COVID19S-REAAD™ Anti-SARS-CoV-2 Total Antibodies ELISA

CATALOG NO.: CE0400SP1 (96 Determinations)
FOR *IN-VITRO* DIAGNOSTIC USE

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com

INTENDED USE

COVID19S-REAAD™ Anti-SARS-CoV-2 Total Antibodies ELISA (**Coronavirus Disease 2019 S** – **RE**combinant **A**ntigen – **A**ntibody **D**etection) is an enzyme–linked immunosorbent assay intended for qualitative and semi-quantitative detection of SARS-CoV-2 total antibodies to the spike protein in human serum and plasma (citrate, heparin, EDTA). COVID19S-REAAD™ is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection, and to determine the antibody levels before and after a COVID-19 vaccination. COVID19S-REAAD™ should not be used as the sole basis for diagnosis of COVID-19.

COVID19S-REAAD™ is intended for professional use in detecting COVID-19 patients or post-vaccination by detecting patient's anti-SARS-CoV-2 antibodies against spike protein. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection or vaccination, although the post-infection or vaccination duration of antibodies presence is not well characterised. Individuals may have detectable virus present for several weeks following seroconversion.

The sensitivity of COVID19S-REAAD™ early after infection is unknown. Negative/Non-reactive results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

SUMMARY AND EXPLANATION OF TEST

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. The disease was first identified in December 2019, and has since spread globally, resulting in the ongoing pandemic[2,3]. Majority of the cases result in mild symptoms, however some progress to pneumonia and multi-organ failure[2,4]. The overall mortality rate for this disease was up to 4.6% (ranging from 0.2% to 15% according to age group and other health problems)[5].

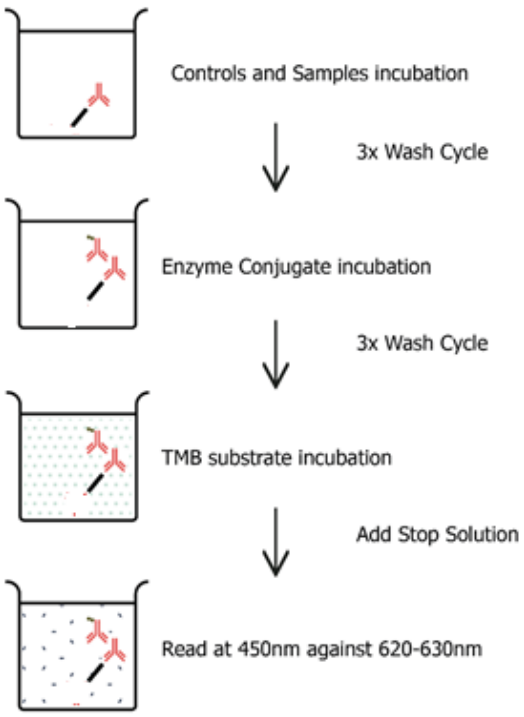
SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons. The incubation time of SARS-CoV-2 is three to seven, maximally 14 days[6].

It has been reported that PCR-confirmed SARS-CoV-2 positive patients may seroconvert and develop antibodies against SARS-CoV-2 antigens anywhere from 6-21 days after the onset of clinical symptoms[7]. The specific and reliable detection of human IgG antibodies to SARS-CoV-2 remains a key method to monitor infections, to effect contact tracing, for serosurveillance, and to monitor antibody titers after vaccination.

COVID19S-REAAD™ detects total antibodies (neutralising antibodies) against SARS-CoV-2 spike 1 receptor-binding domain (S1-RBD) glycoproteins. In conjunction with other diagnostic tests, it can be used to determine if an individual has been exposed to SARS-CoV-2 virus.

PRINCIPLE OF THE PROCEDURE

Recombinant SARS-CoV-2 proteins are bound onto the microwells. Diluted serum/plasma specimens are added to SARS-CoV-2 proteins coated wells and incubated. After incubation and washing, human antibodies against SARS-CoV-2 proteins remain bound to the well surface. Secondary antibodies conjugated to horseradish peroxidase (HRP) targeting human antibodies are then added to each well. After incubation, the microwells are washed once again before a tetramethylbenzidine (TMB) substrate is added. As the assay determination is based on enzymatic reaction, the reaction can be stopped by the addition of 1N sulphuric acid, which will turn the blue coloration to yellow. The wells can then be read on any suitable spectrophotometer or microwell ELISA plate reader. It is always recommended to read the wells at 450 nm against a 620-630 nm reference filter to eliminate any possible causes of interference.



MATERIALS PROVIDED

The amount of reagents is sufficient for 5 optimal separate runs.

Label	Reagent Constituents	Quantity
MICROPLATE	(Ready to use) 12 strips x 8 microwells coated with SARS-CoV-2 spike proteins	1 microplate
20X WASH BUFFER	(20X Concentrate) Phosphate Buffer with 1% Tween 20	50 mL
POSITIVE CONTROL	(100X Concentrate) High level of human chimeric antibody. Preservative: 0.05% Proclin 300	100 µL
NEGATIVE CONTROL	(100X Concentrate) Low level of human chimeric Antibody. Preservative: 0.05% Proclin 300	100 µL
REFERENCE CONTROL	(100X Concentrate) Cut-off level of human chimeric antibody. Preservative: 0.05% Proclin 300	100 µL
DILUENT	(Ready-to-use) Phosphate buffer with 1% Bovine serum albumin, 0.05% Tween 20 and 0.05% Proclin 300	2 x 50 mL
100X HRP CONJUGATE	(100X Concentrate) Anti-human total antibodies coupled with horseradish peroxidase in stabilizing buffer.	200 µL
TMB SUBSTRATE SOLUTION	(Ready-to-use) Solution with 3,3',5,5' tetramethylbenzidine	15 mL
STOP SOLUTION	(Ready-to-use) 1N H_2SO_4 solution	15 mL
-	Reseal-able bag for unused microwells	1 piece
-	Instructions for use	1 copy

PRECAUTIONS

1. For in vitro diagnostic use only.
2. The test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.
3. Some countries may regulate this test to be handled at Biosafety Level 2.
4. All components in the test kit have been quality controlled and tested against a Master Lot Unit. Pooling of any component is strictly not recommended; if there is sufficient balance to carry on testing, they should be use wholly on its own and never be pooled.
5. Reagents are only stable up till date of expiry and the manufacturer is not responsible for usage of expired reagents.
6. Do not use the kit if the packaging of components is damaged.
7. Do not use microwell plates if there is no desiccant inside microplate pouch.
8. Assay set up must be carried out at room temperature. Any balance reagents that have been poured out should not be replaced into their original containers in case of cross contamination. All unused portions should be discarded appropriately.
9. The test must be performed on human serum and plasma. The use of whole blood, or plasma with other anti-coagulants specimen matrices has not been validated.
10. Before opening the Control & Conjugate vials, tap the vials firmly to ensure that the liquids are at the bottom (of the vials).
11. Do not use tap water. Strictly only deionised water can be use whenever required.
12. After each wash, ensure that reagents are added immediately to avoid wells drying up.
13. To avoid cross contamination of reagents, recap containers immediately and change gloves if there are any spills. This is one major reason for false results.
14. Do not mix reagents from different lots within a test run.
15. Do not mix various lots of any kit component within an individual assay.
16. Sodium Azide has been known to inhibit conjugate activity and thus any material that contains the chemical must not be present in the testing area.
17. Over or under washing can result in assay variation and will affect validity of results.
18. Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

ADDITIONAL MATERIALS REQUIRED

1. Measuring containers for wash buffer and diluents.
2. Timer (up to 30 minutes)
3. Calibrated pipettes capable of dispensing 2-20 µL, 20-200 µL and 100-1000uL with less than 3%CV.
4. Deionised or distilled water.
5. Paper towels.
6. Wash bottle, semi-automated or automated wash equipment.
7. Microplate spectrophotometer with dual wave length. Actual reading at 450 nm with reference of 620~630 nm.
8. Dilution tubes / vials
9. 37°C Dry-Heat incubator

STORAGE AND STABILITY

1. All reagents must be stored at 2 ~ 8°C. Refer to the package for kit expiry.
2. Unopened microwells must be sealed in the reseal-able bag provided and stored at 2 ~ 8°C.

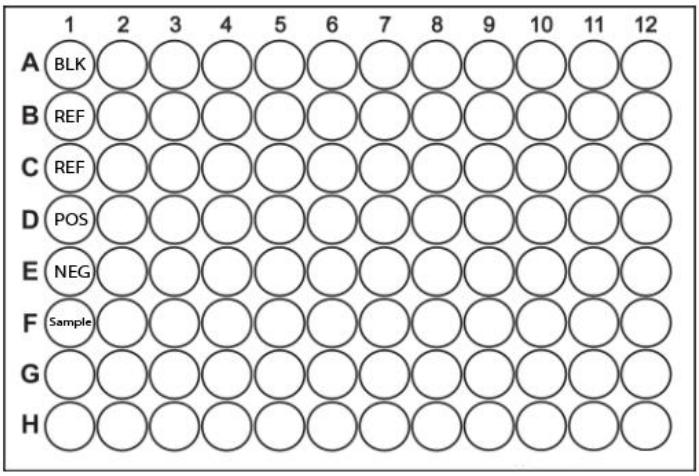
[NOTE: Only with proper and constant storage will kit be stable for the dating period specified.]

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum or plasma (citrate, heparin, EDTA).
2. Handle all blood samples as if of infectious nature.
3. Restalyst only warrants optimal performance if samples are freshly collected that are clear, non-haemolysed, non-lipemic and non-icteric.

PROCEDURE OF TEST

1. Place the desired number of strips into the microwell frame. Recommended to include: 1 well for Diluent Blank (BLK), 2 wells for REFERNCE CONTROL (REF), 1 well each for POSITIVE CONTROL (POS) and NEGATIVE CONTROL (NEG) followed by 1 well each for samples. *If using software for automated system, please check for configuration setup or contact software manufacturer for alternative recommended configuration. Additionally, ensure system is adequately maintained and calibrated appropriately.*



2. Dilute REFERENCE CONTROL, POSITIVE CONTROL, NEGATIVE CONTROL and samples at 1:100 dilution. i.e.
   2 µL of Controls/Samples + 200 µL of DILUENT or
   5 µL of Controls/Samples + 500 µL of DILUENT
3. Pipette 100 µL of the respective Controls/Samples into the designated well.
4. Cover and incubate the microplate at 37°C for 30 minutes.
5. Immediately, prepare diluted HRP Conjugate by diluting 1 volume of 100X HRP CONJUGATE with 100 volumes of DILUENT. i.e.
   10 µL of 100X HRP CONJUGATE + 1000 µL of DILUENT
   Recommended to prepare diluted HRP Conjugate minimally 10 to 20 minutes before adding into well.
   Diluted HRP Conjugate can be prepared 2 hours before adding into well.
   For automated system, prepare diluted HRP Conjugate before the start of the test.
   [CAUTION: too early or too late a preparation may yield unstable results]
6. When incubation time is up, wash the wells thoroughly either manually or by using a semi/fully automated washer. Use ~350µL of 1X Wash Buffer per well. A minimum of 3 wash cycles for manual wash is required while 3-5 cycles may be required for semi/fully automated washers.
   [NOTE: Washing Buffer is prepared by diluting 50mL 20X WASH BUFFER with 950mL distilled water]
   Manual Washing:
   Fill all wells by dispensing 350 µL of 1X Wash Buffer using multichannel pipette. Remove the 1X Wash buffer by flicking out or aspiration. Repeat this step for another 2 times
   Automated Washer Settings:
   Number of cycles: 3
   Dispense Volume: 350 µL
   Dispense Flowrate: 450 µL / Well / Sec
7. Ensure that plate is tapped dry; add 100µL of Diluted HRP Conjugate to each well.
8. Repeat steps 4 and 6.
9. Ensure that plate is tapped dry; add 100µL of ready-to-use TMB SUBSTRATE SOLUTION to each well and incubate at 37°C in the dark for 15 minutes.
10. Then add 100µL of STOP SOLUTION to each well and mix well by tapping gently on the sides.
11. Put the microplate in the OD reader to determine the Optical Density (OD) of each well immediately, with primary filter at 450 nm and reference filter at 620~630 nm.

QUALITY CONTROL

For the assay to be valid:

1. $OD_{450\text{-}630nm}$ of Blank Control should be ≤ 0.200.
2. $OD_{450\text{-}630nm}$ of Negative Control should be ≤ 0.300.
3. $OD_{450\text{-}630nm}$ of Reference Control should be between 0.300 and 0.800.
4. $OD_{450\text{-}630nm}$ of Positive Control should be ≥ 1.200.

CALCULATIONS

The antibody concentration titer [AU/mL] could be calculated as follow:

$$\frac{OD_{sample} - OD_{blank}}{OD_{Reference} - OD_{blank}} \times 1 = \text{Conc. [AU/mL]}$$

INTERPRETATION OF RESULTS

Calculate the antibody concentration titer [AU/mL] for each test sample as described above (section "CALCULATIONS")
Results are interpreted as follows:

Ab Titer < 0.40 AU/mL : Non-Reactive
Ab Titer ≥ 0.40 to 0.50 AU/mL : Borderline
Ab Titer > 0.50 AU/mL : Reactive

In case of a borderline results, a secure evaluation is not possible. It is recommended the samples should be repeat tested in duplicate. If one or more results are reactive, the final interpretation of the specimen is reactive; if the repeat results are borderline or negative, the final interpretation is borderline and another specimen should be collected.

PERFORMANCE EVALUATION

1. Precision

   Intra-assay and inter-assay precision were determined by testing 3 samples – Reactive sample, Weak reactive sample, and Non-reactive sample. The COVID19S-REAAD™ demonstrates an intra-assay precision of 1.27%, 2.71% and 5.23% as well as an inter-assay precision of 3.76%, 5.55% and 8.17% respectively. Precision was determined in accordance with CLSI Document EP05-A3. Samples were assayed in triplicate in 2 runs per day for 12 days.

		Mean Ab	Repeatability		Within-Laboratory Preision	
Specimen Type	N[a]	Titer [AU/mL]	SD[b]	%CV[c]	SD	%CV
Reactive	72	3.9257	0.0498	1.27%	0.1476	3.76%
Weak Reactive	72	1.1674	0.0316	2.71%	0.0647	5.55%
Non Reactive	72	0.1139	0.0060	5.23%	0.0093	8.17%

[a] Number of results
[b] Standard deviation
[c] Coefficient of variation

2. Analytical Specificity

   The following potential interfering substances were spiked using different concentration into reactive or negative serums against SARS-CoV-2 IgG antibodies, and tested in replicates. No false positivity or false negativity was found in the table below:

Substance	Concentration
Haemoglobin	1.310 g/dL
Total Bilirubin	4.42 mg/dL
Triglycerides	369 mg/dL
Cholesterol	289 mg/dL
Glucose	296 mg/dL
Uric Acid	13 mg/dL
Total Protein	12.1 g/dL
Creatine Kinase	332.3 U/L

3. Cross-Reactivity

   Cross-reactivity was determined in accordance with CLSI Document EP07-ED3. COVID19-REAAD™ was evaluated for potential cross-reactivity in specimens with other viral and microbial antibodies and other disease states. No false positive results were detected with most of the potential cross-reactants listed below:

Clinical Category	No. Tested	No. of Reactive
Anti-MERS	10	0
Anti-SARS	10	2
Anti-Coronavirus NL63	10	0
Anti-Coronavirus 229E	10	1
Anti-Influenza A	10	0
Anti-Influenza B	10	0
Anti-RSV	10	0
Anti-Adenovirus	10	0
Anti-EBV	30	0
Anti-HBV	13	0
Anti-HIV	14	0
Rheumatoid factor	30	0

4. Clinical Performance

   A total of 502 samples were tested which included plasma and serum. A total of 150 samples that was derived from individuals that were positively diagnosed with RT-PCR. Samples were collected more than 14 days after diagnosis using RT-PCR.

Methods			RT-PCR		Total
			Pos	Neg	
COVID19S-REAAD™ Anti-SARS-CoV-2 Total Ab ELISA	Reactive	Ab+	140	0	140
	Non-reactive	Ab-	10	352	362
Total			150	352	

This comparison gave the following results:

Positive Percent Agreement (PPA) = 93.33% (140/150)

Negative Percent Agreement (NPA) = 100.00% (352/352)

Overall Rates of Agreement (ORA) = 98.01% (140+352)/(150+352)

LIMITATIONS

1. Optimal assay performance requires the strict adherence to the assay procedure described. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
2. Results obtained from immuno-compromised individuals should be interpreted with caution.
3. The performance characteristics have not been established for visual result determination.
4. The performance characteristics have not been established for matrices other than serum and plasma (K2EDTA).
5. The assay should not be used to diagnose or exclude acute infection. Results are not intended to be used as the sole basis for patient management decisions.
6. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
7. A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present during the stage of disease in which a sample is collected.
8. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity.
9. The product has not been tested with samples positive for human coronavirus and other respiratory pathogens antibodies.
10. The assay has not been evaluated with samples collected <14 days after symptoms onset.

11. Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.
12. The sample stability has not been demonstrated.

BIBLIOGRAPHY

1. World Health Organization (WHO), 2020. Naming the coronavirus disease (COVID-19) and the virus that causes it. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it (assessed 31st March, 2020).
2. Hui, D. S.; I. Azhar E.; Madani, T. A.; Ntoumi, F.; Kock, R.; Dar, O.; Ippolito, G.; Mchugh, T. D.; Memish, Z. A.; Drosten, Christian; Zumla, A.; Petersen, E., 2020. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis*. 91: 264–66.
3. World Health Organization (WHO), 2020. *WHO Director-General's opening remarks at the media briefing on COVID-19.* https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 (assessed 31st March, 2020).
4. World Health Organization (WHO), 2020. https://www.who.int/news-room/q-a-detail/q-a-coronaviruses (assessed 31st March, 2020).
5. www.worldometers.info, 2020 Coronavirus (COVID-19) Mortality Rate https://www.worldometers.info/coronavirus/coronavirus-death-rate/ (assessed 31st March, 2020).
6. Wang G, Jin X. The progress of 2019 Novel Coronavirus (2019-nCoV) event in China. J Med Virol. 2020; 92(5): 468-72
7. Okba, N.M.A, Muller, M.A., Li, W., Wang, C., GeurtsvanKessel, C.H.,Corman, V.M., Lamers, M.M., Sikkema, R.S., de Burin, E., Chandler, F.D., Yazdanpanah.T, le Hingrat, Q., Descamps, D., Houhou-Fidouh, N., Reusken,C.B.E.M., Bosch, B.-J., Drosten, C. Kpopmans, M.P.G., & Haagmans, B.L. (2020). SARS-CoV-2 specific antibody responses in COVID-19 patients. MedRxiv,2020.03.18.20038059. https://www.medrxiv.org/content/10.1101/2020.03.18.20038059v1(assessed 30th June, 2020).

TECHNINCAL PROBLEMS/ COMPLAINTS

Should there be a technical problem/ complaint, please do the following:

1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact Restalyst office or your local distributor.

Restalyst Pte Ltd
50 Loyang Way, Singapore 508743
Tel: +65 6543 3640 Fax: +65 543 3645
Email: restalyst.info@reste-group.com

FOR PROFESSIONAL USE ONLY

EFFECTIVE DATE: 20/05/2021

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com