SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit

Item No. 502820

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
502111	SARS-CoV-2 Spike Glycoprotein RBD Reagent	2 vials/50 dtn	-20°C
401138	SARS-CoV-2 Spike RBD IgG Positive Control (mouse)	1 vial	-20°C
401139	SARS-CoV-2 Spike RBD IgG Negative Control (mouse)	1 vial	-20°C
401140	Anti-Mouse IgG HRP Conjugate (10X)	1 vial/1.5 ml	-20°C
401141	SARS-CoV-2 Spike RBD IgG Standard (mouse)	1 vial/25 ng	-20°C
502114	Streptavidin Precoated 96-Well Strip Plate	1 plate	RT
400108	Immunoassay Buffer D Concentrate (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 on page 3 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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's SARS-CoV-2 Spike RBD IgG (Mouse) ELISA Kit. 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 13)

INTRODUCTION

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped positive-stranded RNA virus, a member of the *Betacoronavirus* genus, and the causative agent of COVID-19.[1-3] The SARS-CoV-2 genome contains approximately 30 kilobases and 14 open reading frames (ORFs) that encode four structural proteins: spike, envelope, membrane, and nucleocapsid, as well as 16 non-structural proteins and nine accessory factors.[4] 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.[5,6] 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.[7-11] In this way, ACE2 acts as the functional receptor for SARS-CoV-2.

In mice, however, differences in ACE2 prevent SARS-CoV-2 binding and viral infection.[12] Mice must be humanized through transgenic expression of human ACE2 or sensitization using a replication-defective adenovirus encoding human ACE2 to facilitate the induction of SARS-CoV-2 infection and pneumonia. These humanized model systems have been used to assess the effects of neutralizing antibodies on SARS-CoV-2 infection, as well as in the development of lipid nanoparticle-based vaccines for the treatment of SARS-CoV-2.[12,13]

About This Assay

Cayman's SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit can be used to assess the presence of antibodies against the SARS-CoV-2 spike RBD (Spike RBD) in mouse plasma and serum. Cayman's SARS-CoV-2 Spike RBD IgG (mouse) ELISA Kit does not differentiate between neutralizing and non-neutralizing antibodies, including those that specifically interrupt the interaction between the spike glycoprotein RBD and the ACE2 receptor on the host cell.

This ELISA is an immunometric (*i.e.* sandwich) assay that can be used either qualitatively or semi-quantitatively to assess mouse Spike RBD IgG. For qualitative analysis, a user-defined cutoff value distinguishes IgG-positive from IgG-negative mouse serum. For semi-quantitative analysis, the standard curve spans the range of 0.078-5 ng/ml, with a lower limit of detection (LLOD) of 0.02 ng/ml.

Principle Of This Assay

Each well of the supplied plate has been coated with streptavidin, which captures biotinylated Spike RBD during the first incubation. During the second incubation, any antibodies in the sample specific for Spike RBD will bind to the immobilized Spike RBD protein on the plate. Spike RBD-specific IgG present in the well is then detected using the supplied Anti-Mouse IgG-HRP Conjugate, which recognizes mouse IgG. The enzymatic activity of HRP is subsequently determined 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 HRP conjugate, which is proportional to the concentration of the antibodies present in the sample against Spike RBD.

$$\text{Absorbance} \propto [\text{anti-mouse IgG HRP}] \propto [\text{Spike RBD IgG (mouse)}]$$

A schematic of this process is shown in Figure 1, on page 10.

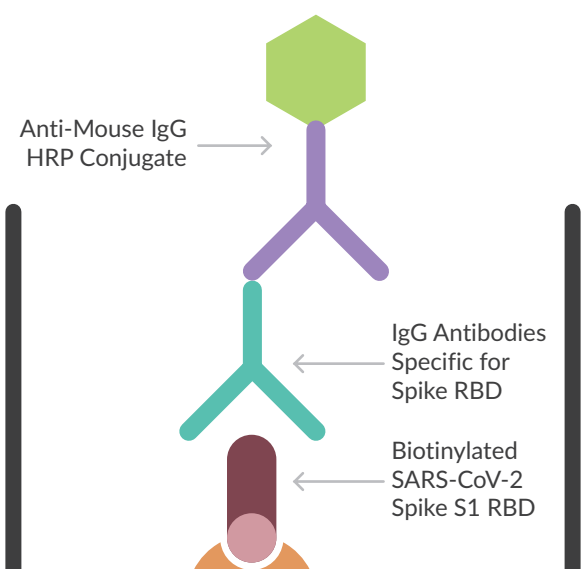


Figure 1. Schematic of the ELISA

Definition of Key Terms

NSB (Non-Specific Binding): non-immunological binding of the HRP conjugate to the well.

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for at least 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 Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D Concentrate (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.

Sample Preparation

This assay has been validated in mouse serum and plasma. Validation data is available at https://www.caymanchem.com/product/502820.

Qualitative Assay

To determine positivity, dilute each sample 1:100 in Assay Buffer prior to testing.

To assess Spike RBD IgG response strength or determine titer, prepare serial dilutions starting at 1:100. Titers may be expressed relative to a user-defined fixed absorbance, with the titer corresponding to the highest dilution that still produces a positive signal.

Semi-Quantitative Assay

To estimate levels of Spike RBD IgG in positive samples, prepare a standard curve using the included SARS-CoV-2 Spike RBD IgG Standard (mouse) (Item No. 401141). As a guideline, mouse samples collected exactly 3 weeks post-immunization were found to require an approximate starting dilution between 1:500 and 1:1,000 to fall within the range of the standard curve. However, optimal dilution will vary between samples and should be determined empirically. The measured IgG concentrations are approximate, as the affinity of various antibodies to Spike RBD may differ from that of the standard.

Preparation of Assay-Specific Reagents

SARS-CoV-2 Spike RBD IgG Standard (mouse) (Semi-Quantitative Assay only)

Reconstitute the lyophilized SARS-CoV-2 Spike RBD IgG Standard (mouse) with 1 ml of Assay Buffer and mix gently. The concentration of this solution (the bulk standard) will be 25 ng/ml. The reconstituted standard will be stable for one month when stored at 4°C.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-7 and NSB. Aliquot 800 µl of Assay Buffer to tube #1 and 500 µl to tubes #1-7 and the NSB tube. Transfer 200 µl of the bulk standard (25 ng/ml) to tube #1 and mix gently. The concentration of this standard, the first point on the standard curve, will be 5 ng/ml. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Repeat this process for tubes #3-7. Do not add any standard to the NSB tube (Assay Buffer only).

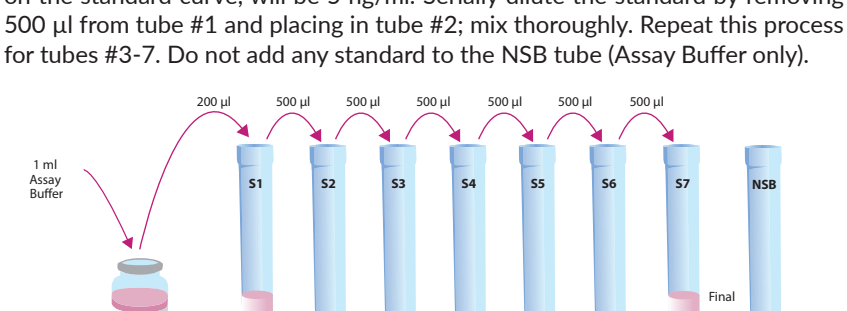


Figure 2. Preparation of the SARS-CoV-2 Spike RBD standards

SARS-CoV-2 Spike Glycoprotein RBD Reagent

Immediately prior to use, reconstitute one vial of lyophilized SARS-CoV-2 Spike Glycoprotein RBD Reagent (Item No. 502111) with 6 ml of Assay Buffer and mix gently. Each vial contains a sufficient amount to assay half a plate. The reconstituted SARS-CoV-2 Spike Glycoprotein RBD Reagent will be stable for two weeks when stored at 4°C.

SARS-CoV-2 Spike RBD IgG Positive Control (mouse) (Qualitative Assay only)

This vial contains lyophilized mouse serum spiked with Spike RBD IgG. Reconstitute the lyophilized SARS-CoV-2 Spike RBD IgG Positive Control (mouse) (Item No. 401138) with 1 ml of Assay Buffer and mix gently. The reconstituted positive control will be stable for two weeks when stored at 4°C.

SARS-CoV-2 Spike RBD IgG Negative Control (mouse) (Qualitative Assay only)

Reconstitute the lyophilized SARS-CoV-2 Spike RBD IgG Negative Control (Item No. 401139) with 1 ml of Assay Buffer and mix gently. The reconstituted negative control will be stable for three weeks when stored at 4°C.

Anti-Mouse IgG HRP Conjugate (1X)

Anti-Mouse IgG HRP Conjugate (10X) (Item No. 401140) is supplied as a concentrated (10X) stock solution of anti-mouse IgG antibody conjugated to HRP. At the time of the assay, thaw the HRP conjugate at room temperature.

To assay half a plate, dilute 0.6 ml of the HRP conjugate into 5.4 ml of Assay Buffer to make a 1X working solution. Scale up or down as needed. Do not dilute HRP-conjugate until immediately before use. Discard any unused HRP-conjugate (1X). Aliquot any unused Anti-Mouse IgG HRP Conjugate (10X) and store at -20°C.

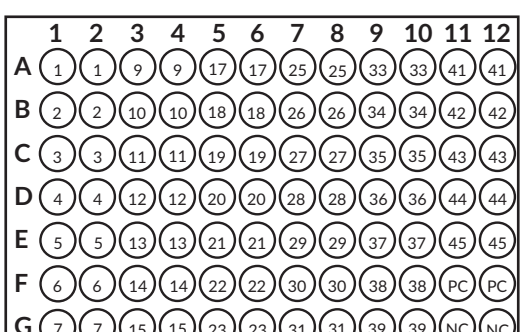
Plate Set Up

The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate 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 store at room temperature. Be sure the plate packet is sealed with the desiccant inside.*

For qualitative analysis, each plate or set of strips must contain a minimum of two NSB wells, two negative control wells, and two positive control wells. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at least in duplicate.*

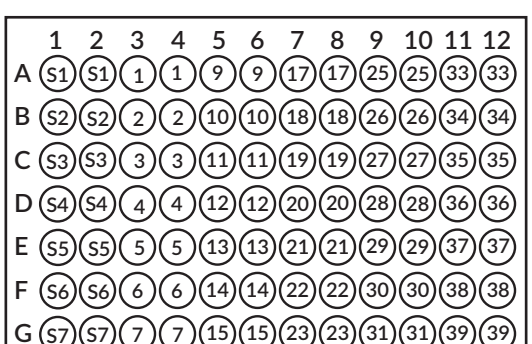
For semi-quantitative analysis, each plate or set of strips must contain a minimum of two NSB wells and a seven-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at least in duplicate.*

Suggested plate formats are shown in Figures 3 and 4, on page 17 and 18. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well be recorded on the template sheet provided (see page 29).



1-45 = Sample Wells
PC = Positive Control Wells
NC = Negative Control Wells
NSB = Non-specific Binding Wells

Figure 3. Qualitative assay sample plate format



NSB = Non-Specific Binding Wells
S1-S7 = Standards Wells
1-40 = Sample Wells

Figure 4. Semi-quantitative assay sample plate format

Performing the Assay

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.

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

1. Pipette 100 µl of the reconstituted SARS-CoV-2 Spike Glycoprotein RBD Reagent into all wells.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012), and incubate for 60 minutes at room temperature on an orbital shaker.

Addition of Controls, Standards, 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 and negative controls (qualitative format only) or standards S1-S7 (semi-quantitative format only) and the samples into the designated wells.
3. Add 100 µl of Assay Buffer to the NSB wells.
4. Cover the plate with the 96-Well Cover Sheet an incubate for 30 minutes at room temperature on an orbital shaker.

Addition of the Anti-Mouse 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-Mouse IgG-HRP Conjugate (1X) to all wells of the plate.
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. Remove the plate cover being careful to keep TMB Substrate Solution from splashing on the cover. *NOTE: Any loss of TMB Substrate Solution will affect the absorbance readings.*
5. 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

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used.

Qualitative Assay

Calculations

1. Calculate the average absorbance of the NSB wells.
2. Calculate the average absorbance of the sample wells and subtract the average NSB. This is the corrected sample.
3. Calculate the average absorbance of the positive control wells and subtract the average NSB. This is the corrected positive control.
4. Calculate the average absorbance of the negative control wells and subtract the average NSB. This is the corrected negative control.
5. Calculate the percentage of positive control for each sample:

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

The positive control supplied is intended to be a reference tool for assessing whether a sample is positive for IgG antibodies against Spike RBD. The positivity cutoff (see Table 1, below) was determined experimentally in Cayman's study of known negative (pre-immunization) sera and sera collected beginning at three weeks post-immunization.

User discretion is advised. The positivity cutoff for mouse sera may change accordingly to align with the end user's experiment.

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

% of Positive Control	Result	Interpretation
≥15%	Positive	Sample contains IgG against Spike RBD

Table 1. Qualitative sample interpretation

Semi-Quantitative Assay

Plot the Standard Curve and Determine the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) versus concentration (linear x-axis) for standards S1-S7 and include the NSB wells as a zero standard. Fit the data with a quadratic fit. Using the equation of the line, calculate the concentration of Spike RBD IgG in each sample, making sure to correct for sample dilution.

Please note that this is a semi-quantitative assay, as polyclonal antibodies present in a sample will have different affinities to the Spike RBD than the antibody standard supplied in this kit. During assay validation, a typical immune response resulted in a SARS-CoV-2 Spike RBD IgG concentration in excess of 2,000 ng/ml.

Performance Characteristics

Representative Data of Semi-Quantitative Assay

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

SARS-CoV-2 Spike RBD IgG Standards (ng/ml)	Absorbance (450 nm)	%CV* Intra-assay Precision	%CV* Inter-assay Precision
5	2.572	4.9	0.1
2.5	1.373	6.4	1.0
1.25	0.723	4.5	2.3
0.63	0.388	5.4	2.2
0.31	0.212	5.7	2.3
0.16	0.132	4.9	4.7
0.08	0.090	14.0	9.5
NSB	0.041	-	-

Table 2. Typical results for semi-quantitative assay
*%CV represents the variation in concentration as determined using a reference standard curve

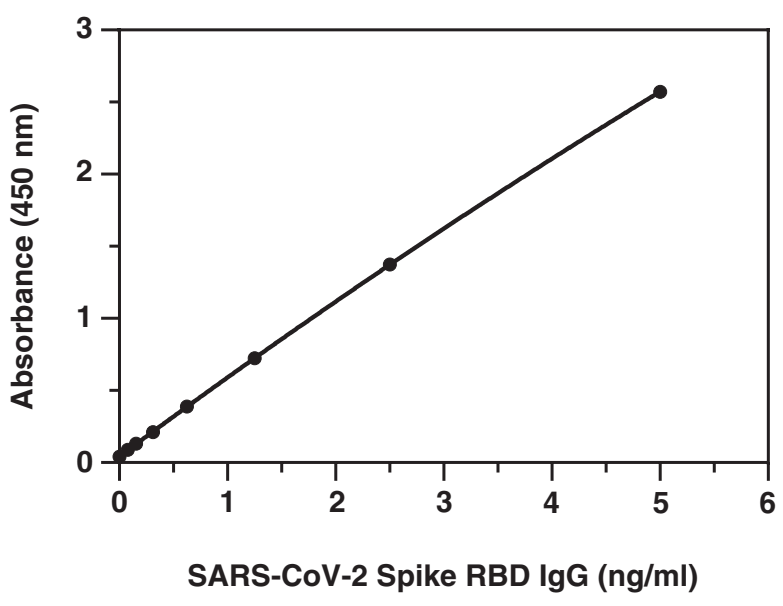


Figure 5. Typical standard curve

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
High NSB (>0.2 O.D.)	A. TMB substrate has been contaminated B. Poor washing; ensure proper washing C. Exposure of wells to controls, standards, or samples D. Polysorbate 20 was not added to the buffer
High negative control wells (>15% Positivity cut-off)	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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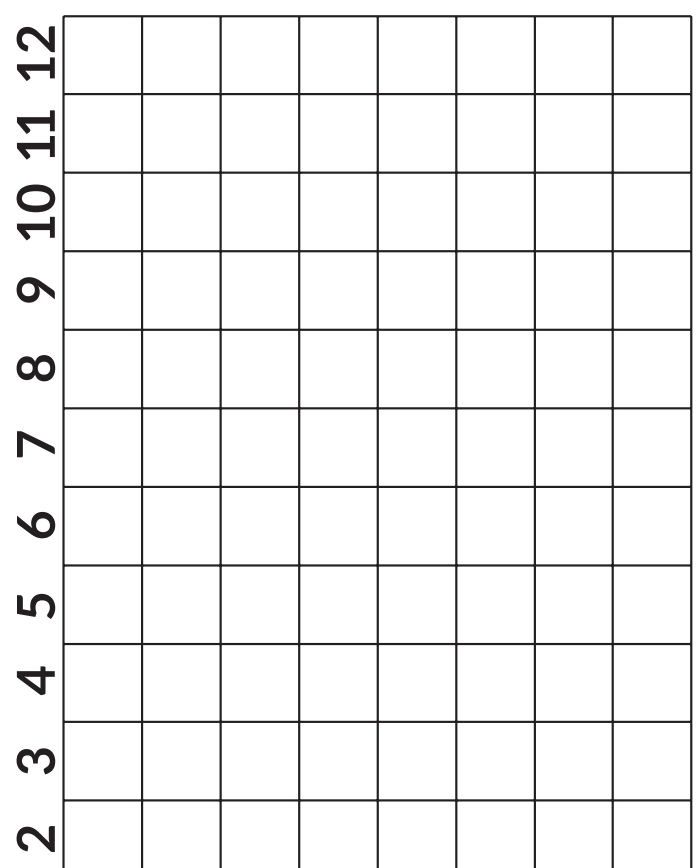
12. Hassan, A.O., Case, J.B., Winkler, E.S., *et al*. *A SARAS-CoV-2 infection model in mice demonstrates protection by neutralizing antibodies*. *Cell* **182**, 744-753 (2025).
13. Swingle, K.L., Hamilton, A.G., Han, X., *et al*. Circular RNA lipid nanoparticle vaccine against SARS-CoV-2. *PNAS* **122(38)**, e2505718122 (2025).

Assay Summary

Procedure	Controls/Standards and Samples	NSB
Prepare Reagents	Mix all reagents gently	
Add SARS-CoV-2 Spike RBD Reagent	100 µl	
First Incubation	Seal plate and incubate for 60 minutes at room temperature on an orbital shaker	
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)	
Add Controls/Standards and Samples	100 µl	--
Add Assay Buffer	--	100 µl
Second Incubation	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker	
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)	
Add Anti-IgG HRP Conjugate	100 µl	
Third Incubation	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker	
Wash	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 room temperature on an orbital shaker, protected from light	
DO NOT WASH. Add HRP Stop Solution	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.