INTENDED USE

The **Human Anti-SARS-CoV-2 Virus Spike 1 [S1] IgG** ELISA Kit is an immunoassay suitable for quantifying IgG antibody activity specific for S1 subunit of the spike protein of the SARS-CoV-2 virus, etiologic agent for the **COVID-19** respiratory disease, in serum or plasma of vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization, and timing;
- Qualifying and standardizing vaccine batches & protocols

The assay is for research use only (RUO) and is not intended nor validated for diagnosing SARS-CoV-2 virus disease. Reagents contain no virus or viral antigens.

GENERAL INFORMATION

SARS-CoV-2 virus (SARS-CoV-2), is a novel coronavirus emerged as a human respiratory pathogen and causing the 2020 pandemic named **COVID-19**. The **SARS-CoV-2** genome is closely related to 2 bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses (88% identity) and more distantly from 2 other human pathogenic coronaviruses, **SARS-CoV** (~79% identity) and **MERS-CoV** (~50% identity).

The genome of the coronavirus encodes 23 putative proteins including 4 major structural proteins: nucleocapsid [**N** protein], spike [**S** protein], membrane [**M**] and small envelope proteins [**E**]. The **S protein** is a glycoprotein essential for viral attachment to the host cell surface receptors and translocation into the infected cells; trimers of the S protein make up the spikes of the virus. The S protein is cleaved in host cells into S1 and S2 subunits; S1 protein binds the host receptor, while S2 mediates membrane fusion. A minimal receptor-binding domain [RBD] located in the S1 protein (aa. 318-510) can combine with the ACE2 receptor on host epithelial cells. While the S1 subunit of **SARS-CoV-2** shares around 70% identity to that of the 2 bat SARS-like CoVs and human SARS-CoV, the core domains of RBD (excluding the external subdomain) are highly conserved.

Recombinant proteins of SARS spike protein have shown to be highly immunogenic as vaccines and produce neutralizing antibodies. Therefore, the spike proteins represent candidates for effective vaccine development.

PRINCIPLE OF THE TEST

The Anti-SARS-CoV-2 S1 IgG ELISA kits are based on the binding of antibodies (IgG) in samples to the recombinant, purified SARS-CoV-2 S1 antigen immobilized on the microwells. Bound antibody is detected by anti-human IgG-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the HRP substrate, which is directly proportional to the amount of anti-SARS-CoV-2 S1 IgG present in the sample. Stop Solution is added to terminate the reaction, and absorbance is then measured using an ELISA reader at 450nm. The presence of antibody (IgG) in samples is determined relative to anti-SARS S1 Calibrators.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8° C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 2-8° C for long term and ambient temperature for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8° C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready To Use: Store as indicated on labels.

Component	Part	Amt	Contents
SARS-CoV-2 S1 Coated Strip Plate	405201	8-well strips (12)	Coated with purified recombinant SARS-CoV-2 S1, and post-coated with stabilizers.
Anti-SARS S1 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	405202B 405202C 405202D 405202E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-SARS S1; in buffer with antimicrobial as stabilizers.
Anti-SARS S1 Positive Control	405202-PC	0.65 ml	Antiserum with anti-SARS S1 activity; [value range on label]
Low NSB Sample Diluent (LNSD)	TBTm Reduces non-specific binding	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required and Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution
Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.
Example: Initial (1:5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1:50): **10**ul initial (1:5) + **90**ul LNSD (1:50)

Assay Design

Review Interpretation of Results (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the **Anti-SARS S1 Positive Control**; value range is on the vial label.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).
- Run a range of sample dilutions for expected higher positives that allows calculation of antibody **Titer** (when specific titer is at least 4-fold higher than non-immune)**. See Method C.**
- Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

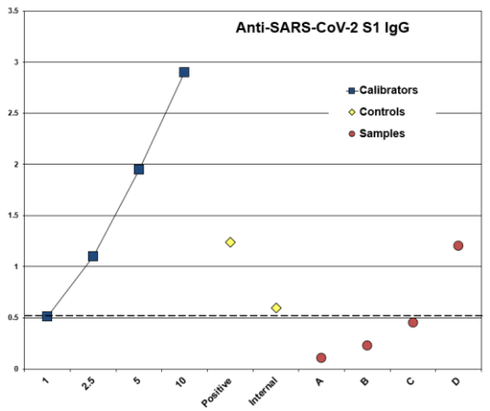
INTERPRETATION OF RESULTS

Method A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

=Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-SARS-CoV-2 S1 IgG, from either natural exposure or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-SARS S1 antibody, derived from S1 immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum reactive to SARS S1; value range is on the vial label. This Control can be used to assess reproducibility and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 2 samples (A,B) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold; 1 sample (C) is borderline.

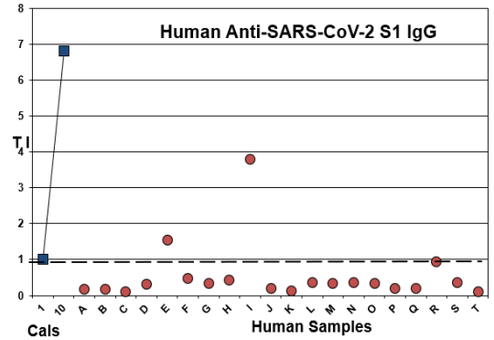
The **1 U/ml** Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p6):

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

ASSAY PERFORMANCE

Example:

Human Serum/Plasma IgG
A panel of sera from individuals of unknown history was tested for anti-SARS-CoV-2 S1 IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-SARS-CoV-2 S1 IgG: one sample (I) was positive (above the 1.0 threshold); 17 samples were negative; 2 samples (E,R) were borderline.

Notes:

1. **Positives** may be due to prior encounter with the virus or non-SARS-CoV-2 proteins with common epitopes; or may be an aspect of the innate immune repertoire.
2. When the **Positive Index** is **above 5.0**, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

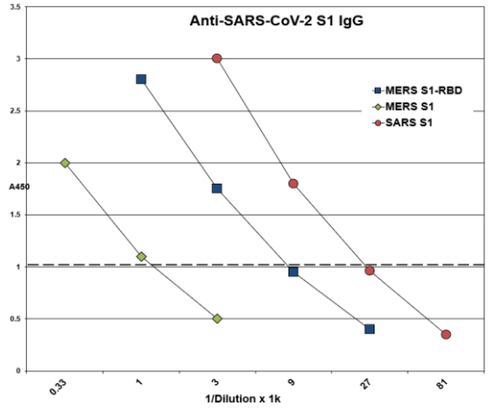
B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.
This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

INTERPRETATION OF RESULTS (cont)

C. Antibody Titer
The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading midrange in the dilution curves of each antibody as **Index.** In the example below, **IgG** titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



Results

MERS S1-RBD: rabbit anti-MERS Spike S1 RBD showing cross-reactivity with the SARS-CoV-2 S1 protein. Titer: **8.4 k**

MERS S1: rabbit anti-MERS Spike S1 showing cross-reactivity with the SARS-CoV-2 S1 protein. Titer: **1.2 k**

SARS S1: rabbit anti-SARS Spike S1 showing reactivity with the SARS-CoV-2 S1 protein. Titer: **25.5 k**

PRODUCT SPECIFICATIONS

Specificity
Recombinant SARS-CoV-2 S1 protein, 681 aa/ 76.5 kDa, was expressed as His-tag fusion protein in HEK293 cells, purified and coated on microwells; stabilizing postcoat contains BSA. The Anti-Human IgG HRP conjugate is specific for IgG; IgM, IgA and IgE class antibodies would not be detected above background.

Sensitivity
The SARS S1-coated plate, anti-human IgG-HRP concentration, and Low NSB Sample Diluent are optimized to differentiate anti-SARS S1 IgG from background (non-antibody) signal with human serum/plasma samples diluted 1:100.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested

Instruction Manual No. M-RV-405200

Recombivirus™ Human Anti-SARS-CoV-2 Virus (COVID-19) Spike 1 IgG ELISA Kit

Catalog # RV-405200, 96 tests

For the Detection and Quantitation of Anti-SARS-CoV-2 S1 IgG in Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

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ELISA Kit Components	Amount	Part
SARS-CoV-2 S1 Coated Strip Plate	8-well strip (12)	405201
Anti-SARS S1 Positive Control	0.65 ml	405202PC
Anti-SARS S1 Calibrator 1 U/ml	0.65 ml	405202B
Anti-SARS S1 Calibrator 2.5 U/ml	0.65 ml	405202C
Anti-SARS S1 Calibrator 5 U/ml	0.65 ml	405202D
Anti-SARS S1 Calibrator 10 U/ml	0.65 ml	405202E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1	RV-405200