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#88005

SARS-CoV-2 Spike Protein Multi-Domain (S1-NTD, RBD, S1, S2) Serological IgG ELISA Kit

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

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

Product Includes	Product #	Quantity	Color	Storage Temp
Spike Multi-Domain Protein Coated Microwells	43037	96 tests		+4C
Anti-Human IgG, HRP-linked Antibody (ELISA Formulated)	94210	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 #88005 Positive Control	57510	1 ea		+4C
ELISA Kit #88005 Negative Control	73072	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 Protein Multi-Domain (S1-NTD, RBD, S1, S2) Serological IgG ELISA Kit is a solid-phase ELISA that detects binding of human IgG to four domains of SARS-CoV-2 spike protein (S-protein): the S1-NTD, RBD, S1, and S2 domains, individually. The four spike protein domains have each been individually coated onto microwells (see plate map for location/color-coding), such that twenty-four tests are provided for each spike protein domain (96 tests total). After incubation with sample, the human IgG specific for each spike protein domain is captured on the plate. The wells are then washed to remove unbound material. Anti-Human IgG, HRP-linked antibody is then used to recognize the bound IgG. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of IgG specific for each spike protein domain.

*Antibodies in this kit are custom formulations specific to kit.

Specificity/Sensitivity

The SARS-CoV-2 Spike Protein Multi-Domain (S1-NTD, RBD, S1, S2) Serological IgG ELISA Kit detects endogenous levels of human IgG directed to the S1-NTD (16-316), RBD (318-541), S1 (16-681), and S2 (686-1208) domains of SARS-CoV-2 spike protein (S-protein).

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.
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#88005

SARS-CoV-2 Spike Protein Multi-Domain (S1-NTD, RBD, S1, S2) Serological IgG ELISA Kit

SARS-CoV-2 Spike Protein Multi-Domain (S1-NTD, RBD, S1, S2) Serological IgG Sampler ELISA Protocol

This ELISA 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. **Spike Multi-Domain 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. Microwell strips are color-coded for each spike protein domain (see plate map below).

	S1-NTD			RBD			S1			S2		
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
B	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
C	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
D	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
E	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
F	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
G	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black
H	Blue	Blue	Blue	Orange	Orange	Orange	Grey	Grey	Grey	Black	Black	Black

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 for reconstitution and dilution of the Anti-Human IgG, HRP-linked Antibody (11 mL provided).
5. **Anti-Human IgG, HRP-linked Antibody (ELISA Formulated):** Supplied lyophilized as a red colored cake or powder. Add 1.0 mL of HRP Diluent (red solution) 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 Antibody to 10.0 mL of HRP Diluent in a clean tube and gently mix. For best results, use this working solution immediately. Unused working solution may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly made solution.
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 at -80°C (there may be some loss of the Positive Control signal if freeze/thawed). Positive Controls are supplied as a control reagent, not as an absolute quantitation measure.

IMPORTANT: This control is intended to give a positive signal for the RBD and S1 coated microwell strips, and is NOT a positive control for the S1-NTD or S2 microwells.

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 at -80°C (there may be some loss of the Negative Control signal if freeze/thawed).

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 should be diluted at least 1:400 with Sample Diluent A and can be further serially diluted if relative quantification is needed by the user. Positive and Negative Controls do NOT need to be diluted after reconstitution. Refer to the datasheet which shows typical results observed for the Positive Control, Negative Control, serum from uninfected individuals, and serum from SARS-CoV-2 patients. When using the cutoff criteria described below to determine if a sample is positive for anti-CoV-2 Spike Protein antibodies, samples diluted 1:400 must be compared to the undiluted Negative Control.

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.

3. Add 100 µL of each diluted sample, Positive Control, Negative Control, and blank (Sample Diluent A only) to the appropriate wells. Seal the plate with the supplied sealing tape and incubate for 1 hour at 37°C.
4. 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.
5. Add 100 µL of reconstituted Anti-Human IgG, HRP-linked Antibody (ELISA Formulated). Seal with tape and incubate the plate for 30 min at 37°C.
6. Repeat wash procedure (Section B, Step 4).
7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 10 min at 37°C.
8. Add 100 µL of STOP Solution to each well. Shake gently for a few seconds.

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

9. 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.

10. Data Analysis:

1. Subtract "blank" well (Sample Diluent A only) absorbance 450 nm values from sample, Positive, and Negative Control values.
2. Positive Control Values should be > 1.0 for S1 and RBD microwell strips.
3. Samples (1:400 dilution) are considered positive, negative, or inconclusive by using the following multipliers relative to the blank subtracted Negative Control absorbance 450 nm values.

	Positive Criteria	Negative Criteria	Inconclusive Criteria
S1-NTD	> 1.7 x Negative Control	< 1.35 x Negative Control	> 1.35 x Negative Control and < 1.7 x Negative Control
RBD	> 3.4 x Negative Control	< 2.7 x Negative Control	> 2.7 x Negative Control and < 3.4 x Negative Control
S1	> 4 x Negative Control	< 3 x Negative Control	> 3 x Negative Control and < 4 x Negative Control
S2	> 4.1 x Negative Control	< 3.1 x Negative Control	> 3.1 x Negative Control and < 4.1 x Negative Control

4. Limitations: Experimental cutoffs were determined by assaying a set of SARS-CoV-2 positive samples (from donors with positive SARS-CoV-2 diagnosis) and uninfected donor serum collected prior to the SARS-CoV-2 pandemic.

NOTE: 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 a positive response against each spike domain protein in this IgG Serological ELISA as differences in disease severity, timing of sample collection relative to disease onset, and patient profiles may affect presence and abundance of antibodies against each spike domain in the reference sample.

Researchers can establish or modify this cutoff using additional samples. Positive or negative results from this assay should not be the sole basis for determining the infection status of a sample. A negative result can occur in SARS-CoV-2 patient samples due to:

- improper sample handling/storage

- timing of sample collection post-infection
- patients having impaired immune function

NOTE: Absorbance 450 nm values should not be directly compared across different spike domain proteins, as they are not absolute values.

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