Actim® ELISA SARS-CoV-2 IgG

INSTRUCTIONS FOR USE

Numbers **[1]** – **[11]** refer to illustrations on page 3.

Intended use

Actim® ELISA SARS-CoV-2 IgG is an immunoenzymometric assay for quantitative detection of specific IgG to SARS-CoV-2 in serum from venous blood samples. The test is intended for professional use to help detect recent COVID-19 infection.

Kit components

Single use only. The components of the Actim ELISA SARS-CoV-2 IgG test 35530ETAC are:

Component	Quantity	Contents
Microtiter plate	1 pc (96 wells)	Microplate coated with recombinant S1 domain of SARS-CoV-2 spike protein.
100x HRP-Conjugate	175 µl	HRP-labeled monoclonal anti-human IgG antibody in a stabilized protein matrix.
50x Calibrators (A-F)	50 µl x6	Calibrators in Tris buffer containing BSA and sodium azide as preservative. The exact concentrations are mentioned in the certificate.
50x Control (Neg-Pos)	50 µl x2	Controls in Tris buffer containing BSA and sodium azide as preservative. The acceptance limits are mentioned in the certificate.
Sample Diluent	50 ml	A ready-to-use sample dilution buffer with BSA and sodium azide as preservative.
25x Wash Solution	100 ml	Concentrated surfactant in a tris-buffered saline with diazolidinyl urea as preservative.
Substrate	20 ml	Tetramethylbenzidine (TMB) in buffer with hydrogen peroxide.
Conjugate Diluent	15 ml	A ready-to-use conjugate dilution buffer with CMIT/MIT as preservative.
Stop Solution	15 ml	0.5 M sulfuric acid
Instruction for use	paper	
Certificate	paper	

Materials and equipment required but not provided in the kit

- Distilled/deionized water
- Precision single channel pipettes capable of delivering 10 µL, 40 µL, 100 µL, and 500 µL
- Precision multi-channel pipettes or dispenser capable of delivering 100 µl
- Microplate reader capable of measuring absorbance at 450 nm
- Plate washer – automated or manual
- Plate shaker, suitable is e.g. a vibration shaker at 1200 rpm
- Glass or plastic tubes for preparation of required dilutions
- A lid/cover or similar to shield the plate from light during incubation with substrate
- Timer

Storage

Store unopened kit at +2…+8 °C, and do not use beyond the expiration date. Unopened kit components are stable until the expiry date. The expiry date of the kit is printed on the outer packaging. Each test component on also have the expire date printed on the labels.

Clinical significance

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a new coronavirus that emerged in December 2019. After spreading rapidly throughout the world in the following months a SARS-CoV-2 pandemic was declared in March 2020. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO. The disease is mainly spread by droplet infection or though close contact with infected patients. The incubation time of SARS-CoV-2 is normally three to seven days but can be as long as 14 days.

The determination of antibodies in serum/plasma samples is used to show if immune system has been in contact with the virus. It is generally accepted that within 19 days after symptom onset, essentially 100% of patients are tested positive for antiviral immunoglobulin. SARS-Cov-2 IgG tests can e.g. be used for serological surveys and/or to monitor and control local outbreaks. The test is useful for determining the real infection rate and infection fatality rate as the presence of the specific antiviral antibodies allow to identity individuals who were infected, also including asymptomatic infections.

Principle of the test

The kit contains a 96-well microtiter plate that is coated with recombinant S1 domain of SARS-CoV-2 spike protein. This protein will capture IgG antibodies against SARS-CoV-2 S1 from samples. Diluted controls, calibrators, and samples are added, and anti-SARS-CoV-2 IgG present will bind onto the plate. After incubation and removal of unbound substances, a diluted horseradish peroxidase (HRP) conjugated anti-human IgG antibody is added. This second, enzyme-labelled incubation is needed for catalyzing a color reaction. After this incubation and removal of unbound conjugate by washing, tetramethylbenzidine (TMB) substrate is added. A blue color develops in proportion to the amount of anti-SARS-CoV-2 IgG present in the well. Stop solution is added and the color changes from blue to yellow. The intensity of the color is measured using a microplate reader.

This kit provides an easy and rapid method to detect the presence of SARS-CoV-2 IgG antibodies in human serum samples. The kit contains all necessary reagents for analyzing 80 single samples or 40 duplicates.

Safety precautions and waste disposal

The reagents are for *in vitro* diagnostic use only.

The kit contains H_2SO_4 (sulfuric acid) (refer to Kit components).

Causes sever skin burns and eye damage (H314). May be corrosive to metal (H290).

Avoid contact with skin or eyes. If contact occurs, immediately flush the area with water. For further information a Material Safety Data Sheet is available.

Wear gloves while performing this assay. Patient sample, sample residue, controls, calibrators, and incubated microtiter plates should always be handled as infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid.

Avoid contact with eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes.

All reagents must be handled as infectious waste and disposed of in accordance with local disposal regulations.

Sample collection and storage

For measurement in duplicate 10 µL of human serum is required. All patient samples should be handled in accordance with National Committee for Clinical Laboratory Standards guidelines for preventing transmission of blood-borne infection. Sample should be tested as soon as possible or stored at +4 °C for up to 24 hours. Samples that cannot be tested immediately should be stored frozen, but repeated freeze/thaw cycles must be avoided.

Procedural notes

- Thoroughly review this procedure.
- Observe Good Laboratory Practice (GLP) and safety guidelines.
- **Important! Reagents are lot specific.** Do not mix or interchange reagents from different lots.
- Allow reagents, samples, and plate/materials to reach room temperature before use.
- Mix all reagents gently but thoroughly immediately prior to use. Avoid foaming. **[1]**
- If liquid is trapped under the cap of the vial, tap the vial or centrifuge it briefly.
- It is recommended that all calibrators, controls and samples are run in duplicate. The average absorbance reading of each duplicate should be used for the calculation of results.

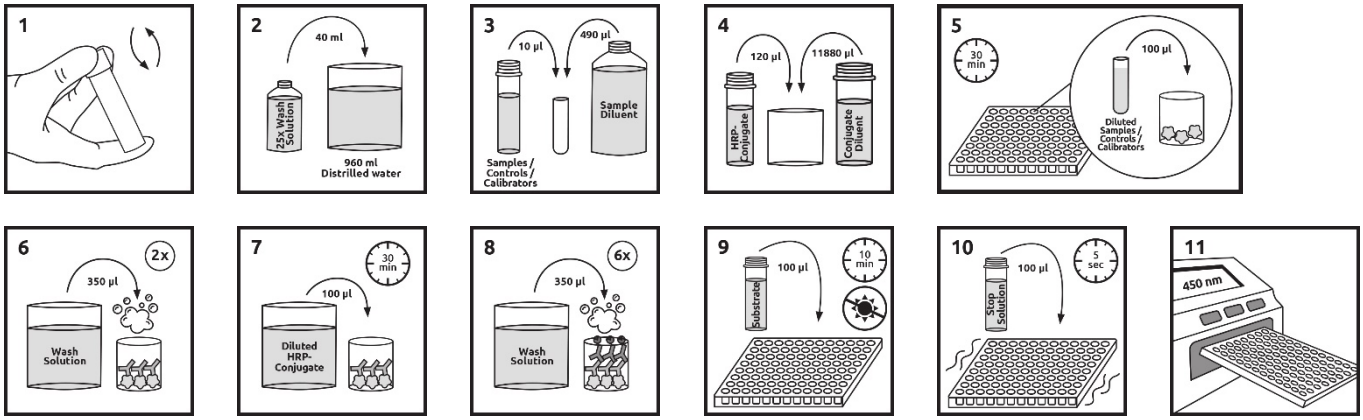
Reagent and sample preparation

1. Dilute 25x Wash Solution 1:25 in distilled/deionized water. **[2]**
2. Dilute calibrators A-F, positive control, negative control, and patient samples 1:50 in Sample Diluent and mix thoroughly. **[3]**
3. Dilute the 100x HRP-conjugate 1:100 in Conjugate Diluent just prior to use. **[4]**

Assay procedure

1. Add 100 µl of diluted Samples/ Controls/ Calibrators to appropriate wells and incubate the plate at room temperature on a shaker for **30 minutes**. **[5]**
2. Wash the plate twice using 350 µl Wash Solution (1x) per well. **[6]**
3. Add 100 µl of diluted HRP-Conjugate to each well and incubate the plate at room temperature on a shaker for **30 minutes** (precisely). **[7]**
4. Wash the plate six times using 350 µl Wash Solution (1x) per well. **[8]**
5. Add 100 µl of Substrate to each well and incubate the plate for **10 minutes** (precisely). No shaking. Cover the plate to protect it from light. A blue color develops in the wells. **[9]**
6. Add 50 µl Stop Solution to each well. Shake the plate for 5 seconds to mix. The solution changes from blue to yellow. **[10]**
7. Read the plate at 450 nm within **10 minutes** after adding the Stop Solution. **[11]**

Illustrations of an example test procedure



Quality control and reference materials

Note that controls and calibrators included in the kit must be used with each run.

Acceptance criteria for controls are determined in the certificate included in the kit. Since there is no Gold Standard concentration available for COVID-19 IgG measurement, the values of the assay calibrators were established by diluting a highly purified human COVID-19 IgG antibody in a matrix.

Calibrator and the Positive and Negative Controls must be within the acceptance criteria specified for the test lot. If these control requirements are not reached, the test results may be inaccurate (invalid) and the test should be repeated. The measuring range is approx. 0.0-1.5 U/ml. If a sample gives absorbance signals outside the calibration curve dilute and re-run the sample, if you need to know the actual concentration. If only a qualitative positive or negative result is needed the samples do not need to be diluted and re-run, if the control requirements of the test otherwise are fulfilled.

The positive control and negative control are intended to monitor for substantial reagent failure but will not ensure precision at the assay cut-off. It is recommended that all assays include the laboratory's own controls in addition to those provided with this kit. Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's Quality Control policy.

Standard curve calculation and test interpretation

Calculate the average absorbance for each pair of duplicate test results.

A standard curve can be drawn from the optical densities of the calibrators by using 4-PL normalization curve fitting. The lot specific calibrator values are defined in the certificate. Appropriate computer assisted data reduction programs should be used for the calculation of results. From the fitted standard curve, the titer in the samples can be determined and results are presented in U/ml.

Clinical decision point is approximately 0.2 U/ml.

A POSITIVE test result in combination with a proven recovery from disease suggests that a previous infection with SARS-CoV-2 is likely. A NEGATIVE result indicates that infection is unlikely. Note that results close to the clinical decision point require further confirmation.

Limitation of the test

- The test is intended for professional use only.
- It is advised to consider the results from test in combination with each individual´s clinical status, results of other diagnostic tests and the background epidemiological information.
- A negative serological test result does not exclude the presence of the recent infection.
- Samples collected at the beginning of infection may not have detectable levels of antibodies.
- Patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead abnormally low IgG levels leading to false negative result results of human COVID-19 IgG.
- The antibody determination does not replace the direct detection by PCR. It is important to note that a positive IgG result against SARS-CoV-2 indicates that an infection has taken place, but this does not necessarily mean that immunity (i.e. protection against infection) is assured.
- Previous infection of SARS or other coronavirus strains may cause IgG positive result due to the similarities of the different strains. Correct performance of sample collection, transport, storage and processing procedures is crucial for the test result.
- The assay is validated manually but can be adapted to an automated instrument.

Notes

- Do not use components if they have been damaged during transportation.
- Use the kit within the expiry period (printed on outer package).
- Do not use reagents from different kit lots or batch codes and do not mix reagents of different kit lots or batch codes.
- Protect the plates from draughts, strong light or direct sunlight during the test procedure.
- Avoid contamination of the reagents. Do not use the same container for several samples! Avoid contamination of reagents by changing pipette tips.
- Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
- Careful aspiration of the wash concentrate is essential for good assay precision.

- Incubation times or temperatures other than those stated in this insert may affect the results.
- Adding of the HRP-Conjugate starts a kinetic reaction that is terminated by washing. Keep the incubation times for each well the same by adding reagents at timed intervals.
- Protected from light, absorbance values are stable for 10 minutes.
- Avoid air bubbles in the microwell as this could result in wrong result.
- Microplate readers measure absorbance vertically. Do not touch the bottoms of the wells.

Performance of the test

Analytical Sensitivity

LoQ and LoD were defined according to the requirements defined in guideline EP17-A2 of the CLSI „Evaluation of Detection Capability for Clinical Laboratory Measurement“ (Clinical and Laboratory Standards Institute, https://clsi.org/). The limit of detection of this test is 0.000 U/ml and the limit of quantification is 0.003 U/ml.

Cross-reactivity (Analytical Specificity)

Due to low homologies of the S1 protein within the coronavirus family, cross reactions to most of the human pathogenic representatives of this virus family can be virtually excluded. However, due to the close relationship of SARS-CoV-1 and SARS-CoV-2, cross-reactions between these two viruses are likely. Cross-reaction to other human viruses was tested. The panel tested is presented in the Table 1. There were no cross-reactions with IgG antibodies produced against other viruses. Two serum samples were just above the cut-off (0.21 U/ml and 0.24 U/ml), but there is no reason to assume that there was an actual cross-reaction to the antibody in any of the samples. The other sample had high levels of anti-Measles virus IgG (1/56 samples), but the other anti-Measles virus IgG positive samples did not give elevated results. The other sample did not differ from the other serum samples, which did not give elevated results. This panel was not tested against the SARS-CoV-1 and its presence cannot be excluded.

TABLE 1. The content of the cross-reactivity panel.

6 x SARS-CoV-2 positive samples (4 donors (2 donors with consecutive samples) with previous positive SARS-CoV-2 PCR test)	
90 x human samples (pre-SARS-CoV-2) measured positive on:	
81 x Adenovirus IgG *66 x Cytomegalie virus (CMV) IgG* *73 x Herpes simplex 1+2 IgG* *86 x Influenzaviruses Type A IgG* *72 x Influenzaviruses Type B IgG* *73 x Mumps virus (Parotitis) IgG* *20 x Mycoplasma pneumoniae IgG* *90 x Parainfluenza 1/2/3 IgG*	*50 x Parvovirus B19 IgG* *89 x Respiratory syncytial virus IgG* *87 x Varicella zoster virus IgG* *87 x Anti-Epstein-Barr-virus-capsid-antigen IgG* *18 x Anti-Enteroviruses IgG* *56 x Anti-Measles Virus IgG* *1 x Anti-Hanta-Virus Pool 1 "Eurasia" IgG* *45 x Haemophilus influenzae B IgG*

Precision

The Intra-assay Precision was evaluated with three (3) serum samples with known spiked levels of positive control. These samples were tested with 16 replicas. The result is summarized below with acceptable results.

Table 2. Intra-assay result summary.

KG1893	OD450	SD	CV%	U/ml	SD	CV%
Sample 1	0.239	0.017	7.1	0.231	0.008	3.6
Sample 2	0.616	0.050	8.1	0.362	0.015	4.2
Sample 3	1.009	0.141	14.0	0.501	0.060	12.0

Inter-assay Reproducibility was evaluated with two (2) different serum samples spiked with known concentration of SARS-CoV-2 spike S1 antibody, and positive and negative control. The samples were tested on sixteen (16) different times. The reproducibility if this kit is acceptable. The result summary is shown below.

Table 3. Inter-assay result summary.

KG1893	OD450	SD	CV%	U/ml	SD	CV%
Spiked serum-1	0.711	0.194	27.2	0.304	0.058	19.2
Spiked serum-2	1.096	0.194	17.7	0.459	0.071	15.5

Interference testing

Interference was determined by spiking (with two different known concentration) serum samples, each made in four (4) replicas. The samples tested are plasma-EDTA, plasma-citrate, hemolyzed serum, lipemic serum, and icteric serum. No interference was found with plasma-EDTA, plasma-citrate, hemolyzed serum and lipemic serum. Icteric serum gave lower than acceptable recovery. When analyzing icteric samples, the result may give a false negative or a too low quantitative result. A qualitative positive result is reliable also with icteric samples.

Clinical Performance

A clinical study was performed to determine the clinical performance of the Actim ELISA SARS-CoV-2 IgG kit. It was investigated by analyzing 670 patient samples, using the Actim ELISA SARS-CoV-2 ELISA IgG test. In these patients, infections with SARS-CoV-2 had been confirmed by RT-PCR tests and days after PCR sampling is known.

Diagnostic sensitivity, specificity, and precision were defined and calculated according to the requirements in guideline of CLSI EP24-A2 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves".

Clinical sensitivity is 96% and specificity is 98% seven (7) days after PCR sampling (Table 4; N=642). The test has high specificity but low clinical sensitivity until Day 7 as expected (Table 5; N=591). Overall results are shown in Table 6 (n=670).

Table 4. Summary of Clinical Performance results.

	≥7 days after PCR sampling	
S1 cut-off 0.2 U/ml	PCR positive	PCR Negative
Positive	76	11
Negative	3	552
Statistic	**Value**	**95& CI**
Sensitivity	96.2%	89.3% to 99.2%
Specificity	98.1%	96.5% to 99.0%
Positive Predictive Value (*)	87.4%	79.4% to 92.6%
Negative Predictive Value (*)	99.5%	98.4% to 99.8%
Accuracy (*)	97.8%	96.4% to 98.8%
(*) These values are dependent on disease prevalence.		

Table 5. Summary of Clinical Performance results.

	< 7 days after PCR sampling	
S1 cut-off 0.2 U/ml	PCR positive	PCR Negative
Positive	16	11
Negative	12	552
Statistic	**Value**	**95& CI**
Sensitivity	57.1%	37.2% to 75.5%
Specificity	98.1%	96.5% to 99.0%
Positive Predictive Value (*)	59.3%	42.7% to 73.9%
Negative Predictive Value (*)	97.9%	96.8% to 98.6%
Accuracy (*)	96.1%	94.2% to 97.5%
(*) These values are dependent on disease prevalence.		

Table 6. Summary of Clinical Performance results.

S1 cut-off 0.2 U/ml	PCR positive	PCR Negative
Positive	92	11
Negative	15	552
Statistic	**Value**	**95& CI**
Sensitivity	86.0%	77.9% to 91.9%
Specificity	98.1%	96.5% to 99.0%
Positive Predictive Value (*)	89.3%	82.3% to 93.8%
Negative Predictive Value (*)	97.4%	95.8% to 98.3%
Accuracy (*)	96.1%	94.4% to 97.5%
(*) These values are dependent on disease prevalence.		

References

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Explanation of symbols

	Use By		Temperature limitation
	Manufacturer		Batch code
	Catalogue number		Single use
	Consult Instructions for use		Contains sufficient for “n” tests
	IVD		CE-marking
	Corrosive substance		


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