SARS-CoV-2 Neutralizing Antibody SimpleDetect ELISA Kit

Item No. 502220

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
502221	SARS-CoV-2 Spike Reagent	1 vial/100 dtn	-20°C
502223	SARS-CoV-2 Neutralizing Antibody Standard	1 vial	-20°C
502222	ACE2-HRP Conjugate	1 vial/100 dtn	-20°C
502224	SARS-CoV-2 Neutralizing Antibody Positive Control	1 vial	-20°C
502225	SARS-CoV-2 Neutralizing Antibody Negative Control	1 vial	-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 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

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 results 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 SimpleDetect 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. The assay has a range of 7.81-1,000 ng/ml, with a midpoint (50% B/B_0) of 75-125 ng/ml, and a sensitivity (80% B/B_0) of approximately 30 ng/ml.

Principle Of This Assay

Cayman's SARS-CoV-2 Neutralizing Antibody SimpleDetect 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 horseradish peroxidase-conjugated, recombinant ACE2 protein (ACE2-HRP conjugate) binds the SARS-CoV-2 spike S1 RBD in the absence of neutralizing antibodies. The plate is washed to remove any unbound reagents and TMB Substrate Solution (which contains the substrate to HRP) is added to the well, followed by the HRP Stop Solution. The final product of this reaction has a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of ACE2-HRP Conjugate bound to the well, which is inversely proportional to the amount of neutralizing antibody in the well. The standard used in this kit is a SARS-CoV-2 neutralizing antibody capable of interfering with the ACE2-HRP Conjugate binding sites on the SARS-CoV-2 spike S1 RBD.

Absorbance $\propto$ [bound ACE2-HRP conjugate] $\propto$ 1/[neutralizing antibody]

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

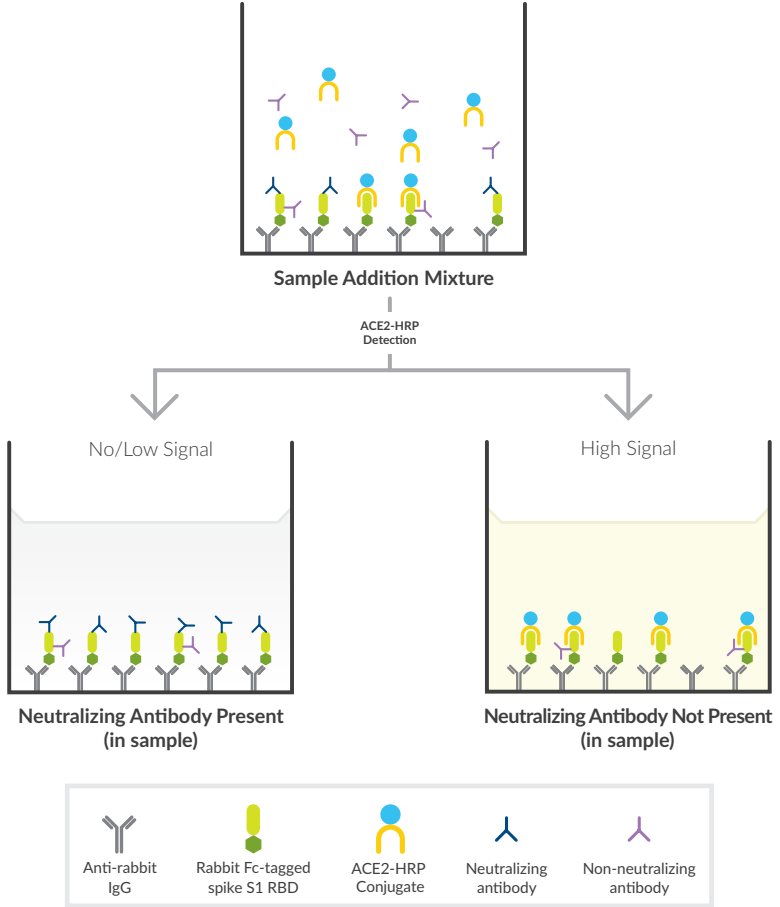


Figure 1. Schematic of the SARS-CoV-2 Neutralizing Antibody SimpleDetect ELISA

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.

Total Activity (TA): total enzymatic activity of the ACE2-HRP Conjugate.

NSB (Non-Specific Binding): non-immunological binding of the ACE2-HRP Conjugate to the well. Even in the absence of the SARS-CoV-2 spike S1 RBD, a very small amount of the ACE2-HRP Conjugate still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the ACE2-HRP Conjugate that the SARS-CoV-2 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): one dtn is the amount of reagent used per well.

LLOD (Lower Limit of Detection): 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 (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 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X). *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.

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 recommmended 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 standard 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 SARS-CoV-2 spike S1 RBD could be different than the standard supplied in the kit.

Sample Matrix Properties

Parallelism

To assess parallelism, plasma and serum positive for SARS-CoV-2 neutralizing antibodies were assayed at multiple dilutions using the SARS-CoV-2 Neutralizing Antibody SimpleDetect ELISA Kit. Measured 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 SARS-CoV-2 spike S1 RBD.

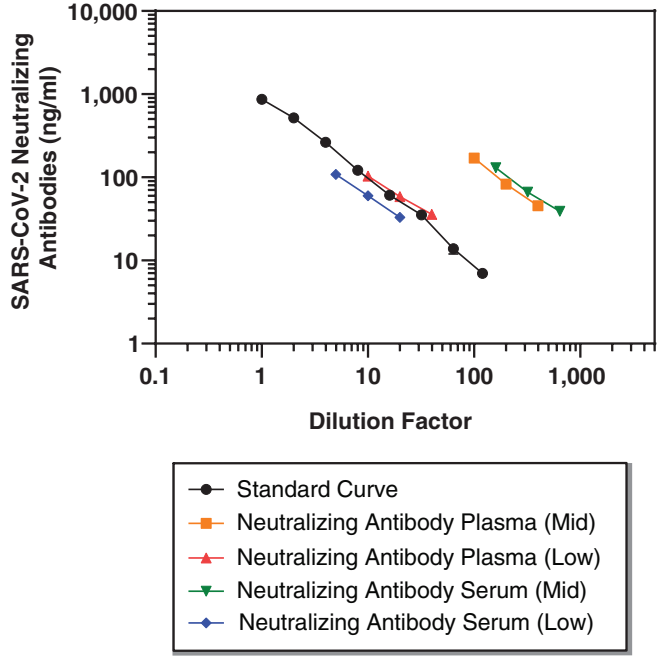


Figure 2. Parallelism of human plasma and serum in the SARS-CoV-2 Neutralizing Antibody SimpleDetect ELISA

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 SimpleDetect 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	Measured Concentration (ng/ml)	Linearity (%)
Plasma (mid response)		
100	17,081	100
200	16,600	97
400	18,174	106
Plasma (low response)		
10	1,033	100
20	1,176	114
40	1,178	114
Serum (mid response)		
160	20,904	100
320	21,214	101
640	25,084	120
Serum (low response)		
5	542	100
10	508	94
20	662	122

Table 1. Linearity in human plasma and serum samples
*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Preparation of Assay-Specific Reagents

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

Reconstitute the lyophilized SARS-CoV-2 Neutralizing Antibody Standard (Item No. 502223) 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 approximately one week when stored at 4°C.

To prepare the standard for use in ELISA: Obtain seven clean test tubes and label them #2-8. Aliquot 200 µl of Immunoassay Buffer D (1X) to tubes #2-8. Transfer 200 µl of the bulk standard (1,000 ng/ml) to tube #2 and mix gently. Next, remove 200 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than four hours.

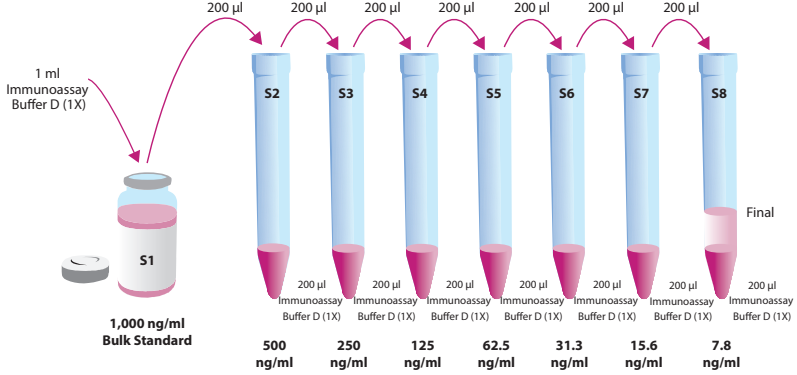


Figure 3. Preparation of the SARS-CoV-2 neutralizing antibody standards

SARS-CoV-2 Spike Reagent

Reconstitute the SARS-CoV-2 Spike Reagent (Item No. 502221) with 6 ml of Immunoassay Buffer D (1X). A 20% surplus of reagent has been included to account for any incidental losses. The reconstituted SARS-CoV-2 Spike Reagent is stable for one week when stored at 4°C.

ACE2-HRP Conjugate

Reconstitute the ACE2-HRP Conjugate (Item No. 502222) with 6 ml of Immunoassay Buffer D (1X). A 20% surplus of reagent has been included to account for any incidental losses. The reconstituted ACE2-HRP Conjugate is stable for one week when stored at 4°C.

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

Reconstitute the SARS-CoV-2 Neutralizing Antibody Positive Control (Item No. 502224) with 500 µl of Immunoassay Buffer D (1X). The reconstituted Positive Control is stable for one week when stored at 4°C.

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

Reconstitute the SARS-CoV-2 Neutralizing Antibody Negative Control (Item No. 502225) with 500 µl of Immunoassay Buffer D (1X). The reconstituted Negative Control is stable for one week when stored at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer (1X) (~300 µl/well) prior to use in the assay. *NOTE: If you do not need to use all the strips at once, place the unwashed 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, and an eight-point standard curve run in duplicate (if running the semi-quantitative assay). *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.

Suggested plate formats for both the qualitative and semi-quantitative assays are show 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. We suggest recording the contents of each well on the template sheet provided (see page 33).

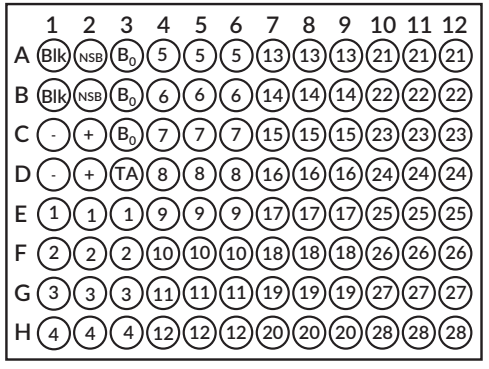


Figure 4. Qualitative assay suggested sample plate format

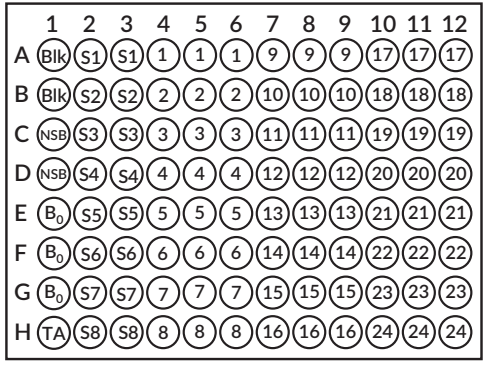


Figure 5. Semi-quantitative assay suggested sample plate format

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.

Plate Preparation

Rinse the plate (or strips to be used) five times with ~300 µl of Wash Buffer (1X).

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

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

   Add 100 µl of Immunoassay Buffer D (1X) to NSB wells. Add 50 µl of 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, including the S1 bulk standard, 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 of positive or negative control to the respective control wells.

4. **Samples**

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

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

   Add 50 µl of reconstituted SARS-CoV-2 Spike Reagent to each well except the TA, 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 RT on an orbital shaker.

Second Incubation: ACE2-HRP Conjugate

1. DO NOT WASH THE PLATE. Add 50 µl of reconstituted ACE2-HRP Conjugate to all the wells except the TA and Blk wells.
2. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at RT 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. Dilute 5 µl of ACE2-HRP Conjugate in 120 µl of Immunoassay Buffer D (1X) and mix. Add 5 µl of this to the TA wells.
3. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature (RT) for 30 minutes.
4. Remove the cover sheet 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/analysisTools/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 Blk 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.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

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 the 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 SARS-CoV-2 spike S1 RBD, blocking the interaction between the SARS-CoV-2 spike S1 RBD and the ACE2-HRP Conjugate. 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 table 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

Approximate 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 SARS-CoV-2 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 samples and identify it on the standard curve, reading the corresponding concentration value 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 a %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 indicate 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

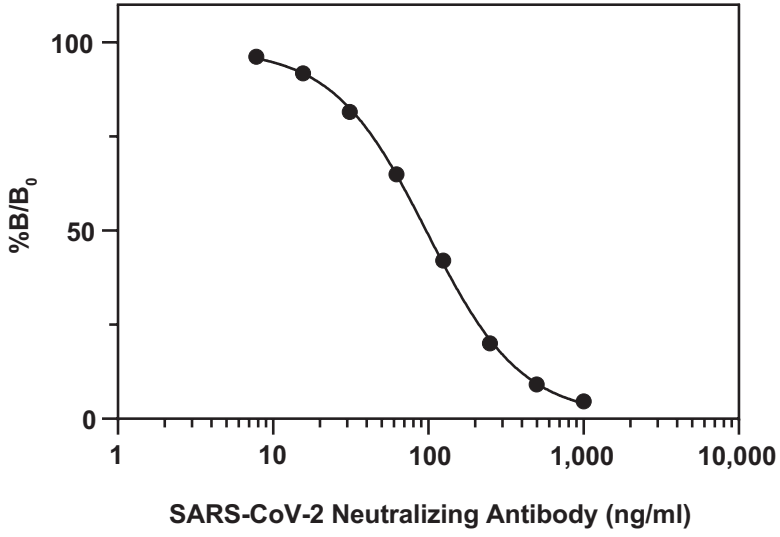
Representative Data

The standard curve 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. Do not use the data below to determine the values of your samples

Absorbance at 450 nm

Neutralizing Antibody Standards (ng/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.011				
B_0	1.574	1.563			
TA	3.749				
1,000	0.084	0.073	4.6	3.0	5.4
500	0.154	0.143	9.1	1.2	8.4
250	0.324	0.313	20.0	1.0	9.1
125	0.669	0.658	42.1	1.6	4.1
62.5	1.027	1.016	65.0	1.6	3.1
31.3	1.286	1.275	81.6	4.0	5.7
15.6	1.446	1.435	01.8	12.2	19.5
7.8	1.514	1.503	96.2	42.7**	44.7**

Table 3. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve
**Evaluate data in this range with caution



Assay Range = 7.81-1,000 ng/ml
Sensitivity (defined as 80% B/B_0) = 30 ng/ml
Mid-point (defined as 50% B/B_0) = 94 ng/ml
LLOD = 7.5 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

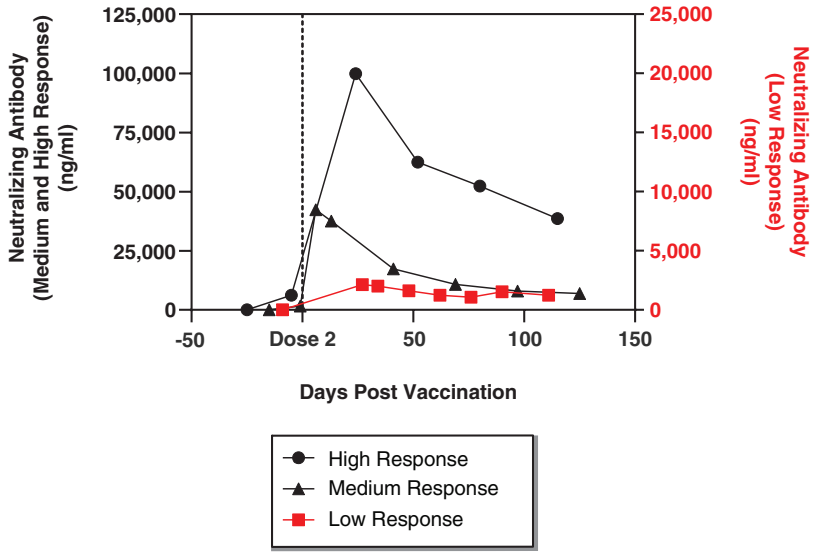


Figure 7. Concentration of neutralizing antibody in vaccinated individuals over time. Samples were chosen to be representative of a vaccine response over time for typical low-, medium-, and high-response individuals.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates/ triplicates	A. Poor pipetting/technique B. Bubble in the well(s) C. Trace organic contaminants in the water source
High NSB (>0.2 O.D.)	A. Poor washing; ensure proper washing is used B. Exposure of NSB wells to spike reagent
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

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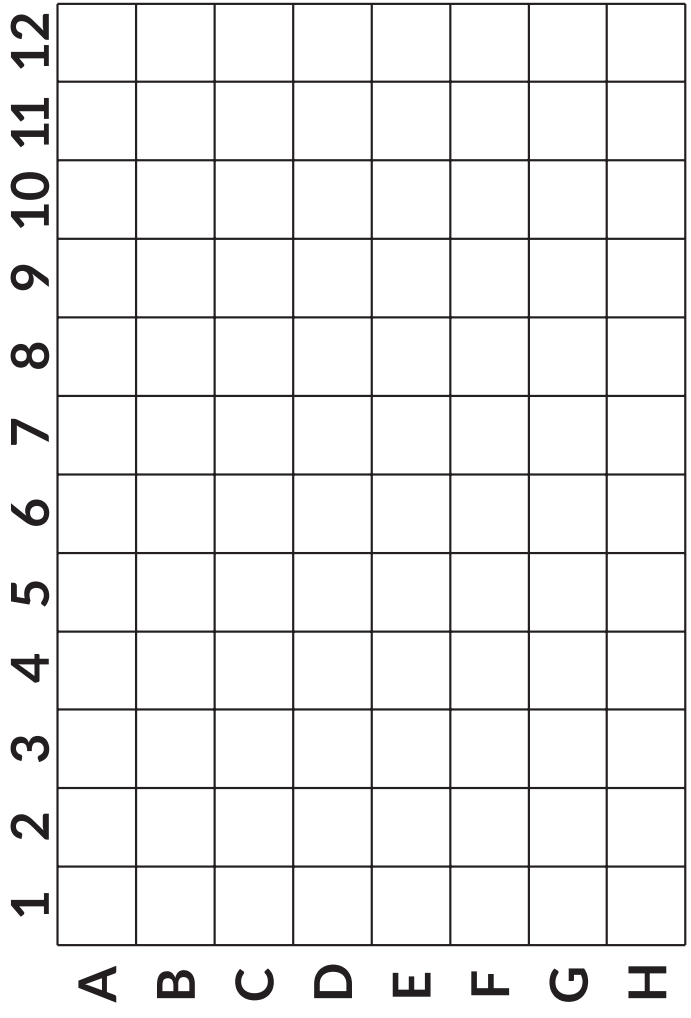
Procedure	Blk	TA	Positive Control	Negative Control	NSB	B_0	Standards/ Samples
Plate Preparation	Rinse strips to be used for the assay 5 x ~300 µl Wash Buffer (1X)						
Reconstitute and mix	Mix all reagents gently						
Immunoassay Buffer D (1X)	--	--	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	--	--	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 the plate and incubate for 60 minutes at RT on an orbital shaker						
ACE2-HRP Conjugate	--	--	50 µl	50 µl	50 µl	50 µl	50 µl
Second Incubation	Seal the plate and incubate for 30 minutes at RT on an orbital shaker						
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)						
Apply TMB Substrate Solution	Apply 175 µl TMB Substrate Solution						
TA - Apply Diluted ACE2-HRP Conjugate	--	5 µl	--	--	--	--	--
Develop	Seal the plate and incubate for 30 minutes at RT on an orbital shaker protected from light						
Apply HRP Stop Solution	Apply 75 µl HRP Stop Solution						
Read	Read absorbance at 450 nm						

Table 4. Assay summary



NOTES

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