INSTRUCTION MANUAL

REF **3940**

September 28, 2020

GA CoV-2 IgG +

- 24 x 4 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to immunodominant antigens of SARS-Coronavirus 2 in human serum and plasma

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Module based Enzyme Immunoassay for the confirmation of positive IgG antibodies against SARS coronavirus 2 (SARS-CoV-2) in the first screening. The test determines the specificity of antibodies against the main immunodominant antigens (Spike Glycoprotein 1, Spike Glycoprotein 2, Nucleocapsid) of SARS-CoV-2 in human serum or plasma.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously known as "2019-nCoV) is a zoonotic single-stranded RNA viruses with positive polarity, belonging to the coronaviruses family. It is classified in the genus beta-coronavirus, which also includes SARS-CoV (2003) and MERS-CoV (2012). Among the coronavirus structural proteins envelope, membrane, spike and the nucleocapsid, the last two are the main immunogens.

An infection with the SARS-CoV-2 can lead to a respiratory syndrome called Corona Virus Disease 2019 (COVID-19). It was emerging in human living since the end of 2019 in the province of Hubei, China, and rapidly spreading with a pandemic trend all over the word.

Patients infected with SARS-CoV-2 may remain asymptomatic or develop only mild upper airways symptoms, similar to those of a cold or flu. Others develop pneumonia and ARDS requiring intubation in ICU, and may undergo complications that can be fatal. It can take up to 14 days after exposure to SARS-CoV-2 before they appear. Currently suitable methods for SARS-CoV-2 diagnosis, examine the genetic material of the virus in oral swabs using the polymerase chain reaction (PCR). But PCR only shows a positive result if the virus is still present. The tests cannot identify individuals who have gone through an infection, recovered and have the virus from their bodies removed.

Enzyme immunoassays, on the other hand, are serological tests that allow the determination of specific antibodies to SARS-CoV-2. For a significant serological result, 2 samples from one patient should be tested, one sample at the onset of symptoms and a second sample collected about 4 weeks later.

1. Zhu N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020. doi:10.1056/NEJMoa2001017 (2020).
2. Lu, R. *et al.* Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565–574 (2020).
3. Corman V.M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045 (2020).
4. Meyer *et al.* Serological assays for emerging coronaviruses: challenges and pitfalls. Virus Res. https://doi.org/10.1016/j.virusres.2014.03.018. (2014).
5. Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506 (2020).
6. WHO pandemic statement. http://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-apandemic. Visited on April 8th, 2020.
7. Liu, Y. *et al.* J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J. Travel Med. https://doi.org/ 10.1093/jtm/taaa021 (2020).
8. Zhang J. *et al.* Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. The Lancet, Infectious Diseases DOI:https://doi.org/10.1016/S1473-3099(20)30230-9 (2020)

PRINCIPLE OF THE TEST

GA CoV-2 IgG + is a reagent kit for the determination of IgG antibodies against immunodominant major antigens of the SARS Coronavirus-2. The test kit consists of modules separately coated with the major antigens of the virus:

		Microtiter strips			
		1	2	3	4
Samples	A	BSA	Spike Glycoprotein 1	Spike Glycoprotein 2	Nucleocapsid
	B				
	C				
	D				
	E				
	F				
	G				
	H				
Antigen Code		**N**	**S1**	**S2**	**C**

The patient samples are pipetted horizontally over the 4 strips of a module (e.g. A1+A2+A3+A4), any antibodies present react with the specific antigens bound to the solid phase in the first reaction step. Unbound sample components are removed by a wash step after 45 minutes incubation at 37 °C.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 45 min at 37 °C, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the added colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature (18-25 °C) turning the solution from blue to yellow. The optical density (OD) of the solution measured at 450 nm is directly proportional to the amount of specific antibodies bound.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma samples (citrate, EDTA, heparin) can be used as well. Hyperlipemic, hemolytic or contaminated samples must not be used and the samples must not contain preservatives.

Samples can be stored up to 5 days at 2 - 8 °C in the primary tubes. For longer storage, sera or plasmas extracted from the primary tubes must be frozen at -20 °C. Repeated freezing and thawing should be avoided. If necessary, aliquots should be prepared before freezing.

Patient samples have to be diluted before their use in the assays **1 + 20** (v/v), i.e. **50 µl sample + 1000 µl sample diluent (C)**. Controls must not be diluted.
The Sample diluent contains a strong virus-inactivating substance, diluted samples may be stored at +2-8°C for only 48 hrs.

TEST COMPONENTS for 24 x 4 DETERMINATIONS

A Ag 96	**Microtiter plate**, 3 modules with 4 strips per 8 wells coated with BSA (Negative control), Spike Glycoprotein 1, Spike Glycoprotein 2 and Nucleocapsid antigens of SARS-CoV 2 (recombinant)	1 vacuum sealed with desiccant, 2 adhesive foils
B BUF WASH	**Concentrated wash buffer** sufficient for 1200 ml solution 20x	60 ml concentrate capped white
C DIL	**Sample diluent**	50 ml ready for use capped black
G START	**Start reagent**	8 ml ready for use capped white
D CONJ	**Conjugate** containing anti-human-IgG coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.3 M sulfuric acid 0.3 M	15 ml ready for use capped yellow
P CONTROL	**Positive control** (diluted serum) +	2.0 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) –	2.0 ml ready for use capped green

Materials required in addition

- calibrated micropipettes (200, 50, 10 µl) with tips
- incubator (37 °C +/- 0.5 °C)
- timer
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620-630 nm (blank)
- absorbent paper
- distilled water
- vortex or similar mixer

Size and storage

GA CoV-2 IgG + has been designed for 24 x 4 determinations.

The expiry date of the test when stored at 2 – 8 °C is indicated on the outer label. Do not use the test kit after the expiration date.

After opening the test kit, it can be used for 6 months.

Preparation before use

The microtiter plate with divisible strips is sealed in an aluminum-coated pouch together with desiccant. Open the package only after reaching room temperature (approx. 1 h). Do not use the plate if the desiccant bag has turned from yellow to dark green. Protect unused wells from moisture and place them back in the bag together with the desiccant and close it.

The 20-fold concentrated Wash Buffer Solution must be dissolved with distilled water and mixed carefully before use. Carefully dissolve any salt crystals that may be present by warming and shaking before diluting. If necessary, dilute the entire amount of the concentrate. Avoid foaming during preparation, as the presence of bubbles could lead to poor washing efficiency.
After dilution the washing solution is stable at +2-8 ° C for about 1 week.

All other reagents are ready for use. Please mix well before use (Vortex).

Store substrate protected from light and oxidizing substances.

ASSAY PROCEDURE

- **Patient sample dilution 1 + 20 (v/v)**
  e.g. 50 µl serum + 1000 µl sample diluent (C)
  after the addition of the sample the color of the sample diluent (C) changes from olive green to dark blue

The samples are applied horizontally into 4 cavities of a module according to the pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1				Sample 9				Sample 17			
B	Sample 2				Sample 10				Sample 18			
C	Sample 3				Sample 11				Sample 19			
D	Sample 4				Sample 12				Sample 20			
E	Sample 5				Sample 13				Sample 21			
F	Sample 6				Sample 14				Sample 22			
G	Sample 7				Sample 15				Sample 23			
H	Sample 8				Sample 16				Sample 24			

In case of use of the kit controls (P, N), those controls can replace two samples in the above scheme.

1. Bring all reagents and the required number of test cavities to room temperature (18-25 °C) before use. Mix gently without causing foam.
2. Add **50 µl of Start reagent (G)** to all wells.
3. Dispense into 4 horizontal wells of one module:
   200 µl diluted samples.
   alternatively 200 µl of Negative control (N) and Positive control (P) instead of 2 samples
4. Cover plate, shake for 30 seconds, incubate **45 minutes** at 37 °C.

5. Decant, then wash each well **5** times using **350 µl** wash solution (made of B), use a soak time of 20 seconds each.
6. Add **100 µl** of conjugate (D) solution to all wells.
7. Cover plate, incubate **45 minutes** at 37 °C.
8. Repeat wash step 5.
9. Add **100 µl** of substrate (E) to each well.
10. Incubate **15 min protected from light** at room temperature (18…25 °C).
11. Add **100 µl** of stop solution (F) to each well and mix gently.
12. Read the OD at **450 nm** versus 620 (630 nm) within 20 min after adding the stop solution.

If the washing process cannot be carried out with a soak time of the washing buffer, the washing process must be extended by one step.

DATA PROCESSING

The test result of each sample is evaluated by calculating a cut-off from the individual sample value on the Negative control row (well N) of the respective module:

$$\textbf{Cut-off (Co)} = 0.250 + OD_{\text{well N}}$$

The binding index BI is calculated by the ratio of OD values of samples to the Cut-off:

$$\textbf{BI} = OD_{\text{sample}} / \textbf{Co}$$

Interpretation is done according to the following table:

BI	SARS-CoV-2 IgG
< 1.0	negative
1.0 – 1.2	borderline
> 1.2	positive

Patients with borderline results should be retested after a period of 1-2 weeks using a freshly collected sample.

Example of Typical Assay Results

sample	OD	BI
Patient 1 Cut-off	0,289	
Patient 1 S1	1,604	5,6 - positive
Patient 1 S2	0,320	1,1 - borderline
Patient 1 C	0,170	0,6 - negative

A confidence index of the confirmation test defines the degree of reliability of the given result and is listed in the table below:

Confidence index	
Very high	antibodies against 3 antigens
High	antibodies to Nucleocapsid and Spike 1
Medium	antibodies to Nucleocapsid only

In case of medium index (only antibodies to Nucleocapsid) it is recommended to follow-up the person by testing a second sample collected after 7-10 days from the first one.

Test validity

The test run is valid if:

- OD 450/620 nm of Negative control < 0.200
- OD 450/620 nm of Positive control > 0.500 at least on the nucleocapsid antigen (strip C)
- OD 450/620 nm of row N < 0.250

If the above mentioned quality criteria are not met, repeat the test and make sure that the procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

PERFORMANCE CHARACTERISTICS

Precision

Using a negative, a low positive and a positive sample, the precision profiles were created in 16 repetitions in three separate runs. Results showed values of CV in the range 4-20% depending on their OD at 450nm in intra- and inter-assay variance. The variability did not lead to an incorrect sample classification.

Sensitivity

Analysis conducted on 80 samples, previously tested positive for IgG in the corresponding ELISA GA CoV-2 IgG, a correlated sensitivity of almost 100% was observed. No false negative result was in fact observed in the confirmation.

When samples, collected during the course of the infection at sequential dates from outset of first symptoms and PCR positivity, were tested with the GA CoV-2 IgG+ Assay, IgG to Spike antigens came out quite later than antibodies to Nucleocapsid and kept on increasing in their BI values together with IgG to Nucleocapsid.

Specificity

The assay specificity, evaluated examining more than one hundred samples collected before and after the outbreak of COVID-19, first tested negative for IgG on the GA CoV-2 IgG ELISA, reached an overall value of almost 100%. No false positive reactions were observed.

As found in the corresponding GA CoV-2 IgG, past infections of SARS-CoV-1 (and MERS) may give a positive result due to the high level of genetic homology between the two viruses. Other Coronavirus strain may give a low response in view of similarity among different strains.

Potential crossreactions with other respiratory infective agents were studied for the corresponding IgG ELISA on samples positive for antibodies to: PIV 1-3, Influenza A and B, H.influenzae, hCoV 229E, hCoV OC43, hCoV HKU1, hCoV NL63, Rhinovirus, RSV, Adenovirus, M.pneumoniae and C.pneumoniae. No crossreactions were observed. Antibodies, commonly present in human sera and plasma, to unrelated infective agents were also studied for the corresponding IgG and IgM ELISA. Antibodies positive to CMV, EBV, HSV1&2, Toxoplasma, Rubella, H.pylori, Malaria sps, Coxsackie virus, Parvovirus B19 and HCV, HIV, Syphilis and HBsAg did not crossreacted.

No interferences were observed in pregnant women, abnormal levels of liver enzymes and other common organ-specific pathologies.

Potentially interfering samples in EIA were studied and found to be non-reactive: Haemoglobin up to 500 mg/dl, Bilirubin up to 20 mg/dl, Triglyceride (milky samples) up to 3000 mg/dl, Serum proteins up to 15g/dl, Rheumatoid factor up to 2500 U/ml (Cobas).

Limitations of Method

Interferences were seen, as for the reference ELISA, when fibrin aggregates, visible particles and lipid layers were present in the sample, giving usually a false positive result. These samples have to be cleared by filtration on a 0.22 µm filter (lipid layer) or centrifuged for 30 min at 4000 rpm (aggregates) before testing, or discarded as not suitable for testing.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

INCUBATION SCHEME

GA CoV-2 IgG + (3940)

Dilute the patient sample (1+20)	e.g. 50 µl serum + 1000 µl sample diluent (C)

1.	Bring all test reagents to room temperature (18…25 °C).		
2.	Dispense	Start reagent (G)	50 µl
3.	Pipette	Controls (P/N) if required	200 µl
		Diluted patient samples	200 µl
4.	Incubate		Shake for 30 sec, 45 min at 37 °C
5.	Wash		5 x 350 µl (soak time 20 sec)
6.	Dispense	Conjugate (D)	100 µl
7.	Incubate		45 min at 37 °C
8.	Wash		5 x 350 µl (soak time 20 sec)
9.	Dispense	Substrate (E)	100 µl
10.	Incubate		15 min at room temperature (18-25 °C) in the dark
11.	Dispense	Stop solution (F)	100 µl
12.	Read		at 450 nm against 620 (630) nm

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. The exchange of individual components is only permitted within the framework of a compatibility list of the manufacturer.
- The kit should be performed by trained technical staff only.
- The test kit or its opened reagents should only be used within the specified shelf life.
- All reagents should be kept at 2 - 8 °C in the original package until use.
- Do not use or mix reagents from different lots. Do not use reagents from other manufacturers.
- Some of the reagents contain small amounts of ProClin 300 (< 1.0 % v/v) und Natriumazid (< 0.1%) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.