



Orders: 877-616-CELL (2355)  
orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com  
cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

#20154 store at +4C

## SARS-CoV-2 Spike Protein Serological IgG ELISA Kit

1 Kit (96 assays)

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 Protein Coated Microwells II	87455	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 #20154 Positive Control	42923	1 ea		+4C
ELISA Kit #20154 Negative Control	92351	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 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 this 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).

### 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

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit [cellsignal.com/trademarks](https://cellsignal.com/trademarks) for more information.

## Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.

# #20154

## SARS-CoV-2 Spike Protein Serological IgG ELISA Kit

### SARS-CoV-2 Spike Protein Serological IgG 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 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:
  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  $\mu$ L 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  $\mu$ L 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  $> 1.5$  and Negative Control Values should be  $< 0.75$ .
  3. Samples (1:800 dilution) are considered positive if they are greater than  $4.1 \times$  Negative Control absorbance 450 nm value.
  4. Samples (1:800 dilution) are considered negative if they are less than  $3 \times$  Negative Control absorbance 450 nm value.
  5. Samples (1:800) are considered inconclusive if they are greater than the  $3 \times$  Negative Control absorbance 450 nm value and less than  $4.1 \times$  Negative Control absorbance 450 nm value.
  6. 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

## SARS-CoV-2 Spike Protein Serological IgA 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 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 IgA, HRP-linked Antibody (11 mL provided).
5. **Anti-Human IgA, 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 1:200 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 1:200 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  $\mu$ L 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  $\mu$ 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.
  3. Clean the underside of all wells with a lint-free tissue.
5. Add 100  $\mu$ L of reconstituted Anti-Human IgA, 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  $\mu$ L 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  $\mu$ L 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  $> 1.0$  and Negative Control Values should be  $< 0.75$ .
3. Samples (1:200 dilution) are considered positive if they are greater than 4 x Negative Control absorbance 450 nm value.
4. Samples (1:200 dilution) are considered negative if they are less than 3 x Negative Control absorbance 450 nm value.
5. Samples (1:200 dilution) are considered inconclusive if they are greater than the 3 x Negative Control absorbance 450 nm value and less than 4 x Negative Control absorbance 450 nm value.
6. 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. 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

posted August 2020