SARS-CoV-2 Spike S1 Protein ELISA Kit

Catalog No. 55030

(Version A1)

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Overview

COVID-19, which is short for coronavirus disease 2019, is the official name of the respiratory disease caused by infection with the novel coronavirus SARS-CoV-2. The virus that causes COVID-19 was named SARS-CoV-2 because it is a coronavirus that is genetically similar to, yet distinct from, the virus that caused the severe acute respiratory syndrome (SARS) outbreak in 2003. Like many viruses, the COVID-19 virus attaches to a cell that it is infecting through an interaction between a spike protein (S-protein) on the surface of the viral particle and a receptor protein on the surface of the cell.

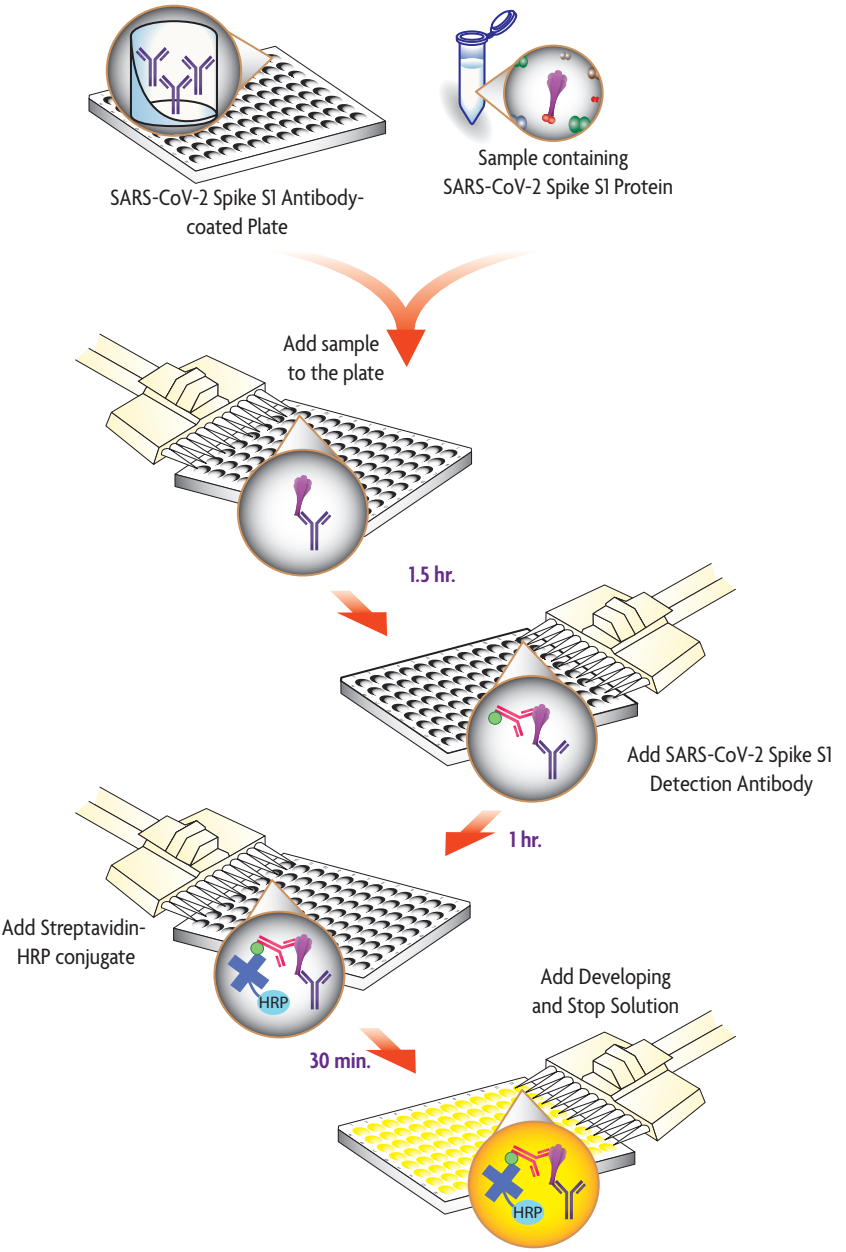
Since the S-proteins are on the surface of the viral particles, antibodies that bind very specifically and with high affinity to the S-protein can be used in immunological assays like ELISAs to detect the presence of the virus in biological samples. Each S-protein, contains two subunits, S1 and S2, with the S1 subunit mainly comprised of the receptor binding domain (RBD). It is the RBD which is responsible for recognizing the cell surface receptor.

The SARS-CoV-2 Spike S1 Protein ELISA Kit is designed for the detection of SARS-CoV-2 Spike S1 (RBD) protein in a high-throughput format. This kit contains enough reagents to be performed in a 96-stripwell plate, enabling you to simultaneously perform up to 96 assays in a single experiment. For added convenience and a more quantitative interpretation of the results, the kit also includes recombinant RBD which enables you to build a reference standard curve.

This ELISA kit is based on the sandwich enzyme immunoassay technique, which uses pre-coated well strips with a recombinant SARS-CoV-2 Spike S1 antibody. Both RBD protein standards and samples are added to the pre-coated wells and the SARS-CoV-2 Spike S1 present in the sample is captured by the antibody. A biotinylated recombinant SARS-CoV-2 antibody is then added, producing the antibody-antigen-antibody "sandwich" immunocomplex. A streptavidin-horseradish peroxidase (HRP) conjugate is then bound to the detection antibodies and developing solutions enable a sensitive colorimetric readout that is easily quantified by spectrophotometry.

Product	Format	Catalog no.
SARS-CoV-2 Spike S1 Protein ELISA Kit	1 x 96 rxns	55030

Flowchart of Process



Kit Performance and Benefits

The SARS-CoV-2 Spike S1 Protein ELISA Kit is for research use only. Not for use in diagnostic procedures.

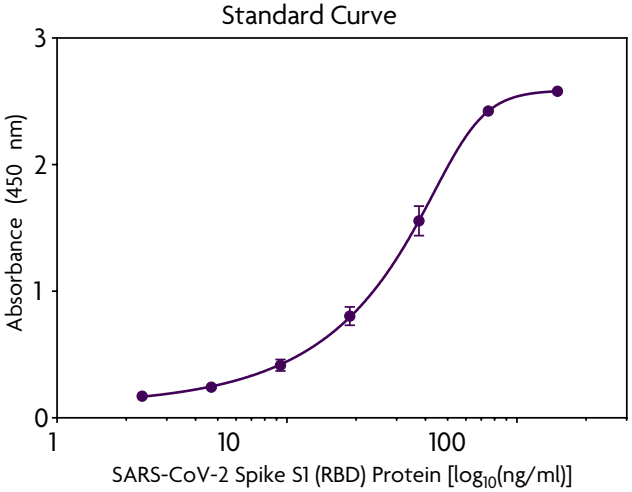


Figure 1: Example Standard Curve
A standard curve was generated by plotting raw absorbance (450 nm) versus concentration of the SARS-CoV-2 Spike S1 (RBD) Protein Standard in Dilution Buffer. The data shown are for four independent experiments with each standard in duplicate. Standard deviation is shown. The development time was 5 minutes. The curve is a 5-Parameter Logistic (5PL) regression fit to the data determined using GraphPad Prism 8 software.

Sensitivity: Sensitivity is the lowest amount of analyte that can be detected. The sensitivity was determined to be 0.58 ng/ml from an average of 4 duplicate standard curves.

Average Intra-assay Precision: Coefficient of variation (CV) = 2.92%
Six replicates of the standard curve were tested on one plate.

Average Inter-assay Precision: Coefficient of variation (CV) = 4.59%
Three independent experiments were performed of the standard curve. Every solution was prepared independently for each run and a single-use aliquot of the SARS-CoV-2 Spike S1 (RBD) Protein was used.

Spike & Recovery: The %recovery for three concentrations of recombinant SARS-CoV-2 Spike S1 (RBD) Protein was evaluated in both plasma and saliva.

Sample Type	Range Recovery (%)	Average Recovery (%)
Plasma	77-116	89
Saliva	91-121	98

Linearity: High concentration of the recombinant SARS-CoV-2 Spike S1 (RBD) Protein was spiked into plasma and saliva, diluted to the range of the standard curve, and evaluated for linearity.

		Plasma	Saliva
1:2	Range (%)	93-109	92-101
	Average (%)	98	95
1:4	Range (%)	90-101	83-95
	Average (%)	94	87
1:8	Range (%)	99-105	87-95
	Average (%)	102	92

Assay time: 3 hours.

Kit Components and Storage

The SARS-CoV-2 Spike S1 Protein ELISA Kit is shipped on dry ice. Upon receipt store the components at the temperatures shown below. Components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
SARS-CoV-2 Spike S1 (RBD) Protein Standard	15 µl	-80°C
SARS-CoV-2 Spike S1 Detection Antibody	15 µl	-20°C
Dilution Buffer	2 x 20 ml	-20°C
Streptavidin-HRP Conjugate	12 ml	4°C
10X Wash Buffer	25 ml	4°C
Developing Solution	12 ml	4°C
SARS-CoV-2 Spike S1 Antibody-coated Plate	1	4°C
Stop Solution	12 ml	RT
Plate Sealer	2	RT
Aluminum Plate Sealer	1	RT

Additional materials required

- Distilled water
- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform/orbital shaker
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength)

Buffer Preparation and Recommendations

1X Wash Buffer

Prepare 1X Wash Buffer by diluting 10X Wash Buffer 1:10 with distilled water. For a half plate, dilute 12 ml 10X Wash Buffer with 108 ml distilled water. For a full plate dilute 24 ml 10X Wash Buffer with 216 ml distilled water. Mix gently by swirling to avoid foaming. 1X Wash Buffer can be stored at 4°C for up to 1 month. Dispose of the 1X Wash Buffer if any particulates are observed.

Dilution Buffer

Supplied read-to-use. Two bottles of Dilution Buffer are supplied and should be stored frozen at -20°C until the day that the assay is conducted. **On the day of the assay thaw Dilution Buffer at room temperature for at least 1 hour before using**. If crystals have formed in the buffer, invert and mix gently until the crystals have completely dissolved. For a half plate one bottle is required. For a full plate, two bottles are required.

Diluted Detection Antibody

Dilute SARS-CoV-2 Spike S1 Detection Antibody 1:1000 in Dilution Buffer for the number of wells to be assayed. For a half plate, dilute 6 µl SARS-CoV-2 Spike S1 Detection Antibody into 6 ml fresh Dilution Buffer. For a full plate dilute 12 µl SARS-CoV-2 Spike S1 Detection Antibody into 12 ml fresh Dilution Buffer. Place on ice and use immediately. Any remaining undiluted SARS-CoV-2 Spike S1 Detection Antibody can be stored at -20°C and is guaranteed until six months after receipt.

Streptavidin-HRP Conjugate Solution

Supplied ready-to-use.

Developing Solution

Supplied ready-to-use. Allow to warm to room temperature for at least 30 minutes before using.

Note: The Developing Solution is light sensitive. Therefore, avoid direct exposure to light during storage. The Developing Solution should be colorless prior to addition to the wells. A green/blue color present in the Developing Solution indicates that it has been contaminated and must be discarded.

Stop Solution

Supplied ready-to-use.

Protocol

Read the entire protocol before use.

Determine the appropriate number of microwell strips required for testing samples, protein standards and blanks in duplicate. Unused microwell strips may be stored in the aluminum pouch at 4°C for up to one month. Use the strip holder for the assay.

Prepare the 1X Wash Buffer and Dilution Buffer as described in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Dilution Buffer, Wash Buffer, Developing Solution and Stop Solution into the wells.

Standard Curve

For creating a standard curve, we suggest placing the serial dilutions of the SARS-CoV-2 Spike S1 (RBD) Protein Standard in duplicate in the first two rows. Up to 40 samples in duplicate can be assayed in the remainder of the plate.

Example Plate Layout

	SARS-CoV-2 Spike S1 (RBD) Protein Standard		SAMPLES									
	1	2	3	4	5	6	7	8	9	10	11	12
A	150 ng/ml	150 ng/ml										
B	75 ng/ml	75 ng/ml										
C	37.5 ng/ml	37.5 ng/ml										
D	18.8 ng/ml	18.8 ng/ml										
E	9.38 ng/ml	9.38 ng/ml										
F	4.69 ng/ml	4.69 ng/ml										
G	2.34 ng/ml	2.34 ng/ml										
H	0 ng/ml	0 ng/ml										

The standard curve should be generated the same day and on the same plate as the samples.

SARS-CoV-2 Spike S1 (RBD) Protein Standard Preparation

1. The recombinant SARS-CoV-2 Spike S1 (RBD) Protein Standard is provided at 1 mg/ml. Thaw on ice. Before using, flick the bottom of the tube gently to mix and quick spin the contents to the bottom of the tube.
2. Make up a 1 µg/ml stock of diluted SARS-CoV-2 Spike S1 (RBD) Protein Standard by adding 2 µl of the 1 mg/ml stock into 2 ml Dilution Buffer and mix by vortexing.

Freeze the remaining undiluted SARS-CoV-2 Spike S1 (RBD) Protein Standard at -80°C. The protein is stable for up to 5 freeze-thaws. The diluted 1 µg/ml stock of SARS-CoV-2 Spike S1 (RBD) Protein Standard can be stored at 4°C for up to a week. Do not used after one week as the signal may be reduced.

Standard Curve Preparation

1. Label eight 1.5 ml microfuge tubes A to H.
2. Into tube A, transfer 15 µl of the diluted 1 µg/ml SARS-CoV-2 Spike S1 (RBD) Protein Standard and 85 µl Dilution Buffer to make a 150 ng/ml solution and mix well.
3. Add 30 µl Dilution Buffer to tubes B through H.
4. Perform a 1:2 serial dilution by transferring 30 µl of the contents in tube A to the tube B.
5. Mix the contents of tube B by pipetting up and down or vortexing.
6. Transfer 30 µl of the contents in tube B to tube C and mix, as previous.
7. Repeat serial dilution in tube C-G. Do not add anything to tube H.

Sample Preparation

Because levels of the SARS-CoV-2 Spike S1 protein may vary between different samples, optimal dilution factors for each sample must be determined by the end-user. It is suggested to dilute saliva at least 1:10 with Dilution Buffer (10 µl saliva: 90 ul Dilution Buffer) as diluting less than 1:10 may result in a matrix affect on the assay. Saliva not used immediately may be stored at -20°C. For long term storage (>2 months) samples should be stored at -80°C. Avoid multiple freeze/thaw cycles.

ELISA Assay

Step 1: Binding of Samples/Controls to the Capture Plate

1. Take out the required number of microwell strips and the tray from the protective pouch containing the SARS-CoV-2 Spike S1 Antibody-coated Plate.
2. Wash the plate/wells/strips 3 times with 200 µl 1x Wash Buffer. Following each wash step, remove the solution from the plate by decanting and tapping the plate on an absorbent material (e.g. paper tissue or towel).
3. For the standard curve wells, dispense 90 µl of Dilution Buffer into each well.
4. Add 10 µl of each diluted standard from tubes A-H to the plate wells as outlined in the plate layout (range: 1.5 ng – 0.023 ng total input). Row H will serve as the blank wells with 10 µl of Dilution Buffer.
5. For the sample wells, add 100 µl of sample diluted in Dilution Buffer.
6. Cover used wells with Plate Sealer and incubate plate for 1.5 hour at room temperature with agitation on an orbital shaker or rocking platform at approximately 700 rpm.

Step 2: Binding of Detection Antibody

7. Before the incubation in Step 1 ends, prepare Diluted Detection Antibody as described in the Buffer Preparation and Recommendations and mix thoroughly. Keep diluted antibody solution on ice until ready to use.
8. After the incubation, remove the adhesive film. Wash the wells 3 times with 200 µl 1x Wash Buffer, tapping the plate dry on paper towels after each wash.
9. Add 100 µl of diluted detection antibody solution to each well.
10. Cover used wells with Plate Sealer and incubate at room temperature for 1 hour with agitation on orbital shaker or rocking platform at approximately 700 rpm.

Step 3: Binding of Streptavidin-HRP

11. After the incubation, wash the wells 3 times with 200 µl of 1x Wash Buffer, tapping the plate dry on paper towels after each wash.
12. Add 100 µl of the Streptavidin-HRP Conjugate solution to each well.
13. Cover used wells with Aluminum Plate Sealer tightly and incubate at room temperature for 30 minutes with agitation on orbital shaker or rocking platform at approximately 700 rpm.

Step 4: Colorimetric Reaction

14. During the incubation, set Developing Solution out on bench to warm to room temperature.
15. After the incubation, wash the wells 3 times with 200 µl of 1x Wash Buffer. Remove as much of the final wash as possible by blotting the plate on paper towels.
16. Add 100 µl of room temperature Developing Solution to each well. Incubate for 5-10 minutes at room temperature in the dark with gentle shaking.
17. Incubate under low light conditions. Monitor the blue color development in the standard curve wells. The Blank wells should remain clear.
18. Following the incubation, stop the reaction by adding 100 µl of the Stop Solution.
19. Read absorbance on a microplate reader at 450 nm within 15 minutes.

    Reading the reference wavelength is optional. Most microtiter plate readers are equipped to perform dual wavelength analysis and with the appropriate software, will automatically subtract the reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, you may read the plate twice, first at 450 nm and then at 655 nm and manually subtract the 655 nm OD from the 450 nm OD values.

Data Analysis

Average the optical density (OD) for the standards, controls and samples and subtract the average OD obtained from the zero standard. If samples generate values higher than the highest standard, repeat the assay with an appropriate dilution.

Generate a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. For a best fit curve use graphing software with 4-Parameter Logistic (4PL) or 5-Parameter Logistic (5PL) regression analysis.

The concentration of SARS-CoV-2 Spike S1 (RBD) Protein in each sample well can be determined from the equation for the standard curve. If the samples have been diluted, the actual concentration of SARS-CoV-2 Spike S1 (RBD) Protein determined from the standard curve must be multiplied by the dilution factor. For example, if the samples were diluted 1:10 in the wells, the actual amount in the original sample would be determined by multiplying by 10.

Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells in the correct order.
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity by mixing a small aliquot of HRP and Developing Solution together.
	Enzyme inhibitor present	Sodium azide will inhibit the peroxidase reaction. Follow our recommendations to prepare buffers.
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader.
	Incorrect assay temperature	Bring Developing Solution and Stop Solution to room temperature before using.
	Inadequate volume of Developing Solution	Check to make sure that correct volume is delivered by pipette.
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the specified wells turn medium-dark blue.
	Concentration of SARS-CoV-2 Spike S1 Detection Antibody is too high	Increase antibody dilutions.
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations .
Uneven color development	Incomplete washing of wells	Ensure all wells are filled with Wash Buffer and follow washing recommendations .
	Well cross-contamination	Follow washing recommendations.
High background in sample wells	Too much sample per well	Decrease amount of sample per well.
	Concentration of SARS-CoV-2 Spike S1 Detection Antibody is too high	Perform antibody titration to determine optimal working concentration. The sensitivity of the assay may decrease.
No signal or weak signal in sample wells	Not enough sample per well	Increase amount of sample if necessary.
No signal or weak signal in SARS-CoV-2 Spike S1 (RBD) Protein Standard	Improper storage. Too many freeze/thaw cycles of protein	Do not use the **diluted** SARS-CoV-2 Spike S1 (RBD) Protein Standard after one week of storage at 4°C. Store the **undiluted** SARS-CoV-2 Spike S1 (RBD) Protein Standard at -80°C and do not freeze-thaw more than 5 times..

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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