SARS-CoV-2 (Covid-19) IgG

ELISA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55109-96

www.zellx.de

Sample Types Validated for:

Serum

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Introduction

Background

A novel coronavirus, named SARS-CoV-2 or 2019-nCoV, has caused an ongoing global pandemic, called COVID-19 in 2020. The pneumonia-like COVID-19 outbreak started in Wuhan City, the capital of Hubei province, in China, and quickly spread around the world within just few months. The SARS-CoV-2 is a human coronavirus that is genetically different from the other common human coronaviruses such as 229E, NL63, OC43, HKU1, which cause seasonal acute respiratory illnesses. It is also genetically distinct from the two newer human coronaviruses, MERS-CoV and SARS-CoV.

The SARS-CoV-2 virus is a positive-sense single stranded RNA virus, and has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The N protein holds the RNA genome, while the S, E, and M proteins together create the viral envelope. The S protein mediates viral entry into host cells by initial binding to the host ACE2 receptor through the receptor-binding domain (RBD) in the S1 subunit and then fusing the viral and host membranes through the S2 subunit. The RBD residues 331 to 524 of the S1 protein elicits the production of antibodies in the host.

The primary and golden standard method to diagnose SARS-CoV-2 infection is a PCR-based assay which detects the RNA of the virus derived from throat and nasal swabs. **For analyzing SARS-CoV-2 using qPCR methods use either our SARS-CoV-2–RT-qPCR Detection kit (N&RP) Cat. No. ZX-22102-100 or Coronavirus (2019-nCoV) Real Time-qPCR Detection Kit Cat. No. ZX-22100-100**.

Serological assays on the other hand, allow the study of the immune response to SARS-CoV-2 in a qualitative and quantitative manner. Serological assays determine the precise rate of infection in an affected area, which is an essential variable to accurately define the infection fatality rate. Serological assays will allow for the identification of individuals who mounted strong antibody responses and who could serve as donors for the generation of serum antibody-based therapeutics. They will also permit health authorities to determine who may be immune and who is not. This information may be very useful for deploying healthcare workers in a strategic manner to limit the risk of exposure and inadvertent spread of the virus. It could also allow proportions of the population that have already acquired immunity to go back to 'normal life'.

Assay principle

The ZellX® SARS-CoV-2 (Covid-19) IgG Immunoassay kit is designed to measure SARS-CoV-2 viral reactive IgG species in human serum samples. Human negative and positive controls are provided to generate negative (low) and positive (high) optical densities (OD) for the assay. The OD readings for these two controls need to fall within the acceptable limits of the assay to be considered valid. Please read comprehensively the kit insert before performing the assay.

The kit includes a 96-well plate that is pre-coated with SARS-CoV-2 recombinant S and N proteins which capture reactive antibodies in blood from people infected with SARS-CoV-2 during the subsequent

detection steps. Initially controls or diluted samples are added to the wells and incubated for half an hour followed by adding the peroxidase-conjugated goat antibody against human IgG. After 30 minutes of incubation, the substrate is added to react with the peroxidase-labeled antibody conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The presence of SARS-CoV-2 IgG in the samples is assessed by comparing with the positive and negative control ODs and the published cut-off ODs provided in the insert.

General information

Materials supplied in the Kit

Component	*Quantity*
SARS-CoV-2 IgG Positive Control	200 µL
SARS-CoV-2 IgG Negative Control	200 µL
Anti-human IgG Peroxidase Conjugate	5 mL
Assay Buffer Concentrate (2x)	24 mL
Wash Buffer Concentrate (20x)	25 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate	1 plate
Sealer	2

Storage instruction

The unopened kit must be stored at -20°C. Once opened, the kit can be stored at 4°C up to the expiration date of the kit, except for the Assay Buffer Concentrate, Positive Control, and Negative Control which must be stored at -20°C.

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker (capable of maintaining shaking at approximately 500 RPM)

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

The Positive and Negative Controls supplied with this kit have been derived from human blood and should be treated as potentially infectious. They have been tested negative for Hepatitis B and C, HIV and other infectious diseases. Appropriate precautions should be taken.

After running the assay, the used assay wells, pipette tips, diluted samples, etc. are biohazardous waste, and should be treated appropriately and disposed of according to local regulations.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The protein-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, which contain sodium azide, will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:2 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 1 part diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Wash Buffer can be stored at room temperature for up to 3 months.

Sample preparation

Biological Safety Cabinet & Personal Protective Equipment (according to local guidelines for working with potentially infectious material, in particular if it is derived from a human or an animal sample) must be used, we recommend to follow the local guidelines for safety.

This assay has been tested and validated for human serum samples.

Collected blood should be allowed to clot at room temperature for 30 to 60 minutes, and then centrifuged to collect serum. The serum can be removed from the clot and stored at 4°C for a short period of time (up to 4 days) or at ≤ -70°C for long term storage.

Avoid multiple freeze-thaw cycles to protect sample integrity. Samples containing visible particulate should be centrifuged prior to use.

We recommend to heat-treat the serum samples at 56°C for 1 hour to inactivate any viral pathogens that may be present prior to analysis. In this regard, we evaluated the effect of one-hour heat treatment at 56°C on sample values; The OD values of PCR positive and PCR negative samples increased 4.4 % and 23.3 % with heat treatment, respectively.

Samples must be diluted in 1x Assay Buffer prior to performing the assay. An initial dilution of 1:100 is made by adding 1 part of sample to 99 parts of 1x Assay Buffer. A minimum volume of 100 µL diluted sample is required for duplicate determinations. We recommend adding 5 µL of serum sample to 495 µL of 1x Assay Buffer

All the samples must be used within 2 hours of preparation.

Assay Procedure

1. Pipette 50 µL of Positive control into duplicate wells in the plate.
2. Pipette 50 µL of Negative control into duplicate wells in the plate
3. Pipette 50 µL of sample into duplicate wells in the plate.
4. Gently tap the sides of the plate to ensure adequate mixing with the coated antigen in the plate.
5. Cover the plate with the plate sealer and shake for 30 minutes (500 RPM) at room temperature (23–25°C).
6. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer. **The aspirated wash solution is a potential biohazard and should be treated accordingly prior to disposal.**
7. Tap the plate on clean absorbent towels to dry.
8. Add 50 µL of Anti-Human IgG Peroxidase Conjugate to each well, using a repeater pipette.
9. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
10. Cover the plate with the plate sealer and shake for 30 minutes (500 RPM) at room temperature (23–25°C).
11. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer. **The aspirated wash solution is a potential biohazard and should be treated accordingly prior to disposal.**
12. Tap the plate on clean absorbent towels to dry.
13. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
14. Incubate at room temperature (23–25°C) for 30 minutes without shaking.
15. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
16. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for controls and sample.
- Verify that the positive and negative control readings fall within acceptable ranges to assess sample readings against cut-off ranges. If they do not, the assay must be repeated.

Control	*Acceptable OD range*
Positive	1.0 – 1.4
Negative	0.12 – 0.30

Results Interpretation

results	*Cut-Off OD ranges*
Negative	≤ 0.329
Needs to be repeated	0.33 – 0.499
Positive	≥ 0.5 – Max

Sensitivity

The sensitivity of ZellX® SARS-CoV-2 (Covid-19) IgG ELISA kit was determined as 100%.

Specificity

The specificity of ZellX® SARS-CoV-2 (Covid-19) IgG ELISA kit was determined as 96.7%.

Precision

Item	*% CV*
Intra assay	4.6, 4.1
Inter assay	7.9, 20.6

Cross Reactivity

Above 95% specificity of negative samples (91) confirms minimal cross reactivity to other viruses.

Protocol summary