylsalicylic acid | 20 µg/mL |
| 3 | Albumin | 5 g/dL |
| 4 | Bilirubin, conjugated | 5 mg/dL |
| 5 | Hemoglobin | 0.1 g/dL |
| 6 | Triglycerides | 500 mg/dL |
| 7 | Na Citrate | 4% (w/v) |
| 8 | Heparin | 3000 U/L |
| 9 | EDTA | 3.4 µmol/L |

**Clinical Performance Studies:**

The clinical performance of ACCEL ELISA COVID-19 kit was validated against a panel of previously frozen samples consisting of 42 SARS-CoV-2 antibody-positive serum samples and 93 antibody-negative serum samples. Positive serum samples were confirmed with a RT-PCR and the presence of antibodies was confirmed by one or more comparator methods. Negative serum samples were taken from the healthy individuals, which confirmed by ELISA, and Rapid test in CLIA-Laboratories.

Positive Percent Agreement (PPA) was calculated for ACCEL ELISA COVID-19 against the comparator method results using a 2 x 2 table. (See Table 1.) Negative Percent Agreement (NPA) was calculated by comparing the ACCEL ELISA COVID-19 result to the expected result (See Table 2.)

***Table 1. ACCEL ELISA® COVID-19 Sensitivity Compared to RT-PCR***

| | RT-PCR Result | | |
|---|---|---|---|
| **ACCEL ELISA® COVID-19 Result** | **Positive** | **Negative** | **Total** |
| Positive | 41 | 0 | 41 |
| Negative | 1 | 0 | 1 |
| Total | 42 | 0 | 42 |
| | | | |
| Positive Percent Agreement (PPA) | 41/42 = 98**%**; 95% CI = 87.7 to 99.6% | | |

**Table 2. ACCEL ELISA® COVID-19 Specificity Compared to Expected**

| | Expected Result | | |
|---|---|---|---|
| **ACCEL ELISA® COVID-19 Result** | **Positive** | **Negative** | **Total** |
| Positive | 0 | 1 | 1 |
| Negative | 0 | 92 | 92 |
| Total | 0 | 93 | 93 |
| | | | |
| Negative Percent Agreement (NPA) | 92/93 = **99%**; 95% CI = 94.2 to 99.8% | | |

**References**


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**Symbol Agenda (EN980/ISO15223)**

| Description | Symbol |
|---|---|
| Manufacturer |  |
| For In vitro diagnostic |  |
| Storage temperature |  |
| Lot number |  |
| Catalogue Number |  |
| Consult Instruction for use |  |
| Enough reagents for n tests |  |
| Authorized Representative<br>In the European Community |  |
| CE mark |  |
| Use by date-indicates the date after which the unopened IVD/Quality Control material can not be used. |  |
| Caution, consult accompanying documents |  |
| Do not Re-use |  |

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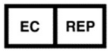

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