User Manual

SARS-CoV-2 IgG ELISA Kits

High sensitivity SARS-CoV-2 IgG ELISA kits for detection of human SARS-CoV-2 IgG in serum and plasma

For Catalog Numbers

K-16026-001 SARS-CoV-2 IgG ELISA Kit, Nucleocapsid, 96-wells

K-16027-001 SARS-CoV-2 IgG ELISA Kit, Spike (RBD), 96-wells

December 14, 2020 | D-05025-007

Important Information

The following instructions are for use with the SARS-CoV-2 IgG ELISA Kits, catalog numbers K-16026-001 and K-16027-001. Please see the Kit Contents section for details.

Storage Information

The SARS-CoV-2 IgG ELISA Kits are stable for at least 6 months when kit components stored properly as indicated on labels.

Warnings and Precautions

- Please read carefully the entire manual to evaluate all steps and familiarize yourself with the details of the protocol. Contact technical service if you require any further clarifications or have any specific questions.
- The SARS-CoV-2 IgG ELISA Kits are for research use only.
- Bring all reagents to room temperature (18–25°C) before use.
- This assay is designed for qualitative detection only. Results should not be the sole basis for clinical diagnosis or treatment. The confirmation of COVID-19 infection must be combined with the patient's clinical symptoms in conjunction with other tests.
- Always wear appropriate Personal Protective Equipment (PPE) such as gloves, goggles and lab coat while working.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Handle all samples and controls as if they are capable of transmitting infectious agents.
- This product is guaranteed to be free of manufacturer defects, and to function as described when the enclosed protocol is followed by properly trained personnel.

Table of Contents

1. Kit Contents

Catalog Number: K-16026-001, SARS-CoV-2 IgG ELISA Kit, Nucleocapsid, 96-wells

• L-07081-001	96-well ELISA Plate	1 plate
• R-03157-025	SARS-CoV-2 Antigen Nucleocapsid Protein	25µg
• R-03155-C12	1X EIA Coating Buffer	12mL
• R-03024-C50	10X AdvanWash	50mL
• R-03728-C50	AdvanBlock-EIA Blocking Solution	50mL
• R-03158-200	Positive Control Serum	200µL
• R-03159-200	Negative Control Serum	200µL
• R-03160-C12	Anti-IgG HRP Conjugate	12mL
• R-03161-C12	TMB Substrate	12mL
• R-03162-C12	Stop Solution	12mL

Catalog Number: K-16027-001, SARS-CoV-2 IgG ELISA Kit, Spike (RBD), 96-wells

• L-07081-001	96-well ELISA Plate	1 plate
• R-03156-025	SARS-CoV-2 Antigen Spike (RBD) Protein	25µg
• R-03155-C12	1X EIA Coating Buffer	12mL
• R-03024-C50	10X AdvanWash	50mL
• R-03728-C50	AdvanBlock-EIA Blocking Solution	50mL
• R-03158-200	Positive Control Serum	200µL
• R-03159-200	Negative Control Serum	200µL
• R-03160-C12	Anti-IgG HRP Conjugate	12mL
• R-03161-C12	TMB Substrate	12mL
• R-03162-C12	Stop Solution	12mL

2. Shipping and Storage Conditions

Components requiring storage at -20°C ship on blue ice. All other components are shipped at ambient temperature. Store at appropriate temperature upon receipt.

The SARS-CoV-2 IgG ELISA Kits are stable for at least 6 months when kit components stored properly as indicated on labels.

3. Additional Items and Materials Required

- 96-well Plate Sealers
- 96-well Dilution Plate (inert non-binding polypropylene plate, preferably conical-bottom)
- Reagent Reservoirs
- Microplate reader capable of measuring absorbance at wavelength of 450 nm
- Multi-well bottle-top microplate washing adapter is strongly recommended, however mult-channel pipette can be also used for washing steps. This protocol is designed assuming manual washing procedure. There are many automated plate washing devices available, which may have different requirements for available washing solutions. If you plan to use an automatic wash station that requires volume of washing solution larger than available in this kit, additional AdavnWash™ washing solution is available as a separate item. Please refer to Related Products section on page 12.

4. Background

In December of 2019, the SARS-CoV-2 virus, which causes the disease COVID-19, was first detected in Wuhan, China. The global COVID-19 pandemic has since caused an unprecedented social and economic disruption to daily life. To aid in the advancement of infectious disease research, Advansta now offers ELISA kits to detect the IgG immune response to both the Nucleocapsid and Spike (RBD) proteins of the virus. The SARS-CoV-2 virus is a single-stranded RNA coronavirus that causes respiratory infections. IgG is an immunoglobulin that is produced in response to an antigen. This assay is designed for the qualitative measurement of human IgG against the Nucleocapsid or Spike (RBD) proteins from the SARS-CoV-2 virus.

5. *ELISA*

ELISA (enzyme-linked immunosorbent assay) is a powerful method for detecting and quantifying a specific protein in a complex mixture. Originally described by Engvall and Perlmann (1971), this method enables analysis of protein samples immobilized in microplate wells using specific antibodies. ELISA is a plate-based assay technique designed for detecting soluble substances such as peptides, proteins, antibodies, or other small molecules. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. This assay is the preferred method to determine the titer of antisera and purified antibodies. In an ELISA, the most important feature that defines the assay's quality is a highly specific antibody-antigen interaction. ELISAs are typically performed in 96-well or 384-well polystyrene plates, which passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. The ability to use high-affinity antibodies and wash away non-specifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a complex sample.

Although many variants of ELISA have been developed and used in different situations, they all follow the same basic steps:

- **Coating/capture**—direct or indirect immobilization of antigens to the surface of polystyrene microplate wells.
- **Plate blocking**—addition of irrelevant protein or other molecules to cover all unsaturated surface-binding sites of the microplate wells.
- **Probing/detection**—incubation with antigen-specific antibodies that affinity-bind to the antigen.
- **Signal measurement**—detection of the signal generated via an enzymatic reaction

The first step of the SARS-CoV-2 ELISA protocol is to immobilize the target antigen (Spike (RBD) or Nucleocapsid protein) to the microplate. After washing away unbound protein, the second step is to add the blocking solution to prevent non-specific binding. After washing away excess blocking solution, the third step is to add diluted plasma or serum to the wells. If the sample contains IgG antibodies against the target protein, they will bind specifically to the plate. After washing away unbound serum proteins, the SARS-CoV-2 specific antibodies will be measured via "indirect" detection, where a horseradish peroxidase (HRP) enzyme conjugated anti-species secondary antibody is added to the plate. After the incubation with this secondary antibody, the wells are washed from the unbound antibody, and then 3,3',5,5'-Tetramethylbenzidine (TMB) substrate is added to detect the HRP enzyme bound to the plate. This produces a colored end product, amount of which which correlates to the amount of IgG present in the original sample. Then, after a short incubation, the reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm. The signal from each well is measured on a plate reader.

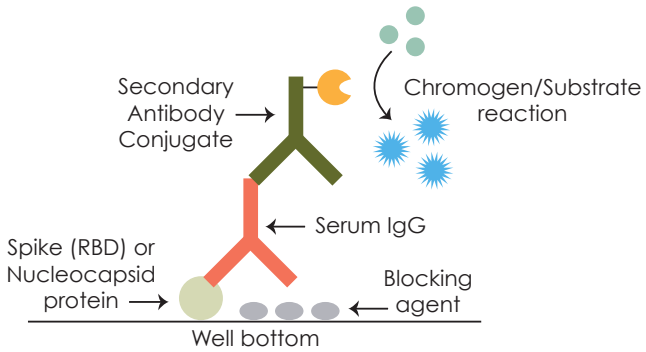
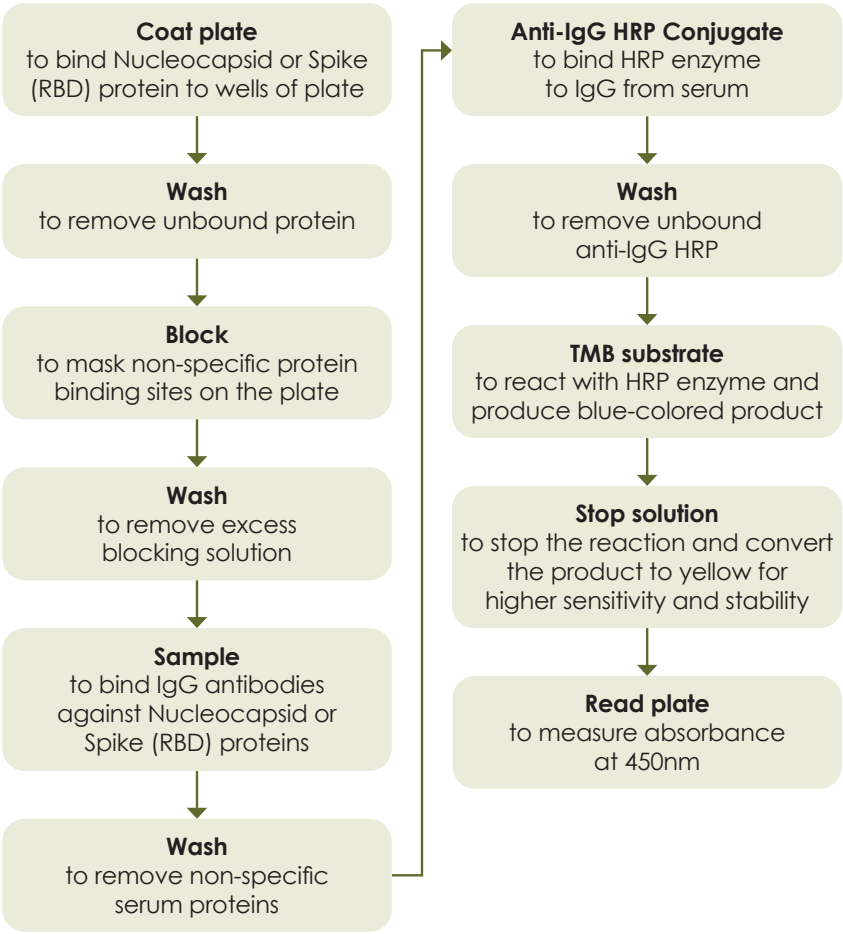


Figure 1. The principle of SARS-CoV-2 indirect ELISA kits.

6. Overview of the protocol for SARS-CoV-2 ELISA Kits



7. Preparation of Solutions

- **1X AdvanWash™ Washing Solution:** Prepare 500mL of wash solution by adding 50mL of 10X AdvanWash to 450mL of high purity water. Mix well prior to use.
- **1X Antigen Coating Solution:** To 12mL of 1X EIA coating buffer add 24µL of Antigen stock solution. Mix well by inversion.

8. Sample Dilution

- **Controls:** Positive and negative serum controls are provided as ready-to-use samples. COVID-19 positive serum has been heat-inactivated for increased safety. Serum samples are pre-diluted 1:50 with AdvanBlock-EIA. With this dilution factor, positive control is expected to generate a signal within linear range of the assay's dynamic range. Up to four wells in the plate can be used for each positive and negative control. Mix well prior to use.
- **Unknown Samples:** Prepare sufficient sample to evaluate at least in duplicate. Dilute serum or plasma samples 1:50 in a 96-well dilution plate with AdvanBlock™-EIA. For example, to 196µL of AdvanBlock™-EIA add 4µL of sample. (Note: Although we recommend screening samples with an initial dilution of 1:50, it is advised that the user determine the optimal dilution by titrating samples or using two-fold serial dilutions ranging from 1:50 to 1:6400).

9. Plate Setup

We strongly recommend using a "dilution plate" to set up all your samples, controls and blanks, according to the layout and design of your experiment. All samples should be transferred to the blocked and washed assay plate in step 5 of the protocol as quickly as possible using a multi-channel pipette. Using this technique greatly improves quality of the assay's results. Do not reuse the tips, use a new set of tips for each sample transfer.

10. Protocol

1. Coat the ELISA plate with 100 µL/well of 1X Antigen Coating Solution and incubate 1h at room temperature (RT).
2. Wash the plate with 1X AdvanWash™ Washing Solution 3 times (250 µL/well per wash).
3. Block the plate with 250 µL/well AdvanBlock™-EIA and incubate 30 minutes at RT.
4. Wash the plate with 1X AdvanWash™ Washing Solution 3 times (250 µL/well per wash). Make sure that washing solution is completely removed from the assay plate.
5. Quickly transfer all samples, controls and blanks from the dilution plate into the assay plate, 50 µL/well and incubate 1h at RT.
6. Wash the plate with 1X AdvanWash™ Washing Solution 3 times (250 µL/well per wash).
7. Add 100 µL/well of Anti-IgG HRP Conjugate and incubate 30 minutes at RT. (Note: The Anti-IgG HRP Conjugate is provided as a pre-diluted ready-to-use solution).
8. Wash the plate with 1X AdvanWash™ Washing Solution 3 times (250 µL/well per wash).
9. Add 100 µL/well TMB substrate and incubate 30 minutes at RT. Protect the plate from light during this step.
10. Add 100 µL/well Stop Solution directly to the wells containing TMB substrate reaction mixture.
11. Measure absorbance at 450nm.

11. Troubleshooting & FAQ

Some common problems that are encountered are addressed below:

Problem	Possible Solutions
No signal	• Reagents added in incorrect order, or incorrectly prepared. Review protocol then repeat the assay. • Reagent contamination. Repeat with fresh reagents.
High background	• Insufficient washing. Increase the number of washes to 4–5 times and add a 30 second soak in-between each wash.
High %CV	• Insufficient plate washing. If using an automated plate washer, check that all ports are clean and free of obstructions. • Plate contamination. Use a fresh plate sealer for each incubation step. Do not reuse pipet tips. • Slow or inaccurate transfer from the dilution plate into the assay plate. • Washing solution not fully removed after the last washing step before adding samples to the plate.
Plate turned uniformly blue after incubation with substrate	• Insufficient plate washing. Increase the number of washes to 4-5 and add a 30 second soak in-between each wash. • Substrate was contaminated. Ensure that the substrate is clear prior to addition to the plate. • Reagent contamination. Repeat with fresh reagents.

12. References

1. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry*. 1971 Sep;8(9):871–4. doi: 10.1016/0019-2791(71)90454-x. PMID: 5135623.

13. Related Products

Catalog Number	Product	Size
R-03151-U10	SARS-CoV-2 Spike (RBD), His Tag (CHO-K1)	100 µg
R-03152-U10	SARS-CoV-2 Spike (RBD) C Tag (CHO-K1)	100 µg
R-03150-U10	SARS-CoV-2 Nucleocapsid, His Tag *(E.coli)*	100 µg
R-03726-E10	AdvanBlock™-Chemi Blocking Solution	1 L
R-03728-E10	AdvanBlock™-EIA Blocking Solution	1 L
R-03024-D50	AdvanWash™ 10X Washing Solution	500 ml
R-03730-D25	10X EIA Coating Buffer	250 ml
K-16025-D25	ELISABright™, sufficient for twenty-five (25) 96-well ELISA plates	250 ml

14. Warranty

This product is warranted to be free of defects of material or workmanship, and to perform as described in the published specifications when stored according to the documentation included with the product, and used according to the accompanying instruction manual by appropriately trained personnel. If the product is found to have a defect upon first use and within 30 days of shipment, the product may be replaced. This warranty extends only to the original purchaser of the product. There is no obligation to replace the product as a result of misuse, improper storage, or negligence of the buyer.

15. User Notes

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Product information: www.advansta.com/products/SARS-CoV-2-Proteins-and-Kits