SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit

Item No. 502070

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
402071	SARS-CoV-2 Spike Reagent	3 vials	-20°C
402072	ACE2 Reagent	3 vials	-20°C
502074	SARS-CoV-2 Neutralizing Antibody Standard	1 vial	-20°C
402054	Anti-His-HRP Conjugate (50X)	1 vial/360 µl	-20°C
502073	SARS-CoV-2 Neutralizing Antibody Positive Control	1 vial/500 µl	-20°C
502075	SARS-CoV-2 Neutralizing Antibody Negative Control	1 vial/500 µl	-20°C
400004/400006	Mouse Anti-Rabbit IgG-Coated Plate	1 plate	4°C
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	2 vials/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.

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

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3640

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).*

INTRODUCTION

Background

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.

ACE2 is expressed in vascular endothelial cells, as well as in the epithelial cells of the kidney, heart, lung, small intestine, and liver.[13] It acts as a negative regulator of signaling through angiotensin II by converting angiotensin II to the vasodilatory and anti-inflammatory peptide angiotensin 1-7.[14] ACE2 is downregulated by SARS-CoV-2 binding, which disrupts the protective effects of angiotensin 1-7. The SARS-CoV-2-ACE2 interaction is a potential target for reducing viral infection. Recombinant human soluble ACE2 inhibits SARS-CoV-2 attachment to cells, and antibodies in convalescent plasma or those raised against the SARS-CoV-2 spike glycoprotein reduce viral entry *in vitro*.[8,15]

SARS-CoV-2 infection can result in the production of neutralizing antibodies, which bind to the SARS-CoV-2 spike RBD preventing further viral entry and infection, starting approximately 4-10 days after symptom onset.[16,17] Plasma levels of SARS-CoV-2 spike glycoprotein-specific IgG antibodies increase for at least four weeks following symptom onset.[16,18] 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.[19]

The detection of neutralizing antibodies to SARS-CoV-2 is important in evaluating the lifetime and efficacy of specific antibodies in the host.

About This Assay

Cayman's SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit is a competitive assay that can be used for qualitative and/or semi-quantitative measurement of neutralizing antibodies in human plasma and serum. When using the neutralizing antibody standard provided in the kit, the assay has a range of 7.81-1,000 ng/ml with a midpoint of approximately 120 ng/ml (50% B/B_0) and a sensitivity (80% B/B_0) of approximately 46 ng/ml.

Principle Of This Assay

Cayman's SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit provides a robust and easy-to-use platform for identifying neutralizing antibodies of the SARS-CoV-2 spike S1 RBD and ACE2 interaction. The assay uses a recombinant rabbit Fc-tagged SARS-CoV-2 spike S1 RBD that binds to a plate pre-coated with an anti-rabbit Fc-specific antibody. A recombinant His-tagged ACE2 protein binds the SARS-CoV-2 spike S1 RBD and the complex is detected with an HRP-conjugated anti-His antibody, which is easily quantified by reading the absorbance at 450 nm. The standard used in this kit is a recombinant SARS-CoV-2 neutralizing antibody capable of interfering with the ACE2 protein for binding sites on the SARS-CoV-2 spike S1 RBD. Any SARS-CoV-2 neutralizing antibodies present will interfere with the interaction between SARS-CoV-2 spike S1 RBD and ACE2.

$$\text{Absorbance} \propto [\text{Bound ACE2}] \propto 1/[\text{neutralizing antibody}]$$

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

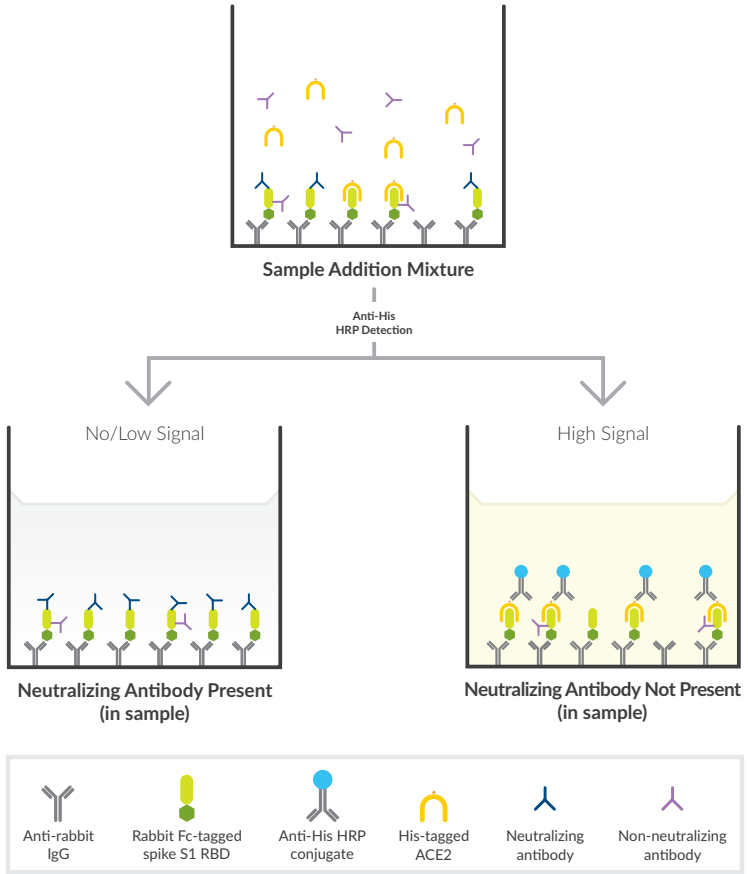


Figure 1. Schematic of the SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit

Definition of Key Terms

Blk (Blank): background absorbance caused by TMB Substrate Solution and the HRP Stop Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

NSB (Non-Specific Binding): non-immunological binding of the Anti-His-HRP Conjugate to the well. Even in the absence of the spike S1 RBD a very small amount of Anti-His-HRP Conjugate still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of ACE2 that the spike S1 RBD can bind in the absence of neutralizing antibodies.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of SARS-CoV-2 neutralizing antibody.

Dtn: determination, where one dtn is the amount of reagent used per well.

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 approximately two months. NOTE: It is normal for the concentrated buffers to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer D (1X) Preparation**

   Dilute the contents of two vials of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 80 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer 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 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding Polysorbate 20 to an end concentration of 0.5 ml/L. *NOTE: 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.*

Sample Preparation

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -20°C.

Testing for Interference

This assay has been validated using human plasma and serum. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve). If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated SARS-CoV-2 neutralizing antibody concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatability in the assay.

Qualitative Assay

For a simple positive or negative result for a sample, dilute human plasma or serum 1:10 in Immunoassay Buffer D (1X) prior to testing in the assay. The resulting data will be an indicator of a positive or negative result based on Table 2 (see page 24). It is recommended to utilize the included positive and negative controls, but the standard curve does not need to be run in a qualitative assay.

Semi-Quantitative Assay

As a secondary test to determine approximate amounts of neutralizing antibodies found in positive samples, run a curve of the included SARS-CoV-2 Neutralizing Antibody Standard. Dilute samples to obtain at least two different dilutions between 20-80% B/B_0, which is the linear portion of the standard curve. The amount of neutralizing antibodies found will be approximate, as the affinity of various neutralizing antibodies to the spike S1 RBD could be different than the standard supplied in the kit.

Sample Matrix Properties

Linearity

Plasma and serum positive for SARS-CoV-2 neutralizing antibodies were serially diluted with Immunoassay Buffer D (1X) and evaluated for linearity using the SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit. The results are shown in the table below. Neutralizing antibodies found in samples may not back-calculate quite as linearly because affinities can differ between different antibodies.

Dilution Factor	Concentration (ng/ml)	Dilutional Linearity (%)
Plasma		
320	54,547	100
640	62,684	115
1,280	66,987	122
Serum High in SARS-CoV-2 Neutralizing Antibodies		
200	44,120	100
400	45,156	102
800	49,150	111
Serum Low in SARS-CoV-2 Neutralizing Antibodies		
10	1,959	100
20	2,117	108
40	2,414	123

Table 1. Dilutional linearity of human plasma and serum samples

Parallelism

To assess parallelism, plasma and serum positive for SARS-CoV-2 neutralizing antibodies were assayed at multiple dilutions in the SARS-CoV-2 Neutralizing Antibody Detection ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below. Results may not be typical, as neutralizing antibodies found in samples may have different affinities to the spike S1 RBD.

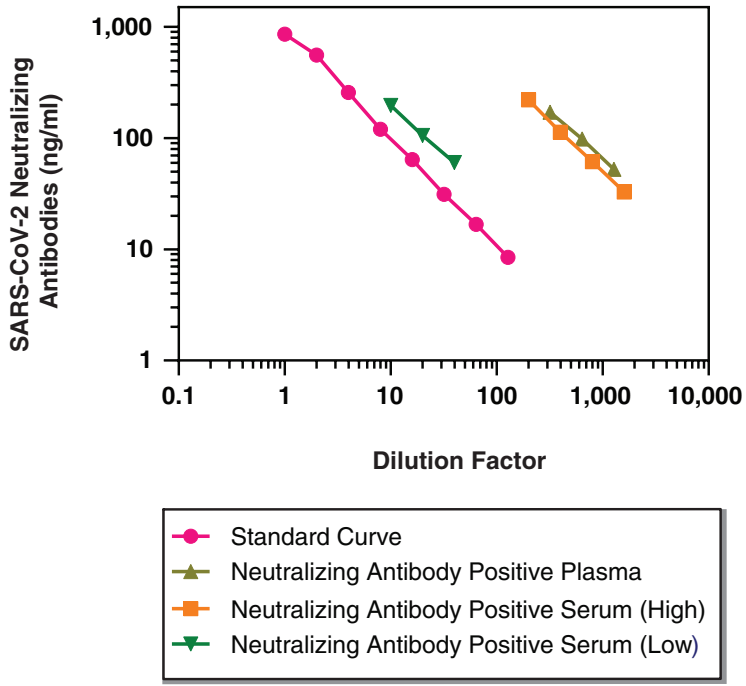


Figure 2. Parallelism of human plasma and serum samples

Preparation of Assay-Specific Reagents

SARS-CoV-2 Neutralizing Antibody ELISA Standard (optional)

To run a semi-quantitative assay, prepare the standard for use in the ELISA:

Reconstitute the lyophilized SARS-CoV-2 Neutralizing Antibody Standard (Item No. 502074) with 1 ml of Immunoassay Buffer D (1X), and mix gently. The concentration of this solution (the bulk standard) will be 1,000 ng/ml. The reconstituted standard will be stable for four hours at room temperature and for at least a week when stored at 4°C.

Obtain eight clean test tubes and label them #1-8. Aliquot 200 µl Immunoassay Buffer D (1X) to tube #2-8. Transfer 200 µl of the freshly prepared bulk standard (1,000 ng/ml) to tube #2 and mix gently. Serially dilute the standard by removing 200 µl from tube #2 and placing in tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored longer than four hours.

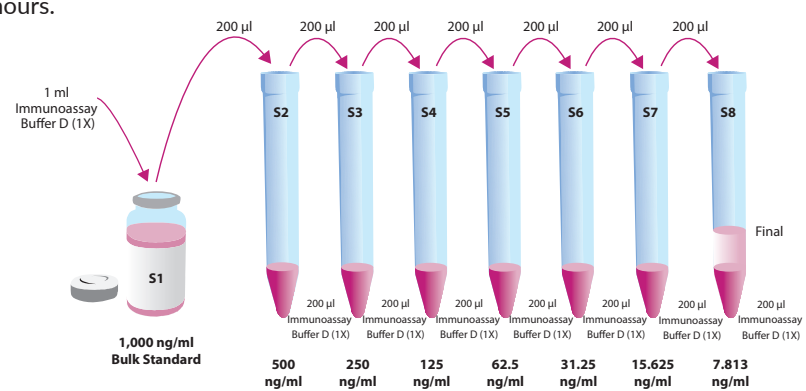


Figure 3. Preparation of the SARS-CoV-2 neutralizing antibody standards

SARS-CoV-2 Spike Reagent

This kit includes three vials of lyophilized SARS-CoV-2 Spike Reagent (Item No. 402071). One vial, after reconstitution, is a sufficient amount of reagent for one-third of a 96-well plate. At the time of the assay, reconstitute one of the vials with 2 ml of Immunoassay Buffer D (1X). Use immediately after reconstitution. Prepare and pool additional vials as needed.

ACE2 Reagent

This kit includes three vials of lyophilized ACE2 Reagent (Item No. 402072). One vial, after reconstitution, is a sufficient amount of reagent for one-third of a 96-well plate. At the time of the assay, reconstitute one of the vials with 2 ml of Immunoassay Buffer D (1X). Use immediately after reconstitution. Prepare and pool additional vials as needed.

Anti-His-HRP Conjugate (1X)

For a full plate, dilute the contents of one vial of Anti-His-HRP Conjugate (50X) (Item No. 402054) with 18 ml of Immunoassay Buffer D (1X). It is recommended that the Anti-His-HRP Conjugate (50X) be diluted immediately prior to performing the assay. If all of the Anti-His-HRP Conjugate (50X) will not be used at one time, store the undiluted conjugate at 4°C where it will be stable for at least 6 months.

SARS-CoV-2 Neutralizing Antibody Positive Control (optional)

The SARS-CoV-2 Neutralizing Antibody Positive Control (Item No. 502073) is ready to use as supplied. If all of the SARS-CoV-2 Neutralizing Antibody Positive Control will not be used at one time, aliquot and store at -20°C where it will be stable for at least 6 months.

SARS-CoV-2 Neutralizing Antibody Negative Control (optional)

The SARS-CoV-2 Neutralizing Antibody Negative Control (Item No. 502075) is ready to use as supplied. If all of the SARS-CoV-2 Neutralizing Antibody Negative Control will not be used at one time, aliquot and store at -20°C where it will be stable for at least 6 months.

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 store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.*

A suggested plate format for both the qualitative and semi-quantitative assays are shown in Figures 4 and 5, on page 19. The user may vary the location and type of wells present as necessary for each particular experiment. The semi-quantitative plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 23 for more details). We suggest recording the contents of each well on the template sheet provided (see page 33).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	NSB	B_0	5	5	5	13	13	13	21	21	21
B	Blk	NSB	B_0	6	6	6	14	14	14	22	22	22
C	-	+	B_0	7	7	7	15	15	15	23	23	23
D	-	+	B_0	8	8	8	16	16	16	24	24	24
E	1	1	1	9	9	9	17	17	17	25	25	25
F	2	2	2	10	10	10	18	18	18	26	26	26
G	3	3	3	11	11	11	19	19	19	27	27	27
H	4	4	4	12	12	12	20	20	20	28	28	28

Blk - Blank
NSB - Non-Specific Binding
B_0 - Maximum Binding
- - Negative Control
+ - Positive Control
1-28 - Samples

Figure 4. Qualitative assay suggested sample plate format

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	B_0	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 5. Semi-quantitative assay suggested 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.

First Incubation: SARS-CoV-2 Spike Reagent and Samples

1. **Immunoassay Buffer D (1X)**

   Add 100 µl Immunoassay Buffer D (1X) to NSB wells. Add 50 µl Immunoassay Buffer D (1X) to B_0 wells.

2. **SARS-CoV-2 Neutralizing Antibody ELISA Standard (optional)**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **SARS-CoV-2 Neutralizing Antibody Positive and Negative Controls (optional)**

   Add 50 µl positive or negative control to the respective control wells.

4. **Samples**

   Add 50 µl diluted sample per well. Each sample should be assayed in duplicate (triplicate recommended).

5. **SARS-CoV-2 Spike Reagent**

   Add 50 µl SARS-CoV-2 Spike Reagent to each well *except* the NSB and Blk wells.

6. **First Incubation**

   Cover the plate with a 96-Well Cover Sheet (Item No. 400012) and incubate for 60 minutes at room temperature on an orbital shaker.

Second Incubation: ACE2 Reagent

1. DO NOT WASH THE PLATE. Add 50 µl of ACE2 Reagent to all wells except the Blk wells.
2. Cover the plate with a 96-Well Cover Sheet and incubate for 60 minutes at room temperature on an orbital shaker.

Third Incubation: Anti-His-HRP Conjugate

1. Empty wells and rinse five times with ~300 µl Wash Buffer (1X). Each well should be completely filled with Wash Buffer (1X) during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbant paper to remove the residual wash buffer.
2. Add 150 µl of Anti-His-HRP Conjugate (1X) to each well except the 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).
2. Add 175 µl of TMB Substrate Solution (Item No. 400074) to each well.
3. Cover the plate with the 96-Well Cover Sheet. Incubate the plate for 15 to 30 minutes at room temperature on an orbital shaker. Allow the wells to turn medium-to-dark blue before stopping the reaction. *NOTE: This reaction can be monitored by reading absorbance at 655 nm prior to stopping the reaction. An optical density (OD) of 0.8-1.0 at 655 nm (OD_{655}) yields an OD_{450} of approximately 2.0-2.5. Do not overdevelop.*
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 75 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. The data should be plotted as either $\%B/B_0$ *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain $\%B/B_0$ for a logistic four-parameter fit, multiply these values by 100.)

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* SARS-CoV-2 neutralizing antibody concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Interpretation of Results

Qualitative Assay

To verify the assay is performing as expected, both a positive and negative control have been included to test on each plate. The positive control supplied contains neutralizing antibodies that will bind to the spike S1 RBD, blocking the interaction between the spike S1 RBD and ACE2. The presence of these antibodies can indicate an ability to suppress progression of SARS-CoV-2 infection. The negative control included in this kit does not contain any neutralizing antibodies. Please use the chart below as guidance for determining positive or negative samples.

Cutoff %B/B_0	Result	Interpretation
≤70	Positive	SARS-CoV-2 neutralizing antibody is present
>70	Negative	SARS-CoV-2 neutralizing antibody is NOT present

Table 2. Qualitative sample interpretation

Semi-Quantitative Assay

Appoximate levels of SARS-CoV-2 neutralizing antibodies can be determined using this assay by running a standard curve. Please note, these levels are only approximate as polyclonal antibodies present in a sample will have different affinities to the spike S1 RBD than the recombinant human antibody standard supplied in this kit.

To determine the neutralizing antibody concentration in a sample, calculate the B/B_0 (or %B/B_0) value for each sample and identify it on the standard curve, reading the corresponding concentration values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition to the well. Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference, which could be eliminated by purification.*

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

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.

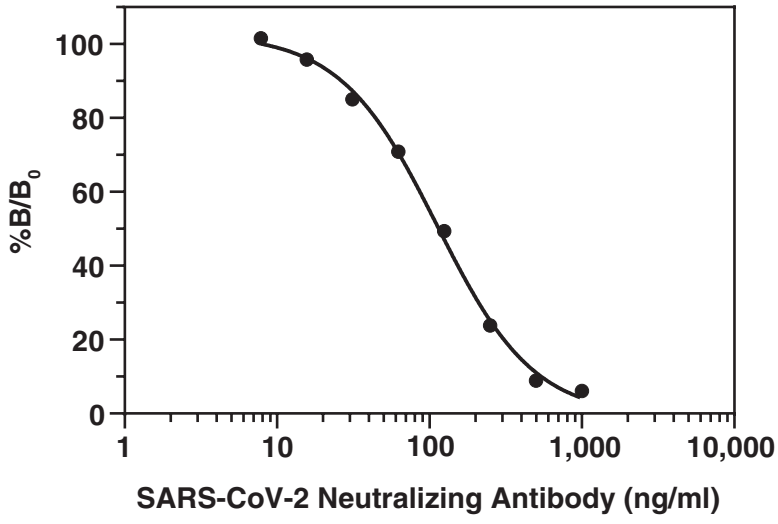
Absorbance at 450 nm (30 minutes)

Analyte Standards (ng/ml)	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
Negative Control	87.5	3.3	8.6
Positive Control	22.1	3.9	13.7

Table 3. Typical results for qualitative assay
*%CV represents the variation in %B/B_0 as determined using NSB and B_0 values

Analyte Standards (ng/ml)	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.12				
B_0	0.95	0.83			
1,000	0.17	0.05	6.1	14.8	12.5
500	0.194	0.074	8.9	4.4	8.4
250	0.319	0.199	23.8	6.7	3.5
125	0.530	0.410	49.3	6.3	1.4
62.5	0.709	0.589	70.9	14.4	3.4
31.25	0.827	0.707	85.0	29.6**	8.2
15.625	0.915	0.795	95.8	55.2**	21.0
7.813	0.965	0.845	101.5	73.7**	41.6

Table 4. Typical results for semi-quantitative assay
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve
**Evaluate data in this range with caution



Assay Range = 7.8-1,000 ng/ml
Sensitivity (defined as 80% B/B_0) = 46 ng/ml
Mid-point (defined as 50% B/B_0) = 121 ng/ml
Lower Limit of Detection (LLOD) = 19 ng/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted in Immunoassay Buffer D (1X).

Figure 6. Typical standard curve

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
High NSB (>0.2 O.D.)	A. Poor washing; ensure proper washing is used B. Exposure of NSB wells to spike reagent C. Immunoassay Buffer D (1X) used to dilute Anti-His-HRP Conjugate.	Careful washing of the plate
No absorbance detected above background in the sample wells	A. Not all reagents added to the well(s) B. Neutralizing antibody concentration too high, further dilution of sample required.	A. Make sure to add all of the reagents to the well(s)
Signal too high or low	The wrong buffer was used to dilute the components	Double-check that reagents were diluted with appropriate buffer

References

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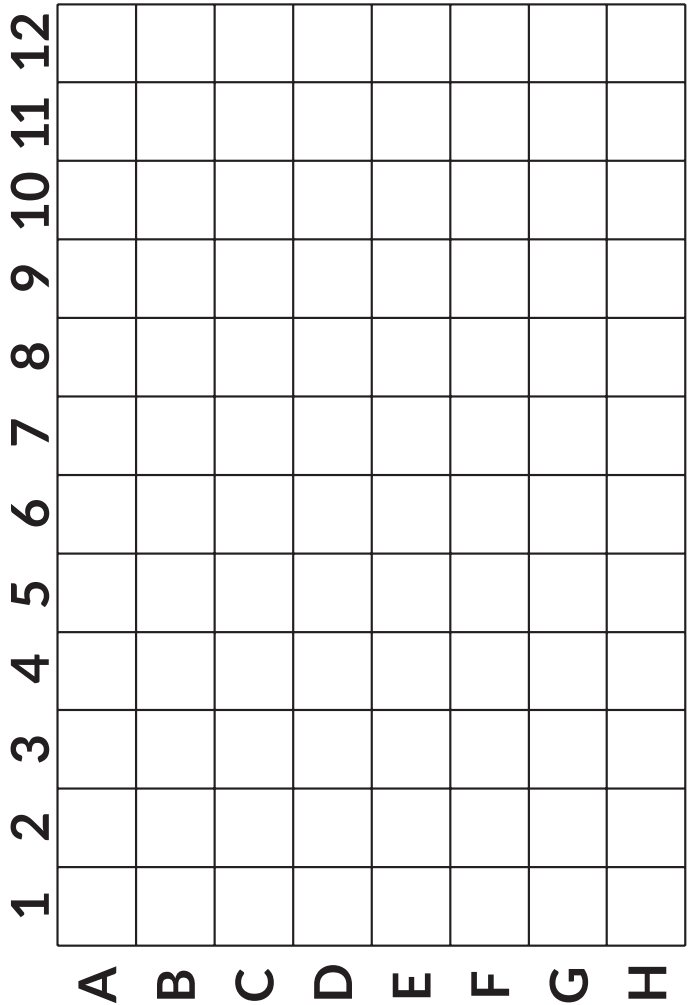
Procedure	Blk	Positive Control	Negative Control	NSB	B_0	Standards/ Samples
Reconstitute and mix	Mix all reagents gently					
Immunoassay Buffer D (1X)	--	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	--	50 µl
Positive or Negative Controls (optional)	--	50 µl	50 µl	--	--	--
SARS-CoV-2 Spike Reagent	--	50 µl	50 µl	--	50 µl	50 µl
First Incubation	Seal plate and incubate plate for 60 minutes at room temperature on an orbital shaker					
ACE2 Reagent	--	50 µl	50 µl	50 µl	50 µl	50 µl
Second Incubation	Seal plate and incubate plate for 60 minutes at room temperature on an orbital shaker					
Aspirate and Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)					
Anti-His-HRP Conjugate	--	150 µl	150 µl	150 µl	150 µl	150 µl
Third Incubation	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker					
Aspirate and Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)					
Apply TMB Substrate Solution	175 µl	175 µl	175 µl	175 µl	175 µl	175 µl
Development	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker protected from light					
Apply HRP Stop Solution	Apply 75 µl HRP Stop Solution					
Read	Read optical density at 450 nm					

Table 5. 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.