ZYMO RESEARCH

Quick COVID-19 Antibody Detection Kit

Accurate ELISA-based detection of SARS-CoV-2 RBD antibodies in 2.5 hours

Highlights

- Highly sensitive and specific detection of IgG antibodies directed against the SARS-CoV-2 receptor-binding domain (RBD).
- Set up to results in only 2.5 hours.
- Included Cut-off Control simplifies test validation.

Catalog Numbers:
D5327

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

Table of Contents

Product Contents

***Quick* COVID-19 Antibody Detection Kit**	**D5327** (96 reactions)
RBD-coated 96-well ELISA plate	1 pc
10X ELISA Wash Buffer[1]	15 ml
Sample Diluent	11 ml
Anti-IgG HRP Antibody	11 ml
TMB Substrate[2]	11 ml
Stop Solution[3]	11 ml
Negative Control[4]	0.5 ml
Positive Control[4]	0.5 ml
Cut-off Control	0.5 ml
96-well Plate Lid	1 pc
Instruction Manual	1 pc

Storage Temperature – Store all kit components (*i.e.*, solutions, controls, plate) at 4 °C.

[1] Prepare the **1X ELISA Wash Buffer** by diluting the **10X ELISA Wash Buffer** 1:10 with deionized water (add 15 ml **10X ELISA Wash Buffer** to 135 ml deionized water). The **1X ELISA Wash Buffer** should be prepared all at once and stored at 4°C for use within one week.
[2] **TMB Substrate** is light sensitive and should be stored in the dark.
[3] Caution: **Stop Solution** contains 0.5 M H_2SO_4 (sulfuric acid). Please use proper safety precautions.
[4] **Negative Control** and **Positive Control** contain heat-inactivated human serum and must be treated as biohazardous material.

Specifications

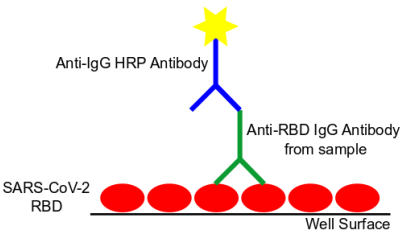
- **Sample Sources –** Human serum or plasma containing anticoagulants such as Citrate, EDTA or Heparin (heat-inactivated or untreated).
- **Processing Time –** 2.5 hours
- **Sample Quantity –** A minimum of 2 µl human serum/plasma per well.
- **Detection –** Absorbance at 450 nm with a reference filter between 560 nm and 650 nm.
- **Required Equipment –** Incubator capable of reaching 37°C; Plate reader; Multi-channel pipette is recommended

Product Description

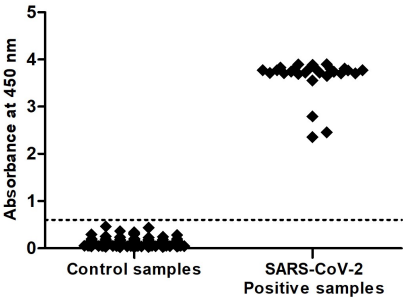
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). Over 20 million COVID-19 cases have been reported in more than 200 countries to date[1] since it was first described in December 2019 in Wuhan, China.

The virus infects host cells in the human respiratory tract by initially binding to the angiotensin-converting enzyme 2 (ACE2). Binding to ACE2 is mediated by the receptor-binding domain (RBD) of the viral spike glycoprotein. Accordingly, recent serological studies imply that the concentration of antibodies against the SARS-CoV-2 RBD correlates with the propensity to neutralize the virus and hence provide immunity[2,3].

The ***Quick* COVID-19 Antibody Detection Kit** provides a straightforward ELISA-based test for determining the presence of IgG antibodies directed against the SARS-CoV-2 RBD in human serum or plasma. Test results are quickly and easily validated using the included Cut-off Control, which has been calibrated with over 200 serum and plasma samples.



***Quick* COVID-19 Antibody Detection Kit** is an indirect ELISA. First, IgG Antibodies from the human sample bind to SARS-CoV-2 RBD that is attached to the bottom of the well. The human IgGs are then recognized by **Anti-IgG HRP Antibody**. Subsequent addition of the **TMB Substrate** and **Stop Solution** will produce a yellow color in wells containing human IgGs against SARS-CoV-2 RBD.



***Quick* COVID-19 Antibody Detection Kit** accurately detects IgG antibodies against SARS-CoV-2 RBD. Serum/Plasma samples taken before October 2019 (Control, n=189) and samples from COVID-19 positive patients (SARS-CoV-2 Positive, n=27) were analyzed. The **Cut-off Control** (dashed line) represents a reliable threshold value between the two cohorts.

[1] World Health Organization: Coronavirus disease (COVID-19) Weekly Epidemiological Update 1, August 17th 2020
[2] Premkumar et al (2020) Science Immunology 5(48), eabc8413
[3] Robbiani et al (2020) Nature 584, 437-442

Protocol

Buffer Preparation and Storage

- ✓ Prepare the **1X ELISA Wash Buffer** by diluting the **10X ELISA Wash Buffer** (1:10) in deionized water (add 15 ml **10X ELISA Wash Buffer** to 135 ml deionized water). The **1X ELISA Wash Buffer** should be prepared all at once and stored at 4°C for use within one week.

Before Starting

- ✓ Allow the **RBD-coated 96-well ELISA plate** to incubate for 30 minutes at room temperature before unpacking to avoid condensation.
- ✓ Allow all reagents, buffers, and controls to incubate for 30 minutes at room temperature and mix thoroughly before starting the ELISA.

ELISA

1. Dilute 2 µl serum/plasma sample in 98 µl of **Sample Diluent** and mix thoroughly by pipetting up and down. Transfer the entire mixture to a well of the **RBD-coated 96-well ELISA plate**[1].

2. Add 100 µl of **Positive Control**, **Negative Control** and **Cut-off Control** to three separate wells.

3. Cover the plate with the provided **96-well Plate Lid** and incubate at 37°C for 1 hour.

4. Discard the samples and controls from the wells by inverting the plate[2].

5. Wash each well with 200 µl of **1X ELISA Wash Buffer** and remove the liquid from each well by inverting the plate. Repeat this wash step 2 more times.

6. Remove excess liquid by tapping the plate onto a paper towel.

---

[1] Samples must be transferred to the plate within 2 hours after dilution.
[2] Serum/Plasma samples and **Negative and Positive Controls** are biohazardous and must be disposed of accordingly.

7. Add 100 µl **Anti-IgG HRP Antibody** to each well. Cover the plate with the provided **96-well Plate Lid** and incubate at 37°C for 1 hour.

8. Discard the antibody from the wells by inverting the plate.

9. Wash each well with 200 µl of **1X ELISA Wash Buffer** and remove the liquid from each well by inverting the plate. Repeat this wash step 2 more times.

10. Remove excess liquid by tapping the plate onto a paper towel.

11. Add 100 µl of **TMB Substrate** to each well and incubate for 10 minutes at room temperature in the dark.

12. After the 10 minute incubation[1], immediately add **100 µl of Stop Solution**[2] to each well containing the **TMB Substrate** and measure the absorbance at 450 nm (with reference filter between 560 nm and 650 nm) in a plate reader. Calculate the mean value if samples were measured in replicate.

[1] The enzymatic reaction has to be stopped right after 10 min, since prolonged incubation can lead to invalid control values.
[2] Upon addition of the **Stop Solution**, precipitate might form for samples containing very high levels of IgGs against the SARS-CoV-2 RBD. This does not effect the validity of the assay.

Interpretation of Results

The following criteria must be met for a valid test:

Control	Absorbance$_{450nm}$
Cut-off Control	0.4-0.8
Negative Control	<0.2
Positive Control	>1

Interpretation of test results for human samples is based on their ratio to the **Cut-off Control,** which is calculated as follows:

$$\textbf{Ratio} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of } \textbf{Cut-off Control}}$$

The following criteria are recommended for interpreting the test results:

Ratio	Test result
> 1	Positive
< 0.8	Negative
0.8 - 1	Indeterminable

Troubleshooting

Problem	Possible Causes and Suggested Solutions
No color development after addition of the TMB Substrate and Stop Solution	**Wrong Buffers used.** Make sure to use the correct Buffers and Reagents to dilute your samples, wash the plate and develop the assay, respectively. **Improper Kit Storage conditions.** After arrival, immediately store all kit components at 4 °C. **TMB Substrate exposed to light.** The TMB Substrate is light sensitive and should therefore be stored and incubated in the dark. Avoid exposure to direct sunlight at all times.
Control values are lower than stated in the protocol	**Non-homogenous reagents.** Make sure that all buffers, reagents, and controls are mixed well before applying to the plate. This particularly applies to the Anti-IgG HRP-Antibody and the Negative, Positive and Cut-off Controls. **Reagents not at room temperature.** Allow all kit components to incubate for 30 minutes at room temperature before starting the ELISA. Lower temperatures might lead to inefficient antibody binding and lower HRP-activity.
Control values are higher than stated in the protocol	**Non-homogenous reagents.** Make sure that all buffers, reagents, and controls are mixed well before applying to the plate. This particularly applies to the Anti-IgG HRP-Antibody and the Negative, Positive and Cut-off Controls. **Dirty plate bottom.** Before inserting the plate into the plate reader, ensure that there are no smudges, dirt, or dust on the bottom of the plate, which could lead to an aberrant measurement. **Wrong conditions for TMB Substrate incubation.** After adding the TMB Substrate to the wells, the incubation must be carried out in the dark, at room temperature and for only 10 minutes. Prolonged incubation or incubation at elevated temperatures can lead to unwanted increase in signal strength.

Ordering Information

Product Description	Catalog No.	Size
Quick COVID-19 Antibody Detection Kit	D5327	96 wells

Individual Kit Components	Catalog No.	Amount
10X ELISA Wash Buffer	D5327-1-15	15 ml
Sample Diluent	D5327-2-11	11 ml
Negative Control	D5327-3-500	0.5 ml
Positive Control	D5327-4-500	0.5 ml
Cut-off Control	D5327-5-500	0.5 ml
Anti-IgG HRP Antibody	D5327-6-11	11 ml
Stop Solution	D5327-8-11	11 ml

Notes

100% satisfaction guarantee on all Zymo Research products, or your money back.

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

Integrity of kit components is guaranteed for up to six months from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

ZYMO RESEARCH

The **BEAUTY** *of* **SCIENCE** *is to Make Things* **SIMPLE**®

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682