SARS-CoV-2 Spike S1 IgG ELISA Kit

FOR THE QUALITATIVE DETERMINATION OF ANTI SARS-COV-2 SPIKE S1 ANTIBODY IN HUMAN SERUM, PLASMA.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR USE IN HUMAN.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	SARS-CoV-2 Spike S1 IgG ELISA Kit
Catalog No.	SK00706-01
Lot No.	
Formulation	96 T
Sample Volume	10 µL
Dilution Factor	100 x (Optimal dilutions should be determined by each laboratory for each application.)
Sample Type	Human Serum or Plasma
Specificity	Anti SARS-CoV-2 Spike S1 and RBD IgG
Positive Control	Humanized Anti SARS-CoV-2 S1-RBD Monoclonal Antibody
Intra-assay Precision	6 - 8%
Inter-assay Precision	6 - 10%
Storage	2 - 8° C for 1 month. For longer storage check page 2-3 for detail.
This kit contains sufficient materials to run 90 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Info@AvisceraBioscience.com
Website: www.AvisceraBioscience.com
Website: www.AvisceraBioscience.net

INTRODUCTION

The spike protein mediates receptor binding and membrane fusion. Spike protein contains two subunits, S1 and S2. S1 contains a receptor binding domain (RBD), which is responsible for recognizing and binding with the cell surface receptor. S2 subunit is the "stem" of the structure, which contains other basic elements needed for the membrane fusion. The spike protein is the common target for neutralizing antibodies and vaccines.

When a human becomes infected with the SARS-CoV-2, it initiates an immune response. After infection, immunoglobulin antibodies such as IgM and IgG appear in blood. The IgM antibody is an early indicator of the infection and the IgG antibody is an important indicator of recent and past infection.

The Anti SARS-CoV-2 Spike S1 IgG ELISA Kit is a qualitative indirect ELISA detection system for evaluation of Human anti-SARS-CoV-2 Spike S1 IgG in Human specimen or blood samples. When the positive control, an humanized monoclonal anti-SARS-CoV-2 Spike S1 IgG, and specimen are added to the capture plate, which was precoated with the purified recombinant the glycosylated SARS-CoV-2 spike protein S1, the positive control and Human Anti SARS-CoV-2 spike protein S1 Antibody IgG in a specimen can be captured on the plate. Other unbound molecules are removed by the washing steps. Then, Detection Antibody Monoclonal Anti human IgG Fc HRP Conjugate is added to the plate. After washing steps, TMB solution is added and the color turns blue. The reaction is stopped by adding stop solution and the color turns yellow which can be read at 450 nm by a microtiter plate reader. The absorbance of the sample is proportional to the concentration of the Anti-SARS-CoV-2 S1 protein IgG.

The result is not to be used as a clinical diagnostic test.

PRINCIPLE OF THE ASSAY

This assay employs the qualitative indirectly enzyme immunoassay technique. A purified the glycosylated SARS-CoV-2 Spike S1 recombinant protein has been pre-coated onto a microplate. Positive control and samples are pipetted into the wells and any Antibody present is bound by the immobilized Spike S1 Proteins. After washing away any unbound substances, an HRP Conjugate Polyclonal Anti Human IgG Fc specific is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Human Anti SARS-CoV-2 Spike S1 IgG bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
S1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified glycosylated Spike S1 His Tag recombinant.	**706-01-01**	**1 plate**
Positive Control –1mL / vial of positive control solution.	**706-01-02**	**1 vial**
Negative Control –1 mL / vial of negative control solution.	**706-01-03**	**1 vial**
Detection Antibody HRP – 0.12 mL per vial, 100-fold concentrate of Monoclonal Detection Antibody HRP Conjugate; liquid.	**AHFC-HRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB02**	**1 bottle**

Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
HRP Diluent Solution-12 ml of buffered solution with preservative	**DB08C**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB03**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 1 month. For longer storage for up to 10 months, unopened Positive Control, Negative Control, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Detection Antibody-HRP Conjugate and TMB Substrate Solution should be stored only at 2 -8 °C. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of bloodborne infection.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human serum or plasma may require 1:100 fold dilution.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Detection Antibody -HRP Conjugate - Pipette 11.88 mL of **Dilution Buffer (DB08C)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution Detection Antibody-HRP conjugate (**protect from light**) should be used within a few hours.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Negative Control to Negative Control wells.
3. Add 100 µL of Positive control to positive control wells, samples per well. Cover with plate sealer. Incubate for 60 minutes on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody-HRP conjugate working solution to each well. Incubate for 30

minutes on microplate shaker at room temperature. **Protect from light.**

6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes on microplate shaker at room temperature. **Protect from light.**
8. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative control											
B	Negative control											
C	Positive Control											
D	Positive control											
E												
F												
G												
H												

QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative Controls. The net optical density (OD450nm) of control must fall in the values listed in the following table. If OD450nm values of controls do not meet the requirements in the following table, the test is invalid and must be repeated.

OD450 values for quality control

	OD450nm	Control of Valid Assay
Quality Control	<0.15	Negative control tested by Detection Antibody
	>0.8	Positive control tested by Detection Antibody

CALCULATION OF RESULTS

Average the duplicate readings for each negative control, positive control and sample 450nm optical density.

RESULT EVALUATION

The positive and negative cutoff values for SARS-CoV-2 Spike S1 antibody IgG detection can be used for interpretation of the sample OD values. The operator can determine the result of the sample by comparing the OD to the following table.

	OD450nm	Results	Interpretation
SARS-CoV-2 Spike S1 IgG Test	< 0.15	Negative	Undetectable SARS-CoV-2 Spike S1 Antibody IgG
	0.15 ~ 0.2	Borderline	Testing should be repeated by an alternative method or another sample should be collected.
	>0.2	Positive	SARS-CoV-2 Spike S1 Antibody IgG Detected

The result is not used as a clinical diagnostic test.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓

Add 100 µL of standard dilutions of positive control, samples, each well. Incubate for 60 minutes on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µL Detection Antibody-HRP conjugate working solution to each well. Incubate for 30 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µL Substrate Solution to each well. Incubate for 5-10 min on plate shaker at RT. **Protect from light.**

↓

Add 100 µL Stop Solution to each well. Read 450nm within 3 min.