GBLISA - COVID-19
S1 RBD IgG ELISA TEST KIT

KIT NAME	KIT SIZE	CAT. NO
GBLISA-COVID 19 S1 RBD IgG ELISA TEST KIT	96T	GBLCOV0096T

GENUINE BIOSYSTEM

INTENDED TO USE

The GBLISA SARS-CoV-2 S1-RBD IgG antibody ELISA is intended for the qualitative detection of IgG antibodies specific for the SARS-CoV-2 Spike S1-RBD protein in human serum or plasma, and for monitoring of recovered patients.

Results from antibody testing should not be used as the sole basis to diagnosis or exclude SARS- CoV-2 infection. It is not intended for screening in general population.

SUMMARY

The outbreak of Coronavirus Disease 2019 (COVID-19) has posed a serious threat to global public health, calling for the development of safe and effective prophylactics and therapeutics against infection of its causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as 2019 novel coronavirus (2019-nCoV). The CoV spike (S) protein plays the most important roles in viral attachment, fusion and entry, and serves as a target for development of antibodies, entry inhibitors and vaccines.1

The spike (S) glycoprotein of coronaviruses is known to be essential in the binding of the virus to the host cell at the advent of the infection process. Most notable is severe acute respiratory syndrome (SARS). The severe acute respiratory syndrome-coronavirus (SARS-CoV) spike (S) glycoprotein alone can mediate the membrane fusion required for virus entry and cell fusion. It is also a major immunogen and a target for entry inhibitors. The SARS-CoV-2 spike (S) protein is composed of two subunits: the S1 subunit contains a receptor-binding domain (RBD) that engages with the host cell receptor angiotensin-converting enzyme and the S2 subunit mediates fusion between the viral and host cell membranes. The S1-RBD protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective

PRICIPLE

Indirect. Total duration of assay: **70 minutes**

Polystyrene microwell strips pre-coated with recombinant SARS-CoV-2 antigens **(Spike S1-RBD protein)** expressed in insect cells. Patient's serum or plasma sample is added, and during the first incubation step, the specific SARS-CoV-2 S1-RBD IgG antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins.

A second set of Mouse Anti-Human IgG antibody 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-anti-IgG(HRP) 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 S1- RBD SARS-CoV-2 IgG remain colorless

KIT CONTENTS:

- **Coated Microplate**, 8 x 12 strips, 96 wells. Pre-coated with recombinant SARS-CoV-2 antigens (S1-RBD).
- **Enzyme Conjugate**, 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled with Mouse Anti- Human IgG antibody. Contains 0.1% ProClin300 preservative.
- **Sample Diluent,** 1 vial, 11 mL, ***ready to use***
- **Positive Control,**1 vial, 0.3 mL.
- **Negative Control,** 1 vial, 0.3 mL
- **Wash Buffer,** 1 vial, 30 mL (20X concentrated), PBS-Tween wash solution.
- **Substrate,** 1 vial, 11 mL. Ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution,** 1 vial, 6 mL.
- **IFU**, 1 copy.
- **Plate Lid**: 2 pieces

MATERIALS REQUIRED (BUT NOT PROVIDED)

- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

PRECAUTIONS AND WARNINGS

- For in vitro diagnostic use only. For professional use only.
- 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 (18-25°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
- Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
- 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.
- 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 assay steps long time interruptions. Assure same working conditions for all wells.
- 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.
- Assure 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 at 450/630nm.
- 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.
- 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.
- 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.
- Don't 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 GLP (Good Laboratory Practices) and the local or national 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 steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
- 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.
- 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.
- 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.

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.

STORAGE

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

SPECIMEN COLLECTION AND PREPARATION

- 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 haemolysis 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.

TEST PROCEDURE

Reagents preparation

Allow the reagents to reach room temperature **(18-25°C)**. 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. All other reagents are **Ready to use**.

- **Preparation**: 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.
- **Adding Sample Diluent:** Add **100 µL** of Sample Diluent into their respective wells except the Blank.
- **Adding Sample**: Add **10 µL** of Positive control, Negative control, and Specimen into their respective wells except the Blank. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
  Note: After adding Sample, the reagents in wells turns Blue color from Green
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** 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.
- **Adding Conjugate:** Add **100µl** of HRP-Conjugate into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** 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.
- **Coloring:** Add **100 µL** of TMB Substrate into each well including 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 S1-RBD SARS-CoV-2 IgG antibody positive sample wells.
- **Stopping Reaction:** Using a multichannel pipette or manually, add **50 µL** of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and S1-RBD SARS-CoV-2 IgG antibody positive sample wells.
- **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 **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.

INTERPRETATIONS OF THE RESULTS

- The A value of the Blank well, which contains only TMB Substrate 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.
- Calculate the average value of the absorbance of the Negative Control (xNC)
- Calculate the cutoffs using the following formulas:

Positive cutoff = 1.1 X (xNC + 0.18)

Negative cutoff = 0.9 x (xNC + 0.18)

- Determine the interpretation of the sample by comparing the OD to the following table:

terpretation	Interval	Results
Negative	Measured value ≤ Negative cutoff	The sample does not contain SARS-CoV-2 S1-RBD antibodies
Positive	Measured value ≥ Positive cutoff	The sample contains SARS- CoV-2 S1-RBD antibodies
Borderline	Negative cutoff < Measured value < Positive cutoff	Retest the sample in conjunction with other clinical tests.

EXPECTED VALUE

Samples from the clinical testing presented ODs of 0.213 – 2.765 for the positive values and 0.040– 0.153 for the negative values. These values should not be in lieu of the interpretation of results calculation.

PERFORMANCE CHARACTERISTICS

Specificity: A study of 756 individuals was tested with GB ELISA kits and found that the specificity of S1-RBD SARS-CoV-2 IgG antibody ELISA kit was 98.15%.

Sensitivity: Among 171 SARS-CoV-2 IgG antibody confirmed were positive when tested with this S1-RBD SARS-CoV-2 IgG antibody ELISA, and 165 samples of which were detected with positive, the sensitivity was 96.49%.

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%.

ANALYTICAL SPECIFICITY

- No cross reactivity observed with samples by RSV IgG and MP IgG 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

- This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other tests.
- In the 1st week of the onset or after four weeks of the infection novel coronavirus patients may be negative for IgM. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgM.
- Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents may cause erroneous results.
- Water de-ionized with polyster resins may inactive the horseradish peroxide enzyme.
- Negative results do not rule out SARS-COV-2 infection, particularly in those who have been contact with the virus.
- Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- 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.
- Positive results may be due to past or present infection with non – SARS-COV -2 Coronovirus strains, such as coronavirus HUKI, NL63, OC43, or 229E

REFERENCES

1. https://www.nature.com/articles/s41423-020-0400-4
2. https://science.sciencemag.org/content/early/2020/04/02/science.abb7269

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