Anti-SARS-CoV-2 Spike Glycoprotein RBD IgG ELISA Kit

Item No. 502110

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
502111	SARS-CoV-2 Spike Glycoprotein RBD Reagent	2 vial/50 dtn	-20°C
502112	SARS-CoV-2 Spike Glycoprotein RBD IgG Positive Control	1 vial	-20°C
502115	SARS-CoV-2 Spike Glycoprotein RBD IgG Negative control	1 vial	-20°C
502113	Anti-Human IgG HRP Conjugate (10X)	1 vial/1.5 ml	-20°C
502114	Streptavidin Precoated 96-Well Strip Plate	1 plate	RT
400108	Immunoassay Buffer D (5X)	2 vials/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.* safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ.cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped positive-stranded RNA virus and a member of the *Betacoronavirus* genus.[1,2] It is the causative agent of COVID-19, a primarily respiratory illness characterized by fever, cough, and shortness of breath that can lead to life-threatening complications.[3-5] The SARS-CoV-2 genome contains approximately 30 kilobases encoding four structural proteins: surface glycoprotein, envelope, membrane, and nucleocapsid.[1,2] The surface glycoprotein, also known as the spike glycoprotein, is located on the outer envelope of the virion.[1] It is composed of S1 and S2 subunits divided by a furin S-cleavage site not found in other SARS-CoVs.[6,7] The S1 subunit contains the receptor binding domain (RBD), which binds to the carboxypeptidase angiotensin-converting enzyme 2 (ACE2) following spike glycoprotein preactivation by furin and TMPRSS2, which cleave at the S1/S2 and S2' sites, respectively, facilitating viral fusion with the host cell membrane.[8-12] In this way, ACE2 acts as the functional receptor for SARS-CoV-2.

SARS-CoV-2 infection results in the production of various antibodies that bind to the SARS-CoV-2 spike glycoprotein RBD starting approximately 4-10 days after symptom onset.[13-15] Plasma and serum levels of total SARS-CoV-2 spike glycoprotein RBD-specific IgG antibodies increase for at least four weeks following symptom onset, and these levels positively correlate with SARS-CoV-2 neutralizing antibody levels in patients with SARS-CoV-2.[13,15,16] SARS-CoV-2 plasma antibody levels begin to decrease 2-3 months post-infection in both symptomatic and asymptomatic individuals, disappearing completely in some asymptomatic individuals.[17]

About This Assay

Cayman's Anti-SARS-CoV-2 Spike Glycoprotein RBD IgG ELISA Kit is an enzyme-linked immunosorbent assay that can be used for the qualitative assessment of the presence of antibodies against the SARS-CoV-2 spike glycoprotein RBD in human plasma and serum. In general, while PCR positivity indicates the presence of viral antigens, this serological assay indicates the presence of IgG antibodies against the SARS-CoV-2 spike glycoprotein RBD, which occurs in the weeks following SARS-CoV-2 infection. The IgG response measured in this kit does not differentiate neutralizing antibodies, which specifically interrupt the interaction between the spike glycoprotein RBD and the ACE2 receptor on the host cell, from non-neutralizing antibodies that bind to the RBD. A positive control is included in this kit to provide a cut-off for seropositivity.

Principle Of This Assay

This immunometric assay is based on a double-antibody "sandwich" technique. Each well of the microwell plate supplied with the kit has been coated with streptavidin, capable of pulling down a biotinylated SARS-CoV-2 spike glycoprotein RBD. Antibodies specific for the SARS-CoV-2 spike glycoprotein RBD, if present in the human plasma or serum sample, will bind to the protein immobilized on the plate during the second incubation. A second monoclonal antibody conjugated to horseradish peroxidase (HRP), which recognizes human immunoglobulin G (IgG), is added to the well forming a "sandwich". The "sandwich" is immobilized on the plate and the excess reagents are washed away. The presence of the Anti-Human IgG in the sample is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound antibody-HRP conjugate, which is proportional to the concentration of the antibodies present in the samples against the SARS-CoV-2 spike glycoprotein RBD.

A schematic of this process is shown in Figure 1, on page 9.

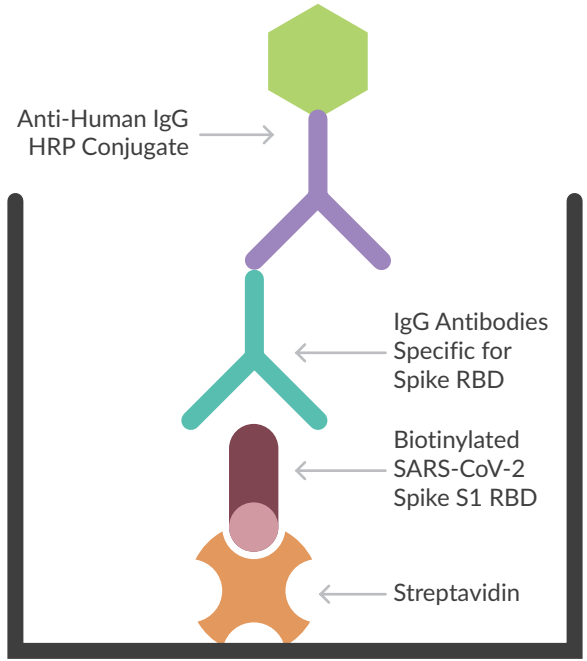

Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by TMB Substrate Solution and the HRP Stop Solution.

NSB (Non-Specific Binding): non-immunological binding of the HRP conjugate to the well. Even in the absence of specific antibody a very small amount of HRP conjugate still binds to the well; the NSB is a measure of this low binding.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water. Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

1. **Assay Buffer (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D (5X) (Item No. 400108) with 40 ml of ultrapure water and add 125 µl of **Polysorbate 20** (Item No. 400035). Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

Sample Preparation

This assay has been validated in human plasma and serum. To determine seropositivity, dilute each sample 1:100 in Assay Buffer (1X) prior to testing in the assay. To determine the relative strength of the IgG response, samples may be diluted further at the user's discretion.

Assay Validation

The Anti-SARS-CoV-2 Spike Glycoprotein RBD IgG ELISA Kit has been validated with both pre-pandemic and SARS-CoV-2 PCR positive plasma or serum. Additionally, samples were tested in an independent assay confirming the results shown below, including one outlier that was PCR positive but seronegative. PCR positivity may not indicate a direct correlation with IgG seropositivity, depending on the time of sample collection.

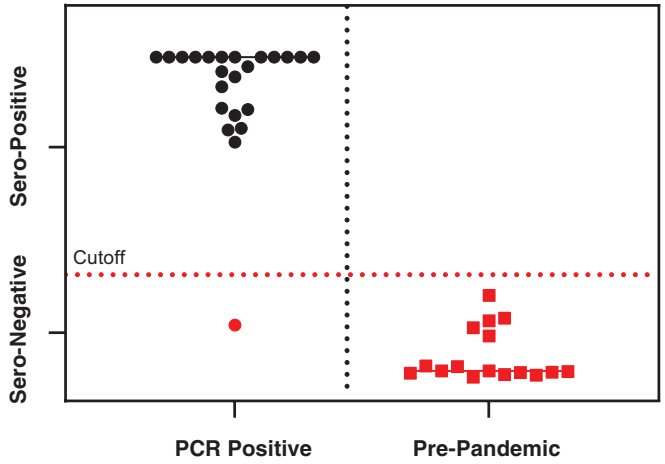

Figure 2. Assay validation

Vaccination Time-Course

Samples from a single donor following vaccination were further diluted in Cayman's Anti-SARS-CoV-2 Spike Glycoprotein RBD IgG ELISA Kit. From this, the positivity titer was back-calculated at the positivity cut-off and then plotted versus time post-vaccination.

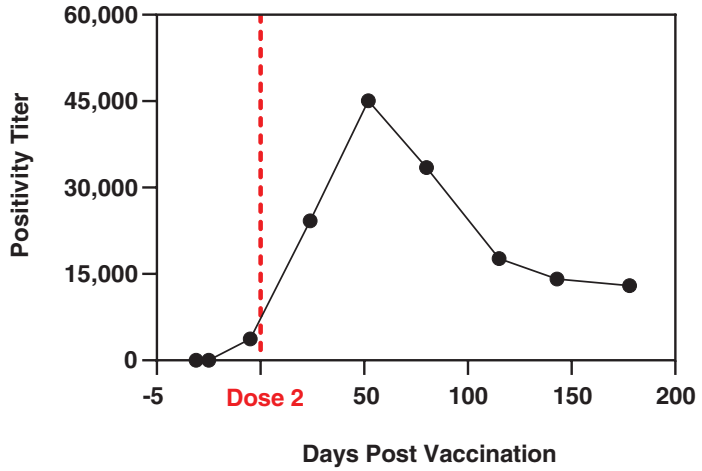

Figure 3. Vaccination time-course

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

SARS-CoV-2 Spike Glycoprotein RBD Reagent

Reconstitute one vial of the lyophilized SARS-CoV-2 Spike Glycoprotein RBD Reagent (Item No. 502111) with 6 ml of Assay Buffer (1X) and mix gently. For a full plate, reconstitute both vials of the provided reagent. The reconstituted SARS-CoV-2 Spike Glycoprotein RBD reagent will be stable for two weeks when stored at 4°C.

SARS-CoV-2 Spike Glycoprotein RBD IgG Positive Control

The SARS-CoV-2 Spike Glycoprotein RBD IgG Positive Control (Item No. 502112) vial contains lyophilized pre-pandemic human plasma or serum spiked with recombinant Anti-RBD IgG. Reconstitute the lyophilized positive control with 1 ml of Assay Buffer (1X) and mix gently. The reconstituted positive control will be stable for four weeks when stored at 4°C.

SARS-CoV-2 Spike Glycoprotein RBD IgG Negative Control

The SARS-CoV-2 Spike Glycoprotein RBD IgG Negative Control (Item No. 502115) vial contains lyophilized pre-pandemic human plasma or serum. Reconstitute the lyophilized negative control with 1 ml of Assay Buffer (1X) and mix gently. The reconstituted negative control will be stable for four weeks when stored at 4°C.

Anti-Human IgG HRP Conjugate

Anti-Human IgG HRP Conjugate (10X) (Item No. 502113) is supplied as a concentrated (10X) stock solution of Anti-Human IgG antibody conjugated to HRP. At the time of the assay, thaw the antibody-HRP conjugate at room temperature.

For a full plate, dilute 1.2 ml of the antibody-HRP conjugate into 10.8 ml of Assay Buffer (1X); for a half plate, dilute 0.6 ml of the antibody-HRP conjugate into 5.4 ml of Assay Buffer (1X) to make a 1X working solution. Do not prepare diluted antibody-HRP conjugate until immediately before use. Discard any unused antibody-HRP conjugate (1X). Store Anti-Human IgG-HRP Conjugate (10X) stock solution at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and seal well with the desiccant inside.*

Each plate or set of strips must contain Blk, NSB, positive control, and negative control wells run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed in duplicate.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest recording the contents of each well on the template sheet provided (see page 25).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	PC	PC
F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	NC	NC
G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39	NSB	NSB
H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40	Blk	Blk

S1-S44 - Sample wells
PC - Positive Control wells
NSB - Non-specific Binding wells
NC - Negative Control wells
Blk - Blank wells

Figure 4. Sample plate format

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Performing the Assay

Addition of SARS-CoV-2 Spike Glycoprotein RBD Reagent and First Incubation

1. Pipette 100 µl of the SARS-CoV-2 Spike Glycoprotein RBD Reagent into all wells except the NSB or Blk wells.
2. Pipette 100 µl of Assay Buffer (1X) into the NSB wells. Leave Blk wells empty.
3. Cover the plate with the 96-Well Cover Sheet (Item No. 400012), tap gently to mix, and incubate for 60 minutes at room temperature on an orbital shaker.

Addition of SARS-CoV-2 Spike Glycoprotein RBD IgG Controls and Samples and Second Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of the positive control, negative control, and samples into designated wells.
3. Add 100 µl of Assay Buffer (1X) to the NSB wells. Leave Blk wells empty.
4. Cover the plate with the 96-Well Cover Sheet, tap gently to mix, and incubate for 30 minutes at room temperature on an orbital shaker.

Addition of the Anti-Human IgG HRP Conjugate and Third Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of the Anti-Human IgG-HRP Conjugate (1X) working solution to all wells of the plate except Blk wells.
3. Cover the plate with the 96-Well Cover Sheet and incubate for 30 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes, protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Calculation of the Data

1. Calculate the average absorbance of the sample wells.
2. Calculate the average absorbance of the negative control wells.
3. Calculate the average absorbance of the positive control wells.
4. Calculate the percentage of positive control:

$$\left[\frac{A450_{\text{sample}} \text{ or } A450_{\text{negative control}}}{A450_{\text{positive control}}}\right] \text{ X } 100\% = \textbf{\% of Positive Control}$$

Please see the table below for guidance on interpretation of sample results. *NOTE: It is possible for samples to be >100%*

% of Positive Control	Result	Interpretation
≥ 10%	Positive	Sample contains IgG against the SARS-CoV-2 Spike RBD
7.5 - 9.99%	Borderline, cannot determine	Retest or collect new sample
< 7.5%	Negative	Sample does not contain IgG against the SARS-CoV-2 Spike RBD

Table 1. Interpretation of results

Performance Characteristics

Representative Data

The data presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. Do not use the data below to determine the values of your samples.

Controls	Absorbance	%CV* Intra-assay Precision	%CV* Inter-assay Precision
Positive Control	1.053	2.9	11.7
Negative Control	0.073	2.5	11.5
NSB	0.044	1.7	6.4
Blk	0.044	--	--

Table 2. Typical data
*%CV represents the variation in absorbance as determined using the included positive and negative controls.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High Blk (>0.1 O.D.)	A. TMB Substrate has been contaminated
High NSB (>0.15 O.D.)	A. Poor washing; ensure proper washing B. Exposure of NSB wells to controls or samples C. Contaminated buffer
High negative control wells (>0.1 O.D.)	A. Negative control has been contaminated
Low positive control wells (<0.8 O.D.)	A. Components may have degraded B. Dilution error in preparing reagents

References

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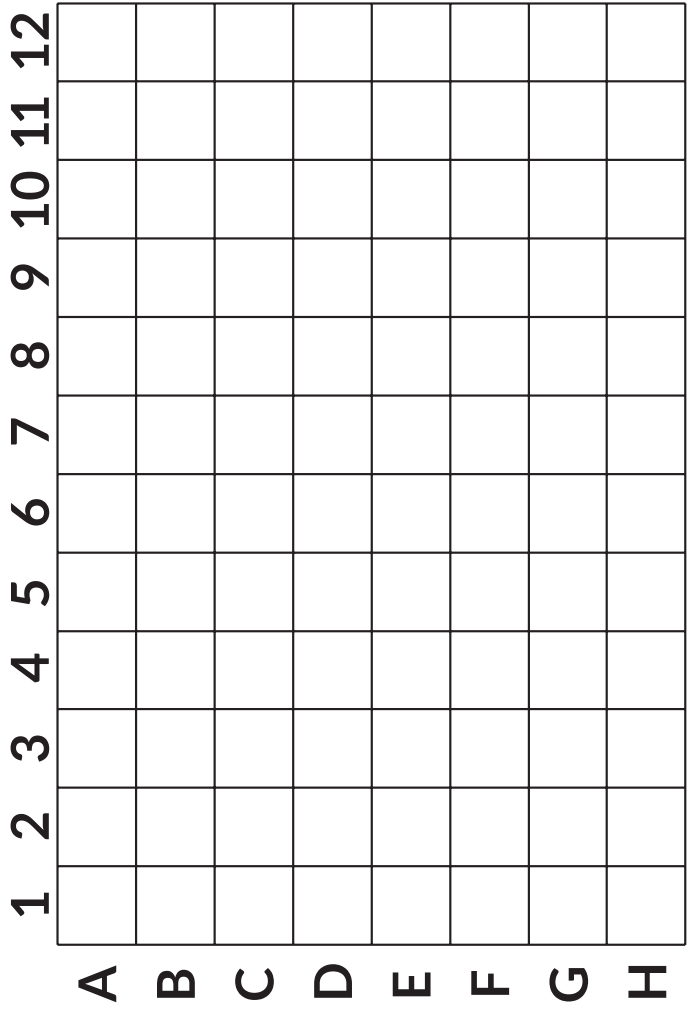
Procedure	Controls/ Samples	Blk	NSB
Reconstitute and mix	Mix all reagents gently		
Assay Buffer	--	--	100 µl
SARS-CoV-2 Spike Reagent	100 µl	--	--
First Incubation	Seal plate and incubate plate for 60 minutes at room temperature on an orbital shaker		
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)		
Assay Buffer	--	--	100 µl
Samples and Controls	100 µl	--	--
Second Incubation	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker		
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)		
Anti-IgG HRP Conjugate	100 µl	--	100 µl
Third Incubation	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker		
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)		
Apply TMB Substrate	100 µl		
Development	Seal plate and incubate for 30 minutes at RT on an orbital shaker, protected from light		
Apply HRP Stop Solution, do not wash	100 µl		
Read	Read absorbance at 450 nm		

Table 3. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.