Store at 4°C

#20154

SARS-CoV-2 Spike Protein Serological IgG ELISA Kit

1 Kit
(96 assays)

Support: +1-978-867-2388 (U.S.)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: The SARS-CoV-2 Spike Protein Serological IgG ELISA Kit is a solid phase ELISA that detects binding of human IgG to full-length SARS-CoV-2 spike protein (S-protein). Full-length spike protein has been coated onto microwells. After incubation with sample, human IgG specific for spike protein 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 spike protein.

*Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: The SARS-CoV-2 Spike Protein Serological IgG ELISA Kit detects endogenous levels of human IgG directed to full-length 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).

Product Includes	Item #	Kit Quantity	Color
Spike Protein Coated Microwells II*	87455	96 tests	
Anti-Human IgG, HRP-linked Antibody (ELISA Formulated)	94210	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 #20154 Positive Control	42923	1 each	
ELISA Kit #20154 Negative Control	92351	1 each	

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

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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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Patient Testing: Patient samples were tested using the SARS-CoV-2 Spike Protein Serological IgG ELISA Kit #20154. Serum or plasma was obtained from donors who had been diagnosed with SARS-CoV-2 (diagnosed positive n=28) or from presumed uninfected donors collected prior to the SARS-CoV-2 outbreak (presumed negative n=62). Samples were heat-inactivated (56°C for 30 min) and diluted 1:800 prior to running the assay, as described in the protocol. Samples were considered positive, negative, or inconclusive based on the criteria described in the "Data Analysis" section of the protocol for the kit. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated from these data.

	CST SARS-CoV-2 Spike Protein Serological IgG ELISA Results					
Reference Samples	**Positive**	**Negative**	**Inconclusive**	**Total**	**PPA**	**NPA**
Positive	28	0	0	28	100%	—
Presumed Negative	2	59	1	62	—	95.2%

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 #20154 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 Protein Serological IgG ELISA #20154. Intra-assay CV (%) was calculated for each sample, and each replicate was correctly identified as being positive or negative when compared to the Negative Control using the cutoff criteria described in the attached protocol.

Sample ID	Average OD (450 nm)	Results	CV (%)
Positive Serum-1	2.312	16/16 are Positive	2.8%
Positive Serum-2	1.660	16/16 are Positive	2.8%
Negative Serum-3	0.268	16/16 are Negative	5.8%

Inter-Assay Precision: Six different assay kits from one lot of material were tested using 3 different serum samples run in duplicate wells and Positive and Negative Controls run in 4 replicate 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 Negative Control using the cutoff criteria described in the attached protocol.

Sample ID	Average OD (450 nm)	Results	CV (%)
Positive Serum-1	2.094	6/6 are Positive	7.5%
Positive Serum-2	1.451	6/6 are Positive	7.4%
Negative Serum-3	0.250	6/6 are Negative	9.5%
Positive Control	2.545	6/6 are Positive	4.9%
Negative Control	0.180	6/6 are Negative	8.3%

#20154

SARS-CoV-2 Spike Protein Serological IgG ELISA Protocol

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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. **Spike Protein Coated Microwells II:** 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 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.
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 1:800 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 1:800 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:
   a. Discard plate contents into a receptacle.
   b. 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.
   c. 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:
   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.
10. Data Analysis:
    a. Subtract "blank" well (Sample Diluent A only) absorbance 450 nm values from sample, Positive, and Negative Control values.
    b. Positive Control Values should be > 1.5 and Negative Control Values should be < 0.75.
    c. Samples (1:800 dilution) are considered positive if they are greater than 4.1 x Negative Control absorbance 450 nm value.
    d. Samples (1:800 dilution) are considered negative if they are less than 3 x Negative Control absorbance 450 nm value.
    e. Samples (1:800) are considered inconclusive if they are greater than the 3 x Negative Control absorbance 450 nm value and less than 4.1 x Negative Control absorbance 450 nm value.
    f. Limitations: Experimental cutoffs were determined by assaying a set of confirmed SARS-CoV-2 positive samples and uninfected donor serum collected prior to the SARS-CoV-2 pandemic. 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