SARS-CoV-2 Spike-ACE2 Binding Assay Kit (CAT NO: 41A249R)

For quantitative detection of the neutralizing antibodies against SARS-CoV-2 in serum, plasma or other samples

(This assay has been calibrated with WHO International Standard for anti-SARS-CoV-2 immunoglobulin, NIBSC code:20/136)

This package insert must be read in its entirety before using this product

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BACKGROUND

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is an enveloped virus with a positive-sense RNA genome and a nucleocapsid of helical symmetry. The SARS-CoV-2 entry into host cells is mediated by the transmembrane spike (S) glycoprotein, which contains a receptor binding domain (RBD). S1RBD is responsible for binding with angiotensin converting enzyme-2 (ACE2), leading to endocytosis into the host cells and viral replication. S1RBD is the main target of neutralizing antibodies upon infection and the focus of the therapeutic drug and vaccine design. Molecules that inhibit formation of the S1RBD-ACE2 complex *in vitro* could be potential treatment for COVID-19 infection.

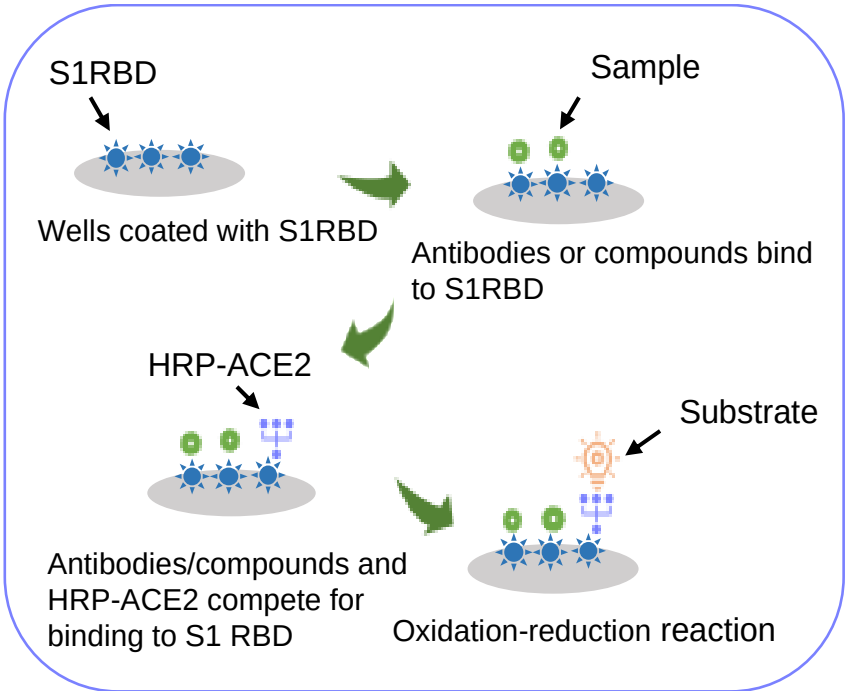
INTENDED USE

The ImmunoDiagnostics SARS-CoV2 S1RBD-ACE2 Binding Assay is designed to measure the interaction between the receptor binding domain of the viral spike glycoprotein (S1RBD) with the cell surface receptor ACE2. The assay can detect the presence of neutralizing antibodies in human plasma or serum after infection or vaccination.

This product is intended for professional use only.

ASSAY PRINCIPLE

The SARS-CoV2 S1RBD-ACE2 Binding Assay is a competitive ELISA assay, which can mimic the virus binding process. 96-well plates are coated with SARS-CoV-2 S1RBD protein that captures neutralization antibodies or compounds which bind to SARS-CoV-2 S1RBD protein in the sample. After washing away unbound materials, captured neutralization antibodies or compounds can block the binding between horseradish peroxidase (HRP) conjugated recombinant human ACE2 and SARS-CoV-2 S1RBD. After washing step, the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added. Color reaction is stopped by 2M H_2SO_4. The presence of neutralization antibodies or compounds against SARS-CoV-2 S1RBD captured inside the wells is inversely proportional to the color density generated in the coupled oxidation-reduction reaction.



REAGENTS SUPPLIED

Each kit is sufficient for 96 tests and contains the following components:

1. One aluminum pouch with a Microwell plate (12 strips of 8 wells each) coated with SARS-CoV-2 S1RBD protein, sealed. The microwell strips can be used separately.
2. 10×Wash buffer-40 ml.
3. 5×Assay buffer-20 ml.
4. 100×Detection reagent: HRP-conjugated human ACE2, 0.12 ml.
5. 10×Standard solution: 1000 IU/ml, 33 ul (Calibrated with WHO International Standard for anti-SARS-CoV-2 immunoglobulin, NIBSC code:20/136).
6. Blank Control: 1 ml, ready for use.
7. Substrate solution, 12 ml, ready for use.
8. Stop solution, 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. Beakers, flasks, cylinders necessary for preparation of reagents.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Statistical calculator with program to perform regression analysis.

STORAGE

- The kit should be stored at 2-8°C, and all reagents should be equilibrated to room temperature before use. Immediately after use remaining reagents should be returned to cold storage (2-8°C).
- Expiry of the kit and reagents is stand on labels.
- Once opened, the strips may be stored at 2-8°C for up to one month.

SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

Handle serum or plasma sample in accordance with National Committee for Clinical Laboratory Standards guidelines for preventing transmission of blood-borne infection.

- Do not use grossly hemolyzed or lipemic samples.
- Serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. **When the human serum is tested, the recommended dilution factor is 10-fold.**
- Plasma: Treat blood with anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000g within 30 minutes for plasma collection. **When the human plasma is tested, the recommended dilution factor is 10-fold.**
- Samples cannot be tested immediately should be aliquoted and must be stored frozen below -20°C. Avoid repeated freeze-thaw cycle.
- The dilution factor and concentrations of other testing samples (such as inhibitors and antibodies) should be determined by individual users.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before use

1. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. I**f precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear**. The 1×Wash buffer may be stored at 2-8°C for up to one month.

2. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5x assay buffer (20 ml) with 80 ml of distilled water or deionized water. **If precipitates are observed in the 5x assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear.** The 1×Assay buffer may be stored at 2- 8°C for up to one month.

3. 1×Detection solution.

Spin down the 100×Detection solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of 1×Detection antibody solution is required per well. Prepare only as much 1×Detection solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is pipetted.

PREPARATION OF SAMPLES AND STANDARD

Preparation of standard

The 10×Standard solution we use in this assay has been calibrated using WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136), and its concentration is equivalent to 1000 IU/ml.

Centrifuge the standard tube briefly before opening the cap. Add 300 µL 1×Assay buffer into 10×Standard solution to generate the first standard (100 IU/ml). Prepare serially diluted standards using 1×Assay buffer as follow:

	Standard Volume	Volume of 1 x assay buffer	Concentration
1	100 IU/ml	-	100 IU/ml
2	50 µl of 100 IU/ml	450 µl	10 IU/ml
3	225 µl of 10 IU/ml	225 µl	5 IU/ml
4	225 µl of 5 IU/ml	225µl	2.5 IU/ml
5	225 µl of 2.5 IU/ml	225 µl	1.25 IU/ml
6	225 µl of 1.25 IU/ml	225 µl	0.625 IU/ml

Preparation of samples

Serum or plasma sample is generally required a **10-fold dilution** in the 1×Assay buffer. A suggested dilution step is to add 22 µL of sample to 200 µL of 1×Assay buffer. Dilution factor can be adjusted based on the titer of the antibodies in the samples. 1×Assay buffer can be used for dilution of other compounds/antibodies to desired concentrations.

ASSAY PROCEDURE

It is recommended that all samples be assayed in duplicate.

1. Add 100µl of samples, Blank Control and Standard into their respective wells, and incubate at room temperature for 1 hour, preferably with shaking at 600 rpm. Duplicate test is recommended.

***Note**: If the plate is not shaken the bound of the signals will be ~20% lower.*

2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total of 3 times.

3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 40 minutes.
4. Wash each well 3 times as described in step 2.
5. Add 100 µl of Substrate solution to each well, incubate at room temperature for 10 minutes. Protect from light.
6. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Determine the optical density of each well at 450 nm immediately.

SUGGESTED MICROPLATE LAYOUT

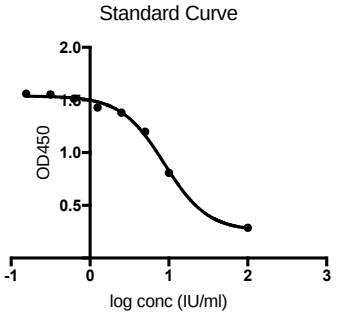
BLK	BLK	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
STD1	STD1	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
STD2	STD2	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
STD3	STD3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
STD4	STD4	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
STD5	STD5	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
STD6	STD6	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41

CALCULATION AND INTERPRETATION OF RESULTS

The test results can be expressed as the international unit or percentage of inhibition.

- Calculation of the titre of antibodies in the samples:

1. Use the curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit.
Standard curve (example):



2. Calculate the concentrations for unknown samples using the standard curve, expressed as IU/ml. Multiply calculated value with respective dilution factor to obtain the final concentration of the sample.
Note: *If the OD450 value of the sample is lower than that of the highest standard in 1×Assay buffer, further dilution is recommended.*
The test result was deemed to be positive when the final concentration of neutralizing antibodies in a sample was higher than 25 IU/ml, suggesting the presence of neutralizing antibodies in the sample.
The test result was deemed to be negative when the final concentration of neutralizing antibodies in a sample was lower than 25 IU/ml, suggesting the absence of neutralizing antibodies in the sample.

- Calculation of the percentage of inhibition of antibodies in the samples

The neutralizing activities of antibodies against SARS-CoV-2 in the serum/plasma can also be calculated as the percentage of inhibition for the binding of S1RBD to ACE2 in each sample using the following formula:

Percentage of Inhibition= (1-OD450$_{sample}$/OD450$_{Blank\ control}$) × 100

TYPICAL DATA

17 serum samples from vaccinated subjects and 14 serum samples from non-vaccinated individuals were tested in this assay. The serum samples from vaccinated subjects were collected 14-16 days after the second injection. The results showed that the presence of neutralizing antibodies against SARS-CoV-2 only can be detected in samples from COVID-19 patients, but not in samples from healthy people.

Sample	OD450	IU/ml	Inhibition
Vaccinated individuals	0.455	636.763	72.565
	0.877	143.426	47.129
	0.334	1094.992	79.838
	0.608	355.404	63.335
	0.719	242.216	56.657
	0.610	352.731	63.207
	0.416	752.362	74.947
	0.739	226.645	55.466
	0.742	224.512	55.296
	0.527	958.211	68.226
	0.816	175.142	50.787
	0.250	1735.596	84.943
	0.494	544.723	70.225
	0.828	168.743	50.106
	0.690	267.199	58.401
	0.380	880.298	77.074
	0.364	949.080	78.052
Non-vaccinated individual	1.576	6.461	5.019
	1.539	8.613	7.231
	1.599	5.249	3.615
	1.488	12.114	10.293
	1.653	2.902	0.383
	1.751	UD	-5.530
	1.667	2.361	-0.510
	1.642	3.317	1.021
	1.473	13.312	11.229
	1.571	6.733	5.317
	1.437	16.333	13.399
	1.488	12.167	10.336
	1.616	4.442	2.595
	1.437	16.333	13.399

Note: *For majority of serum/plasma samples from vaccinated individuals, the percentage of inhibition is equal or greater than 50% at 1:10 dilution.*

PRECISION

Intra-assay: Three different known levels of positive control were spiked into sample buffer as test samples. All samples were tested on the same plate to evaluate intra-assay precision of the kit. The Intra-assay precision of this kit is less than 10%.

Inter-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested in 3 separate assays to evaluate intra-assay precision of the kit. Inter-assay precision of this kit is less than 12%.

PRECAUTIONS AND SAFETY

1. The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (20-25°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. When adding specimens, do not touch the well's bottom with the pipette tip.
10. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600~650nm.
11. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
12. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
13. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
14. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
15. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
16. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: the Stop solution, the Substrate solution, and the Wash buffer.
17. The Stop solution 2M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of sample or control to each well.

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Incubate at room temperature for 1 hour, preferably with shaking at 600 rpm.

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Aspirate and wash each well three times.

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Add 100 µl of 1×Detection solution to each well.

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Incubate at room temperature for 40 minutes.

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Aspirate and wash each well three times.

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Add 100 µl of Substrate solution to each well.

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Incubate at room temperature for 10 minutes.

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Add 100 µl of Stop solution to each well.

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Measure absorbance of each well at 450 nm.

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Interpretation of result