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#69486

SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit

1 Kit
(96 assays)



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New 12/20

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: All

Description: The SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit allows for the detection of antibodies (or small molecules) that block the interaction between the host receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and ACE2. In this assay, SARS-CoV-2 spike RBD protein linked to HRP (RBD-HRP) is pre-incubated with the sample and controls, allowing blocking antibodies present in the sample to bind to the RBD-HRP. These pre-incubated mixtures are then added to microwell plates coated with ACE2 protein. The RBD-HRP is captured by ACE2 in the well to varying degrees depending on the blocking activity present in each sample. The wells are then washed to remove unbound material. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is inversely proportional to the ability of the sample to block the interaction between SARS-CoV-2 spike RBD and ACE2 proteins. For example, if no blocking activity is present in the sample, the RBD-HRP will be able to bind to ACE2 on the microwell plates, resulting in high signal. Conversely, samples with high blocking activity will prevent the RBD-HRP from binding to ACE2 on the microwell plates, resulting in low signal. This kit is designed to detect blocking antibodies present in human serum/plasma samples. However, this kit may also be used to assess the blocking activity of non-human antibodies and small molecules. In the latter scenarios (for non-human antibodies and small molecules), the user will have to determine the appropriate dilution/concentration of their samples to use, along with running the proper controls.

Specificity/Sensitivity: The SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit detects endogenous levels of antibodies that block the interaction between the SARS-CoV-2 spike RBD (318-541) protein and human ACE2 (18-615). This kit is designed to detect blocking antibodies present in human serum/plasma, however, it may also be used to assess blocking activity of non-human antibodies and small molecules.

Background: The cause of the COVID-19 pandemic is a novel and highly pathogenic coronavirus, termed SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). SARS-CoV-2 is a member of the Coronaviridae family of viruses (1). The genome of SARS-CoV-2 is similar to other coronaviruses, and is comprised of four key structural proteins: S, the spike protein, E, the envelope protein, M, the membrane protein, and N, the nucleocapsid protein (2). Coronavirus spike proteins are class I fusion proteins and harbor an ectodomain, a transmembrane domain, and an intracellular tail (3,4). The highly glycosylated ectodomain projects from the viral envelope surface and facilitates attachment and fusion with the host cell plasma membrane. The ectodomain can be further subdivided into host receptor-binding domain (RBD) (S1) and membrane-fusion (S2) subunits, which

Product Includes	Item #	Kit Quantity	Color
ACE2 Protein Coated Microwells*	30924	96 tests	
SARS-CoV-2 Spike RBD Protein, HRP-linked	42530	1 each	Red (Lyophilized)
Sample Diluent A	71637	25 ml	
HRP Diluent	13515	11 ml	Red
ELISA Wash Buffer (20X)	9801	25 ml	
TMB Substrate	7004	11 ml	
STOP Solution	7002	11 ml	
Sealing Tape	54503	2 each	
ELISA Kit #69486 Positive Control	70532	1 each	
ELISA Kit #69486 Negative Control	81889	1 each	

*12 8-well modules -Each module is designed to break apart for 8 tests.

are produced upon proteolysis by host proteases at S1/S2 and S2' sites. S1 and S2 subunits remain associated after cleavage and assemble into crown-like homotrimers (2,4). In humans, both SARS-CoV and SARS-CoV-2 spike proteins utilize the angiotensin-converting enzyme 2 (ACE2) protein as a receptor for cellular entry (5-7). Spike protein subunits represent a key antigenic feature of coronavirus virions, and therefore represent an important target of vaccines, novel therapeutic antibodies, and small-molecule inhibitors (8,9).

Background References:

- (1) Zhou, P. et al. (2020) *Nature* 579, 270-3.
- (2) Tortorici, M.A. and Veesler, D. (2019) *Adv Virus Res* 105, 93-116.
- (3) Li, F. et al. (2006) *J Virol* 80, 6794-800.
- (4) Li, F. (2016) *Annu Rev Virol* 3, 237-61.
- (5) Shang, J. et al. (2020) *Nature* 581, 221-4.
- (6) Wrapp, D. et al. (2020) *Science* 367, 1260-3.
- (7) Yan, R. et al. (2020) *Science* 367, 1444-8.
- (8) Yuan, Y. et al. (2017) *Nat Commun* 8, 15092.
- (9) Amanat, F. and Krammer, F. (2020) *Immunity* 52, 583-9.

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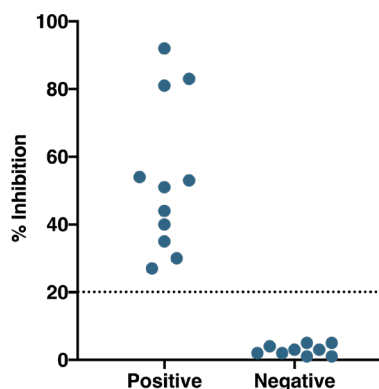
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Patient Testing: Patient samples were tested using the SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit #69486. Serum/plasma samples were heat-inactivated (56°C for 30 min) and diluted 1:10 prior to running the assay, as described in the protocol. Percent inhibition of the SARS-CoV-2 spike RBD-ACE2 interaction (% Inhibition) is plotted for each serum/plasma sample, with values corresponding to samples obtained from donors who had been diagnosed with SARS-CoV-2 (diagnosed positive n=11*) on the left, and values corresponding to samples from presumed uninfected donors collected prior to the SARS-CoV-2 outbreak (presumed negative n=9) on the right. Samples are considered positive or negative for SARS-CoV-2 blocking antibodies based on the 20% inhibition cutoff criteria (dotted line) described in the "Data Analysis" section of the kit protocol.



*Positive reference samples were from patient donors with a positive SARS-CoV-2 diagnosis. However, a positive SARS-CoV-2 diagnosis will not always correlate with the presence of blocking antibodies, as differences in disease severity, timing of sample collection relative to disease onset, and patient profiles may affect presence and abundance of antibodies in the reference sample that block the interaction between SARS-CoV-2 spike RBD and ACE2.

Note: We are continuing to test more samples as available. For the most up-to-date set of data, always refer to the product page for #69486 on the website.

Intra-Assay Precision: Three different serum samples were each tested in 16 replicates using a single assay kit of SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit #69486. Intra-assay CV (%) was calculated for each sample, and each replicate was correctly identified as being positive or negative when compared to the RBD-HRP only control using the cutoff criteria described in the attached protocol.

Sample ID	Average OD (450 nm)	Results	CV (%)
Positive Serum-1	0.136	16/16 are Positive	13.4%
Positive Serum-2	0.966	16/16 are Positive	8.3%
Negative Serum-3	2.190	16/16 are Negative	5.8%

Inter-Assay Precision: Five different assay kits from one lot of material were tested using 3 different serum samples run in duplicate wells and Positive and Negative Controls also run in duplicate wells. Inter-assay CV (%) was calculated for each sample, and each assay kit correctly identified the samples as being positive or negative when compared to the RBD-HRP only control using the cutoff criteria described in the attached protocol.

Sample ID	Average OD (450 nm)	Results	CV (%)
Positive Serum-1	0.098	5/5 are Positive	9.0%
Positive Serum-2	1.146	5/5 are Positive	12.7%
Negative Serum-3	2.337	5/5 are Negative	3.8%
Positive Control	0.831	5/5 are Positive	13.9%
Negative Control	2.344	5/5 are Negative	3.3%

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SARS-CoV-2 Spike RBD-ACE2 Blocking Antibody Detection ELISA Kit

This Kit Is Intended For Research Use Only. Not For Use in Diagnostic or Clinical Procedures.

A Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent. Prepare only as much reagent as needed on the day of the experiment.

- 1. ACE2 Protein Coated Microwells:** Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
- 2. 1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
- 3. Sample Diluent A:** Diluent provided for dilution of samples and for reconstitution of Positive and Negative Controls included in kit.
- 4. HRP Diluent:** Red colored diluent provided for reconstitution and dilution of SARS-CoV-2 Spike RBD Protein, HRP-linked (11.0 mL provided, only 8.0 mL is needed).
- 5. SARS-CoV-2 Spike RBD Protein, HRP-linked:** Supplied lyophilized as a red colored cake or powder. Add 1.0 mL of HRP Diluent to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 mL volume of reconstituted HRP-linked protein to 7.0 mL HRP Diluent in a clean tube and gently invert several times to mix. Do NOT vortex the HRP-linked protein as this may adversely affect its performance. For best results, use this working solution immediately. Unused working solutions may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly made solutions.
- 6. Positive Control:** Reconstitute the vial of lyophilized Positive Control with 1.0 mL Sample Diluent A. Mix thoroughly and gently, hold at room temperature for 1 minute and then follow the steps outlined below in the "Test Procedure" section. Positive Controls are recommended to be used immediately after reconstituting, however remaining material may be stored for up to 4 weeks at 4°C (there may be some loss of the Positive Control blocking activity compared to freshly made solutions). Positive Controls are supplied as a control reagent, not as an absolute quantitation measure.
- 7. Negative Control:** Reconstitute the vial of lyophilized Negative Control with 1.0 mL Sample Diluent A. Mix thoroughly and gently, hold at room temperature for 1 minute and then follow the steps outlined below in the "Test Procedure" section. Negative Controls are recommended to be used immediately after reconstituting, however remaining material may be stored for up to 4 weeks at 4°C.
- 8. TMB Substrate (#7004):** Bring to room temperature before use.
- 9. STOP Solution (#7002):** Bring to room temperature before use.

B Test Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Human-sourced samples should be handled in accordance with accepted safety practices. Samples (human serum or plasma) should be diluted at least 1:10 (6.5 µL sample + 58.5 µL Sample Diluent A) with Sample Diluent A and can be further serially diluted if the user needs relative quantification. Positive and Negative Controls do NOT need to be diluted after reconstitution. When using the cutoff criteria described below to determine if a sample is positive for anti-SARS-CoV-2 Spike RBD Protein blocking antibodies, samples diluted 1:10 must be compared to wells containing a mixture of Sample Diluent A and RBD-HRP (RBD-HRP only control), while the Positive and Negative Controls are used undiluted. An equation to calculate the percent inhibition is described at the end of this protocol.

NOTE: Sample storage/handling, including heat-inactivation of samples, can potentially affect observed signals. Therefore, it is strongly recommended that in addition to the Positive and Negative Controls included with the kit, the user includes their own negative and positive patient samples as controls when running the assay in order to establish an appropriate cutoff value. In addition to testing human serum/plasma, this kit may be used to assess blocking activity of non-human antibodies and small molecules. In this scenario, the user will have to determine the appropriate dilution/concentration of their samples to use, along with running the proper controls.
3. Pre-incubate diluted sample, Positive Control, Negative Control, and Sample Diluent A (for RBD-HRP only control) with the SARS-CoV-2 Spike RBD Protein, HRP-linked in a separate uncoated assay plate or clean microtubes. To do so, in the separate uncoated wells/tubes, mix 65 µL of each sample (diluted 1:10), Positive Control, Negative Control, and Sample Diluent A (for RBD-HRP only control) with 65 µL of the reconstituted SARS-CoV-2 Spike RBD Protein, HRP-linked. For the blank control, mix 65 µL of Sample Diluent A with 65 µL of HRP Diluent in another well/tube (diluent only). Seal the plate with the supplied sealing tape or close the microtubes and incubate for 1 hour at 37°C.
4. Gently remove the tape and transfer 100 µL of the pre-incubated mixtures from the previous step to the appropriate wells of the supplied ACE2 Protein Coated Microwell plate. When finished transferring, seal the plate with the supplied sealing tape and incubate for 1 hour at 37°C.

5. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X ELISA Wash Buffer, 200 μ L each time for every well. After each wash, aspirate or decant from wells. Invert the plate and blot it against clean paper towels to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - c. Clean the underside of all wells with a lint-free tissue.
6. Add 100 μ L of TMB Substrate to each well. Seal the plate and incubate in the dark for 10 min at 37°C.
7. Add 100 μ L of STOP Solution to each well and shake gently for a few seconds.

NOTE: Initial color change is blue, which changes to yellow upon addition of STOP Solution.

8. Read results:
 - a. **Visual Determination:** Read within 30 min after adding STOP Solution.
 - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.
9. Data Analysis:
 - a. Subtract “blank” well absorbance 450 nm values from sample, RBD-HRP only control, Positive Control, and Negative Control values.
 - b. The RBD-HRP only control absorbance values should be > 1.0.
 - c. Positive and Negative Controls should fall within the accepted % inhibition cutoff criteria described below.
 - d. To calculate % inhibition use the following equation:
 $100 - [(OD \text{ value of Sample} \div OD \text{ Value of RBD-HRP only control}) \times 100\%]$
 - e. Interpretation of results*:

$\geq 20\%$ inhibition- Positive result, SARS-CoV-2 blocking antibody detected.

$< 20\%$ inhibition- Negative result, no detectable SARS-CoV-2 blocking antibody.

* Experimental cutoffs were determined by assaying a set of confirmed SARS-CoV-2 positive samples (from donors with positive SARS-CoV-2 diagnosis and seropositive) and uninfected donor serum collected prior to the SARS-CoV-2 pandemic. Researchers can establish or modify this cutoff using additional samples.