PROEL BIOTECH

## SARS-CoV-2 IgG ANTIBODY DETECTION KIT ELISA

# USER'S MANUAL

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# INTENDED USE

This kit is intended for qualitative detection of human IgG antibodies produced after SARS-CoV-2 infection. The kit is for in vitro diagnostic use only.

# CLINICAL SIGNIFICANCE

SARS-CoV-2 is a single stranded RNA virus that caused a global health emergency that is unprecedented in the last 100 years.[1] The virus belongs to the family of coronaviruses, and causes a wide range of symptoms in humans (respiratory, gastrointestinal and even neurological[2]). The mortality rate of SARS-CoV-2 infection is strongly age-dependent, with 2.5 million deaths and 112 million infections worldwide as of 25 February 2021. The virus is mainly thought to spread through close human-human contacts in respiratory droplets, and new evidence points to possible longer-range transmission through air.[3] The majority of symptomatic individuals produce an immune response that causes the appearance of antibodies specific to virus proteins. IgM type antibodies appear 5-7 days after the onset of symptoms, while IgG antibodies can be detected after 1-2 weeks the earliest. The detection of specific IgG antibodies should not be used therefore to diagnose an acute SARS-CoV-2 infection. Recent data suggests that IgG levels decline a few months after infection, and this decline is correlated with the severity of the illness.[4] The current ELISA kit is formulated in order to be applicable for measurement of immune response due to vaccination, as the microplate is coated with a combination of antigens that are prime targets for vaccine development. Current vaccines authorized in the EU have the SARS-CoV-2 spike protein as target antigen, the kit detects anti-Spike 1 and anti-nucleocapsid IgG antibodies. The specific IgG status of a person can be used to establish past exposure to SARS-CoV-2, and can be monitored over time to evaluate the likelihood of protection against reinfection for post-COVID patients or vaccinated individuals.

1 Zhu N, Zhang D, Wang W, et al. "A Novel Coronavirus from Patients with Pneumonia in China." *N Engl J Med.* 382(8):727-733 (2020). https://doi.org/10.1056/NEJMoa2001017

2 Ellul, Mark, et al. "Neurological Associations of COVID-19." *The Lancet* 19(9), 767-783 (2020).

3 Ralph, Robyn, et al. "2019-nCoV (Wuhan virus), a novel Coronavirus: human-to-human transmission, travel-related cases, and vaccine readiness." *The Journal of Infection in Developing Countries* 14.01 (2020): 3-17. https://doi.org/10.3855/jidc.12425

4 Ma, Huan, et al. "Decline of SARS-CoV-2-specific IgG, IgM and IgA in convalescent COVID-19 patients within 100 days after hospital discharge." *Science China Life Sciences* (2020): 1-4. https://doi.org/10.1007/s11427-020-1805-0

# PRINCIPLE OF THE METHOD

The test is based on selective detection of human IgG antibodies that bind to SARS-CoV-2 antigens coated on the wells of the microplate by sandwich Elisa (Enzyme Linked Immunosorbent Assay). After incubation with a 1:101 diluted human serum sample, the unbound serum components are washed away and the well is incubated with an anti-human IgG antibody labelled with horseradish peroxidase (HRP). After a subsequent washing step, addition of a substrate solution (TMB) initiates a chromogenic reaction, dependent on the quantity of immobilized HRP-labelled antibodies on the surface of the well, which in turn depend on the quantity of specific SARS-CoV-2 antibodies captured from the sample. The reaction is stopped with an acidic solution, and the developed yellow colour is measured photometrically at 450/620 nm. The intensity of the yellow colour is proportional to the quantity of SARS-CoV-2 IgG antibodies in the sample.

# MATERIALS PROVIDED

1. Microwell plate: 12x8 wells microplate strips coated with a combination of recombinant SARS-CoV-2 antigens (spike 1 and nucleocapsid proteins); ready to use.
2. Enzyme conjugate: 1 vial of 7 ml; HRP-labeled anti-human IgG antibody; ready to use.
3. Wash Buffer (10x): 10x concentrate; 2 vials of 50 ml; phosphate buffered saline containing Tween-20; the content must be diluted 1:10 with distilled water and mixed well before use.
4. Sample diluent: 2 vials of 50 ml; ready to use.
5. TMB Substrate Solution: 1 vial of 7 ml; ready to use.
6. Stop Solution: 1 vial of 7 ml; 0.25 mol/l $H_2SO_4$; ready to use.
7. Negative control: 1 vial of 1 ml; diluted human serum; ready to use.
8. Positive control: 1 vial of 1 ml; diluted human serum containing anti-SARS-CoV-2 IgG antibodies; ready to use.

# MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated micropipettes with disposable tips of 1 µl, 50 µl and 1 ml
- Freshly distilled/deionized water
- Graduated cylinders for wash buffer dilution

- Vortex mixer
- Disposable gloves
- Absorbent paper
- Photometer/Spectrophotometric microplate reader capable of reading absorbance at 450/620 or 450/630 nm.

## PREPARATION OF REAGENTS

Allow all reagents to reach room temperature for at least 30 minutes prior to use. Wash buffer (10x) concentrate must be diluted to working concentration prior ro use. Note: If crystals are present in the 10x Wash buffer, warm up the solution at 37°C until all crystals are dissolved.

## STORAGE AND STABILITY

The kit should be stored at 2-8°C. The unopened kit is stable until the expiry date printed on the kit label. Once opened, each component is stable for three months. Unused microplates should be resealed in the original packaging containing desiccant.

## SAMPLE HANDLING AND SAFETY

As with all potentially infectious specimens, appropriate precautions should be taken, according to good laboratory practice guidelines. Visibly lipaemic or hemolyzed serum/plasma specimens should not be processed. The controls included in the kit tested negative for anti-HCV, HBsAg, anti-HIV and RPR. Components of the kit should be handled by trained laboratory personnel only. The STOP solution contains sulphuric acid.

# PROCEDURE

1. Remove required microwell strips from the pouch and carefully reseal the pouch with the desiccant to protect unused strips from moisture. Store unused strips at 2-8°C.
2. **Sample dilution:** add 1 µl sample to 100 µl Sample diluent (human serum or plasma), mix by vortexing, or add 10 µl serum to 1 ml Sample diluent, mix by vortexing.
3. Add 50 µl **undiluted** positive and negative control into the first two wells, and the diluted samples into consecutive wells. Incubate at room temperature for 1 hour in the dark. For higher precision, it is recommended to work in duplicates.
4. Wash each well 5 times with 350 µl Wash buffer by dispensing 350 µl of diluted Wash buffer into each well, and then completely aspirate the contents.
5. Add 50 µl Enzyme conjugate into the microwells and incubate at room temperature for 15 minutes in the dark.
6. Wash each well 5 times with 350 µl Wash buffer as described above (Step 4).
7. Add 50 µl TMB Substrate solution into the microwells and incubate at room temperature for 20 minutes in the dark.
8. Add 25 µl Stop solution into each microwell and read the optical density (OD) values at 450 and 620 nm with a microplate/microstrip reader. The corrected OD value ($OD_{450}$-$OD_{620}$) should be used to determine the presence or absence of SARS-CoV-2 IgG antibodies.

## Assay scheme

| Step | Reagents | Reagent quantity | Undiluted controls | Diluted samples | Incubation time | Washing method |
|---|---|---|---|---|---|---|
| 1 | - | - | 50 µl | 50 µl | 60 min | 5x350 µl |
| 2 | Enzyme conjugate | 50 µl | | | 15 min | 5x350 µl |
| 3 | TMB substrate | 50 µl | | | 20 min | - |
| 4 | Stop solution | 25 µl | | | - | - |

# INTERPRETATION OF RESULTS

Absorbance values greater by 0.1 units than the measured OD of the negative control should be considered positive for the detection of SARS-CoV-2 IgG antibodies. Lower values should be reported as inconclusive or negative, according to the table below:

| Measured OD | Reported result |
|---|---|
| $> OD_{neg\ ctr} + 0.1$ | positive |
| $< 0.9 \times (OD_{neg\ ctr} + 0.1)$ | negative |
| Between $0.9 \times (OD_{neg\ ctr} + 0.1)$ and $OD_{neg\ ctr} + 0.1$ | inconclusive, re-test after a few weeks |

The result can be given quantitatively as well, in arbitrary units AU/ml, using the formula $OD_{sample}$/*cutoff*. Positive results will be greater than 1 AU/ml, inconclusive results will be between 0.9 and 1 AU/ml, while negative results will be lower than 0.9 AU/ml.

One has to keep in mind that the level of circulating SARS-CoV-2 IgG antibodies decreases significantly a few months after infection, therefore negative results do not exclude the presence of such antibodies at low levels. The likelihood of reinfection does not depend only on the quantity of such antibodies, but on their neutralising capabilities and on other defence mechanisms as well.[5,6] Given the very small number of well-documented reinfected individuals,[7] it is still not possible to establish any correlation between antibody levels and reinfection probability.

# QUALITY CONTROL

To ensure the validity of the results, each assay must include both negative and positive controls. The measured OD of the negative control should not exceed 0.1. The measured OD of the positive control should not be lower than 0.2.

5 Le Bert, N., Tan, A.T., Kunasegaran, K. et al. "SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls." *Nature* 584, 457–462 (2020). https://doi.org/10.1038/s41586-020-2550-z

6 Habel, Jennifer R., et al. "Suboptimal SARS-CoV-2-specific CD8+ T-cell response associated with the prominent HLA-A* 02: 01 phenotype." *PNAS* 202015486 (2020). https://doi.org/10.1073/pnas.2015486117

7 To, Kelvin Kai-Wang, et al. "COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing." *Clinical Infectious Diseases* (2020). https://doi.org/10.1093/cid/ciaa1275

# ASSAY VALIDATION DATA

The **speci□city** of the assay was evaluated on 88 serum samples of various pathologies acquired from January 2018 to February 2019 at a partner diagnostics and research laboratory, stored at -80℃. The determined OD values for these samples were 0.038 ± 0.016. The diagnostic specificity determined according to the above scheme is 100%.

The **sensitivity** of the assay was evaluated using samples taken from patients with confirmed positive SARS-CoV-2 RT-PCR tests that were symptomatic and a few symptomatic direct contacts of these, all of which tested positive after symptom resolution using a commercial assay based on chemiluminescence. In total 30 such samples were tested. The determined OD values for these samples were 0.444 ± 0.200, ranging from 0.139 to 0.863. All 30 samples tested positive with this assay, therefore the diagnostic sensitivity determined according to the above scheme is 100%.

No cross-reactivity was observed to SARS-CoV-2 IgM, as determined on samples that tested positive for SARS-CoV-2 IgM and negative for SARS-CoV-2 IgG using a commercially available assay based on chemiluminescence.

# PRODUCT WARRANTY AND SUPPORT

The test kit is guaranteed to work as intended if all instructions are followed and the kit is stored according to specifications. It is very important to reseal the unused wells in the original pouch containing desiccant. In case you encounter performance problems with this test kit, please contact us by email at info@proelbiotech.com with information about the type of problem (e.g. false positives, false negatives, too weak absorbance for the positive control etc.). We will replace any faulty products free of charge. This does not affect your statutory rights. We welcome any feedback that helps us further improve our product, especially performance data of the kit in external quality control schemes.

# SYMBOLS USED

| | |
|---|---|
| CE | European conformity |
| IVD | In vitro diagnostic device |
|  | Consult instructions for use |
|  | Consult accompanying documents |
|  | Manufacturer |
|  | Date of manufacture |
| REF | Catalogue number |
| LOT | Lot No. / Batch code |
|  | Expiration date |
|  | Storage temperature |