Anti-SARS-CoV-2 IgA ELISA

Enzyme Immunoassay for Qualitative Detection of IgA Antibodies against

SARS-CoV-2-S1 (RBD)

English

For Research Use Only.
Not for use in diagnostic procedures.

+2°C +8°C

96 wells

REF **E113**

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1. INTENDED USE

Mediagnost Anti-SARS-CoV-2 IgA ELISA E113 is a highly specific enzyme immunoassay for the detection of IgA antibodies directed against SARS-CoV-2-S1 Receptor Binding Domain (RBD) in human blood.
For research use only. For Professional use only.

2. INTRODUCTION

In December 2019 a novel coronavirus SARS-CoV-2 was identified in Wuhan, China and was announced as the causative agent for COVID-19 disease. The incubation period of the disease is 2-14 days. Symptoms of COVID-19 include i.a. fever, fatigue and cough, shortness of breath, muscle pain and tiredness. Most patients have a good prognosis; some severe cases may develop pneumonia, have severe acute shortness of breath, or even succumb to the disease.
The entry process of SARS-CoV-2 to the host cell is mediated by the envelope-embedded surface-located spike glycoprotein S. The protein is cleaved by host proteases into the S1 and S2 subunits, which are responsible for receptor recognition and membrane fusion, respectively[1].
As target antigen of the assay the recombinant Receptor Binding Domain (RBD) of SARS-CoV-2 S1 spike protein, which binds the ACE2 receptor, is used. The use of RBD increases the specificity of the assay since the domain is identical with SARS-CoV but not with MERS-CoV for example. Antibodies directed against the RBD neutralize both virus strains SARS-CoV and SARS-CoV-2[2].

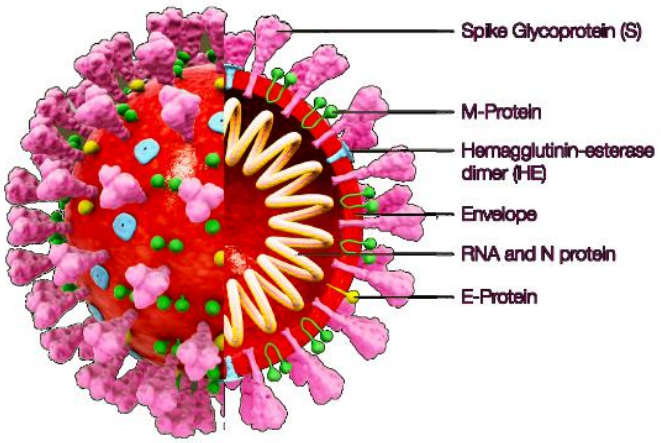


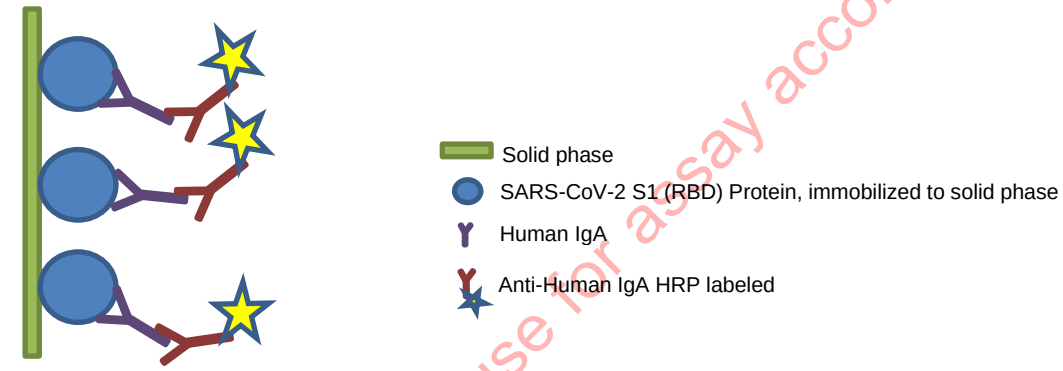
Fig.1: Structural features of Coronavirus[3]

Up to now there are seven coronaviruses described which can infect humans. Two of them are alpha coronaviruses (229E, NL63) and five are beta coronaviruses (OC43, HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2)[4].

[1]Wang et al., Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2, Cell (2020), https://doi.org/10.1016/j.cell.2020.03.045
2Tai et al. https://doi.org/10.1038/s41423-020-0400-4
[3]Jean-Yves Sgro (2020): https://static-bcrf.biochem.wisc.edu/tutorials/booklets/SARS-COV-2_COVID-19_A_Coloring_Book-v1.0.pdf
[4]CDC: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases
[3]Jean-Yves Sgro (2020): https://static-bcrf.biochem.wisc.edu/tutorials/booklets/SARS-COV-2_COVID-19_A_Coloring_Book-v1.0.pdf
[4]CDC: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases

3. ASSAY PRINCIPLE

Mediagnost Anti-SARS-CoV-2 IgA ELISA E113 is a two-step enzyme-linked immunosorbent assay. Wells of a 96-well microtiter plate are coated with recombinant SARS-CoV-2-S1 Receptor Binding Domain (RBD). After addition of human serum or plasma samples anti-S1 IgA antibodies from the sample bind to the immobilized antigen during a two hours incubation followed by several washing steps in order to remove unbound components. The bound anti-SARS-CoV-2 IgA antibodies are detected by incubation with horseradish peroxidase (HRP)-conjugated anti-human IgA for 30 minutes. Subsequently, a HRP substrate solution containing 3,3',5,5'-Tetramethylbenzidine (TMB) is added resulting in the formation of a blue colour. The reaction is terminated by the addition of 0.2 M H_2SO_4 changing the blue colour into yellow signals which are measured by an absorbance microtiter plate reader at 450 nm. The extinction increases with the amount of the captured antibodies directed against SARS-CoV-2-S1 (RBD) from the patient's sera.



4. KIT COMPONENTS

MTP	Microtiter Plate, coated with SARS-CoV-2 S1(RBD) protein, wells are separately breakable, ready for use	8 x 12 wells
DET	Antibody-HRP-Conjugate, goat anti-human IgA-antibody, ready for use	14 mL
PC	Positive Control: anti-SARS-CoV-2 positive control serum, human serum, ready for use	1 mL
NC	Negative Control: anti-SARS-CoV-2 negative control serum, human serum, ready for use	1 mL
DIL	Dilution Buffer, ready for use	100 mL
WB	Washing Buffer, 20-fold concentrated solution	50 mL
S	Substrate Solution (TMB), HRP substrate, ready for use	14 mL
STP	Stop Solution, 0.2 M H_2SO_4, ready for use	14 mL
	Sealing Tape for covering microtiter plate	2
	Instructions for use	1

5. MATERIALS REQUIRED BUT NOT PROVIDED

Aqua dest. or deionized water for dilution of Washing Buffer WB
Precision pipettes with disposable plastic tips
Microtubes, buffer and reagent reservoirs
Incubator 37°C
Vortex-Mixer
Microtiter plate washer, alternatively manual washing
Microtiter plate reader capable of reading absorbency of 450 nm (reference filter ≥ 590 nm)

6. SAMPLES

Serum, EDTA- and Heparin Plasma.
Multiple freeze-thaw cycles should be avoided.
The use of hemolytic, lipemic or icteric samples should be validated by the user.

7. STORAGE and STABILITY

Upon receipt store the kit at 2-8°C.
The shelf life of the components **after initial opening** is warranted for **4 weeks**, store the unused strips and microtiter wells **airtight** together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The 1:20 diluted Washing Buffer **WB** is stable at 2-8°C for 4 weeks.

8. PREPARATION of REAGENTS

Before use bring all reagents to room temperature 20-25°C.

8.1. Washing Buffer WB

Dilute WB 1:20 in Aqua dest., i.e. 50 mL WB + 950 mL Aqua dest.

8.2. Samples

Dilute samples 1:201 in Dilution Buffer DIL, e.g. 5 µL Sample + 1 mL DIL.

9. ASSAY PROCEDURE

Step 1	Addition of controls and samples
	Add 100 µL of each Dilution Buffer DIL as blank, Positive Control (PC), Negative Control (NC). Blanks in double determination. Positive and Negative Controls in triple determination. Samples either in single or double determination (double as a general recommendation to indicate certain faults in individual assay runs) as indicated in the following pipetting scheme.

Pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S1										
B	Blank	S1										
C	PC	S2										
D	PC	S2										
E	PC	S3										
F	NC	S3										
G	NC	S4										
H	NC	S4										

Add 100 µL Dilution Buffer DIL as blank in wells A1 and B1
Add 100 µL Positive Control PC in wells C1, D1, and E1
Add 100 µL Negative Control NC in wells F1, G1 and H1
Add 100 µL of sample 1 (S1) diluted 1:201 in DIL in wells A2 and B2
Add 100 µL of sample 2 (S2) diluted 1:201 in DIL in wells C2 and D2, and so on.

Step 2	Incubation
	Cover the plate with sealing tape and incubate for 2 h at 37°C
Step 3	**Washing**
	Remove the sealing tape from the plate and aspirate the contents of the wells. Wash 3 x with 300 µL Washing Buffer WB per well
Step 4	**Addition of Conjugate and Incubation**
	Add 100 µL Antibody-HRP-Conjugate DET to each well, cover the plate with sealing tape and incubate for 30 min at 37°C
Step 5	**Washing**
	Remove the sealing tape from the plate and aspirate the contents of the wells. Wash 3 x with 300 µL Washing Buffer WB per well
Step 6	**Addition of Substrate and Incubation**
	Add 100 µL of Substrate Solution S to each well and incubate 10 min at 20-25°C in the dark
Step 7	**Addition of Stop Solution**
	Add 100 µL Stop Solution STP to each well
Step 8	**Measurement**
	Measure the absorbance within 30 min at 450 nm (reference filter ≥ 590 nm)
Step 9	**Evaluation of results**
	The test is valid if a P/N ratio of >5 is achieved
Step 10	**Cut-off determination**
	The cut-off is calculated 3 x and 5 x mean values of negative controls.
Step 11	**Interpretation of results**
	Values under 3 x cut-off are negative, Values above 5 x cut-off are positive Values in between both cut-offs are borderline.

10. TYPICAL RESULTS

Sample	OD 450 - ≥590 nm mean	Interpretation of results
Blank	0.008	should not exceed values of the Negative Control
Positive Control	3.494	positive
Negative Control	0.084	negative
Sample 1	1.103	positive
Sample 2	0.186	negative
Sample 3	0.382	borderline

EXAMPLE: CALCULATION P/N RATIO

$$\text{P/N Ratio: } \frac{\text{mean Positive Control } 3.494}{\text{mean Negative Control } 0.084} = 41{,}59$$

EXAMPLE: CALCULATION CUT-OFF

cut-off: 3 x mean negative control = 0.252
cut-off: 5 x mean negative control = 0.420

The values shown in the table above result in the cut-off (3x) of 0.252 and cut-off (5x) value of 0.420.

Samples > 5 x cut-off

All samples of which signals are higher than OD 0.420 are positive, i.e. contain anti-SARS-CoV-2-S1 (RBD) antibodies.

Samples < 3 x cut-off

All samples of which signals are lower than OD 0.252 are negative, i.e. anti-SARS-CoV-2-S1 (RBD) antibodies are not detectable in the sample.

Samples in between 3 x cut-off and 5 x cut-off

All samples which show OD values in between are borderline. Since IgA antibodies to SARS-CoV-2 generally become detectable beginning 10-14 days following infection the borderline samples may indicate the beginning of seroconversion, i.e. the patient is possibly developing antibodies. Therefore, it is strongly recommended to repeat sample drawing and testing around 7 days after the first sample drawing. The IgA antibodies reach rapidly peak levels in contrast to the IgG antibodies which increase more slowly.

Interpretation of results

The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations.
Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.

11. LIMITATION OF PROCEDURE

The influence of the heterophilic antibodies, rheumatoid factors and anti-species antibodies is reduced, but cannot be completely excluded.

12. PERFORMANCE EVALUATION

Diagnostic Specificity and Sensitivity

	Anti-SARS-CoV-2 IgA ELISA E113				
Serum samples of persons with positive PCR results and / or Covid-19 disease	**n**	**pos**	**border-line**	**neg**	**Sensitivity**
0 - 12 d*	27	20	1	6	77,8%
13 - 21 d*	32	31	-	1	96,9%
≥ 22 d*	57	54	3	-	100%
					Specificity
Blood donors	55	-	1	54	98,2%
total	171	105	5	61	-
Accuracy (no. of correct assessments: 164) / (no. of all assessments: 171) = 95,9%					

*days after onset of symptoms or positive PCR results.

To date there are no reference standard SARS-CoV-2 antigen or anti-SARS-CoV-2 antibodies available, accordingly absolute analytical sensitivity cannot be calculated.

Precision

Intra Assay Variance

3 Serum samples were measured 10-fold within one assay.
Mean variance was < 8%.

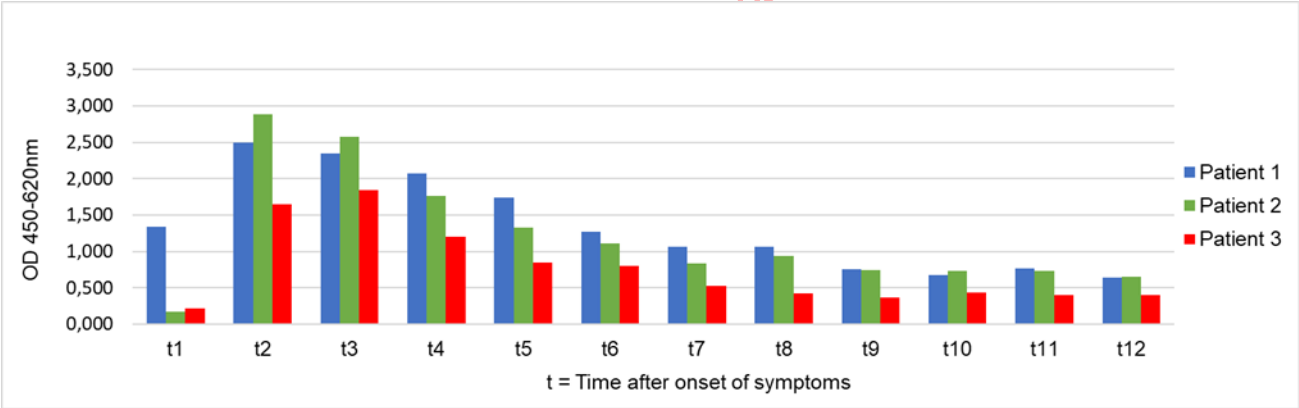
	Number of determinations	Mean value OD	Standard deviation OD	VC (%)
Sample 1	10	0.133	0.010	7.4
Sample 2	10	0.599	0.025	4.1
Sample 3	10	1.756	0.049	2.8

Samples

The influences of anti-coagulants on measurements were investigated in 4 corresponding serum, EDTA and Heparin plasma samples. In comparison to the ODs of the serum samples recovery was measured for Heparin and EDTA plasma samples on average 95.7% and 95.9 %, respectively.

	Serum OD	%	EDTA-Plasma OD	%	Heparin-Plasma OD	%
Sample 1	0.602	100	0.587	97.5	0.588	97.7
Sample 2	2.235	100	2.233	99.9	2.118	94.8
Sample 3	0.157	100	0.144	91.7	0.159	101.3
Sample 4	0.238	100	0.223	93.7	0.214	89.9
mean	-	-	-	**95.7**	-	**95.9**

Fig. 2: Time course of IgA-antibody development against SARS-CoV-2-S1 (RBD) protein of three clinically ill patients[6]



	t = days after onset of symptoms											
Sampling at day	**t1**	**t2**	**t3**	**t4**	**t5**	**t6**	**t7**	**t8**	**t9**	**t10**	**t11**	**t12**
Patient 1	13	20	25	33	40	48	60	74	109	137	165	193
Patient 2	10	17	22	30	37	45	57	71	106	134	162	190
Patient 3	11	18	23	31	38	46	58	72	107	135	163	191

Figure 2 shows the time course of antibody development of three clinically ill COVID-19 patients. The IgA antibodies peak around t2. They decrease between t3 and t4. Therefore, the anti-SARS-CoV-2 IgA ELISA E113 is qualified to detect the entire existing antibody concentration range, even low levels at the onset of an immune response can be detected.

The data have been published:
Flehmig et al.: Longitudinal analysis of virus load, serum antibody levels and virus neutralizing activity in vitro in cases with less severe COVID-19
medRxiv 2020.08.20.20174912; doi: https://doi.org/10.1101/2020.08.20.20174912

13. PROTOCOL

Protocol at a glance

Add 100 µL of controls (2 x blank, 3 x PC, 3 x NC) in A1 to H1 wells

↓

Add 100 µL of samples 1:201 diluted in DIL in wells starting with A2

↓

Incubate at 37°C for 2 hours

↓

Aspirate well contents and wash each well 3 x with 300 µL 1 x WB

↓

Add 100 µL DET to each well

↓

Incubate at 37°C for 30 min

↓

Aspirate well contents and wash each well 3 x with 300 µL 1 x WB

↓

Add 100 µL S to each well

↓

Incubate for 10 min at 20-25°C in the dark

↓

Add 100 µL STP to each well

↓

Measure absorbance at 450 - ≥ 590 nm within 30 min

↓

Calculation and Interpretation

14. WARNINGS AND PRECAUTIONS

For research use only. For in-vitro use only. For Professional use only.

The Mediagnost kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Mediagnost will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.

Do not use obvious damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents and samples. Follow the general practice of serology for sample storage and collection. The disposal of the kit components must be made according to the local regulations.

The test plate MTP is coated with recombinant Antigen.

Human Serum

Following components contain human material: **PC, NC**

Source human serum for the Control Sera provided in this kit was tested by recommended methods and found negative for Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known test methods can offer total assurance of the absence of infectious agents; therefore, all components and patient's specimens should be treated as potentially infectious.

Reagents: NC, PC, DIL, DET, WB

Contain as preservative a mixture of **5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one** (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)

The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Stop Solution (STP)

The Stop Solution contains 0.2 M, Sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+ P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P305+P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing, spread the eyelids.