Revision 13

Store at +4C

#69486

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

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1 Kit (96 assays)

Species Cross Reactivity: All
UniProt ID: #P0DTC2
Entrez-Gene Id: #43740568

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
ACE2 Protein Coated Microwells	30924	96 tests		+4C
SARS-CoV-2 Spike RBD Protein, HRP-linked	42530	1 ea	Red (Lyophilized)	+4C
Sample Diluent A	71637	25 ml		+4C
HRP Diluent	13515	11 ml	Red	+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
TMB Substrate	7004	11 ml		+4C
STOP Solution	7002	11 ml		+4C
Sealing Tape	54503	2 ea		+4C
ELISA Kit #69486 Positive Control	70532	1 ea		+4C
ELISA Kit #69486 Negative Control	81889	1 ea		+4C

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

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 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.

Trademarks and Patents

Limited Uses

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

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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 (diluents 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:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X ELISA Wash Buffer, 200 µL 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.
    3. Clean the underside of all wells with a lint-free tissue.
6. Add 100 µL of TMB Substrate to each well. Seal the plate and incubate in the dark for 10 min at 37°C.
7. Add 100 µL 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:
    1. **Visual Determination**: Read within 30 min after adding STOP Solution.
    2. **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:
    1. Subtract “blank” well absorbance 450 nm values from sample, RBD-HRP only control, Positive Control, and Negative Control values.
    2. The RBD-HRP only control absorbance values should be > 1.0.
    3. Positive and Negative Controls should fall within the accepted % inhibition cutoff criteria described below.
    4. To calculate % inhibition use the following equation:
    100 - [(OD value of Sample ÷ OD Value of RBD-HRP only control) x 100%]

    5. Interpretation of results*:
    ≥ **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.

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