INSTRUCTION MANUAL

REF **3930**

June 08, 2020

GA CoV-2 IgM

- 96 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgM antibodies to 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

Enzyme Immunoassay for the determination of IgM antibodies to SARS-Coronavirus 2 (SARS-CoV-2) in human serum and plasma for the monitoring of immune response in COVID-19 disease.

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 IgM is used for the determination of IgM antibodies to SARS-Coronavirus-2.

Antibodies of the controls and diluted patient samples react with antigens immobilized on the solid phase of microtiter plates. Use of recombinant antigens guarantees the specific binding of antibodies of the specimen under investigation. Following an incubation period of 45 min at 37°C, unbound sample components are removed by a wash step.

The bound IgM antibodies react specifically with anti-human-IgM 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 + 40** (v/v), i.e. **10 µl sample + 400 µl sample diluent (C)**. Controls should not be diluted.

TEST COMPONENTS for 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells coated with spike und 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**	100 ml ready for use capped black
G START	**Start reagent** for blocking interference of rheumatoid factors	8 ml ready for use capped white
D CONJ	**Conjugate** containing anti-human-IgM coupled with HRP	15 ml ready for use capped green
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 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) –	2 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 IgM has been designed for 96 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 (6 test preparations in total).

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

- Ensure that no fingerprints are present on the bottom of the microtiter plate before OD measurement. Fingerprints could lead to false positive results.
- **Patient sample dilution 1 + 40 (v/v)**
  i.e 10 µl serum + 400 µl sample diluent (C)
  after the addiction of the sample the color of the sample diluent (C) changes from olive green to dark blue

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. Leave the first well empty for BLANK.
   Dispense 50 µl of start reagent (G) in each well except the BLANK
3. Dispense into the respective wells:
   200 µl negative control (N) in triplicate
   200 µl positive control (P)
   200 µl diluted samples into all patient wells.
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 except BLANK.
7. Cover plate, incubate **45 min** at 37 °C.
8. Repeat wash step 5.
9. Add **100 µl** of substrate (E) to each well including BLANK.
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.

Pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BK	S4										
B	N	S5										
C	N	S6										
D	N	S7										
E	P											
F	S1											
G	S2											
H	S3											

DATA PROCESSING

The test results are evaluated by calculating a cut-off from the mean value of the Negative Control (N).

Cut-off (Co) = 0.250 + OD $_{\text{Negative control}}$

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

BI = OD $_{\text{sample}}$ / Co

Interpretation is done according to the following table:

BI	SARS-CoV-2 IgM
< 0.9	negative
0.9 – 1.1	borderline
1.1 – 2.0	weak positive
> 2.0	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
Positive control	1.558	
Negative control (mean)	0,039	
Cut-off	0,289	
Patient **1**	1,604	**5,5 - positive**
Patient **2**	0,561	**1,9 - weak positive**
Patient **3**	0,170	**0,6 - negative**

Test validity

The test run is valid if:

- OD 450/620 nm of BLANK < 0.100
- OD 450/620 nm of Negative control (mean) < 0.150 after BLANK subtraction
- OD 450/620 nm of Positive control > 0.500.

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 and a weakly positive sample, the precision profiles were created in 16 repetitions in three separate runs. The variability was found in the range of 5-20% and did not lead to an incorrect sample classification.

Sensitivity

A multicenter study using samples from a cohort of infected patients after the onset of symptoms and in follow-up showed a sensitivity of > 98%. A good correlation of IgM positivity in PCR positive patients tested at the onset of symptoms was observed.

Specificity

The test was performed on about 1000 samples collected before and after the COVID-19 outbreak. A specificity of > 98% was found.

Interference

No cross-reactions were observed by other common infective agents: Influenza virus, Herpes Viruses (CMV, EBV and HSV), Toxoplasma and Rubella, H.pylori, E.coli. No false positive results were observed with antibodies to HCV, HIV, HBsAg, Syphilis, Plasmodium species, and others.

Common autoantibodies (thyroid, liver, ANA and ENA mostly) did not generate false positive results.

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)

Pregnant women, patients with abnormal levels of liver enzymes and other common organ-specific pathologies were tested with expected results.

Interferences were found when fibrin aggregates, visible particles and lipid layers were present in the sample, giving usually a false positive result.

Limitations of Method

Very high titers of rheumatoid factor might lead to false positive results.

In some cases IgM were revealed later than IgG, as reported by the specific references for COVID-19 seroconversions.

In internal studies, all the samples that were detected positive for IgM were confirmed for positive reaction to the Nucleocapsid antigen. In some cases IgM were also positive to Spike antigens, but no single Spike reactivity was found, confirming that seroconversion to antibodies starts up first to Nucleocapsid Core.

In later stages of disease and after full recovery of patients, IgM antibodies show the tendency to remain positive even for several weeks, but with tendency to decrease in their BI ratio. IgG at contrary have the tendency to increase in such a timeline.

Some cases are reported of PCR negative patients with clear clinical symptoms of infection and COVID-19 specific seroconversion.

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 IgM (3930)

Dilute the patient sample (1+40)	i.e. 10 µl serum + 400 µl sample diluent (C)

			Controls (P, N)	Patient samples	Blank
1.	Bring all test reagents to room temperature (18…25 °C).				
2.	Dispense	Start reagent (G)	50 µl	50 µl	
3.	Dispense	Controls (P/N)	200 µl		
		Diluted 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	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	100 µl	100 µl
10.	Incubate		15 min at room temperature (18-25 °C) in the dark		
11.	Dispense	Stop solution (F)	100 µl	100 µl	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) and sodium azide (< 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.