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ab277286 SARS-CoV-2 (COVID-19) IgA ELISA Kit

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SARS-CoV-2 (COVID-19) IgA ELISA Kit datasheet:
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For the qualitative measurement of SARS-CoV-2 (COVID-19) Human IgA in serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1 Overview

SARS-CoV-2 (COVID-19) IgA ELISA Kit (ab277286) is intended for the qualitative determination of IgA class antibodies against SARS-CoV-2 in human serum or plasma (citrate, heparin) to support the diagnosis of COVID-19 disease and constitutes a supplement to direct pathogen detection. In addition, serology can be used to collect epidemiological information on the prevalence of SARS-CoV-2.

Microtiter plates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450/620 nm is read using an ELISA Microtiter plate reader.

2. Protocol Summary

Prepare all reagents and samples as instructed.

Add 100 µL control or sample to designated wells.
Incubate 1 hour at 37°C. Wash.

Add 100 µL conjugated antibody to designated wells.
Incubate 30 minutes at room temperature. Wash.

Add 100 µL TMB Substrate Solution.
Incubate 15 minutes at room temperature in the dark.

Add 100 µL Stop Solution to each well.
Read at 450/620 nm within 30 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

The entire ELISA kit may be stored at +4°C. The opened reagents are stable up to the expiry date stated on the label when stored at +4°C.

Observe the storage conditions for individual prepared components in the Reagent Preparation Section 9.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
SARS-CoV-2 coated Microplate	1 unit	4°C
IgA Sample Dilution Buffer	100 mL	4°C
Stop Solution	15 mL	4°C
20X Wash Buffer	50 mL	4°C
HRP-human IgA Antibody	20 mL	4°C
TMB Substrate Solution	15 mL	4°C
Positive Control	2 mL	4°C
Cut-off Control	3 mL	4°C
Negative Control	2 mL	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing Microtiter plate wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, positive control and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or positive control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 SARS-CoV-2 coated Microplate:

Twelve break-apart 8-well snap-off strips coated with SARS-CoV-2 antigens; in resealable aluminum foil, store at +4°C.

9.2 IgA Sample Dilution Buffer:

Ready to use as supplied.

9.3 Stop Solution:

Ready to use as supplied.

9.4 20X Wash Buffer:

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20-25°C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

9.5 HRP-human IgA Antibody:

Ready to use as supplied.

9.6 TMB Substrate Solution:

Ready to use as supplied. Store in the dark. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

9.7 Positive Control:

Ready to use as supplied.

9.8 Cut-off Control:

Ready to use as supplied.

9.9 Negative Control:

Ready to use as supplied.

10. Sample Preparation

- Use human serum or plasma (citrate, heparin) samples with this assay.
- If the assay is performed within 5 days after sample collection, the samples should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.

10.1 Human serum:

Before assaying, all samples should be diluted 1+100 with IgA Sample Dilution Buffer.
For example: Dispense 10 µL sample and 1 mL IgA Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents and samples as directed in the previous sections.
- We recommend duplicate samples.
- Establish a clear plate layout.

11.1 Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.

11.2 Cover wells with the foil supplied in the kit.

11.3 Incubate for 1 hour ± 5 mins at 37 ± 1°C.

11.4 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 secs. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Δ Note: Washing is important. Insufficient washing results in poor precision and false results.

11.5 Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.

11.6 Incubate for 30 minutes at room temperature (20-25°C). Do not expose to direct sunlight.

11.7 Repeat step 11.4.

11.8 Dispense 100 µL TMB Substrate Solution into all wells.

11.9 Incubate for exactly 15 minutes at room temperature (20-25°C) in the dark. A blue color occurs due to an enzymatic reaction.

11.10 Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.

11.11 Measure the absorbance at 450/620 nm within 30 minutes after addition of the Stop Solution.

12. Measurement

12.1 Adjust the ELISA Microtiter plate reader to zero using the Substrate Blank.

12.2 If - due to technical reasons - the ELISA Microtiter plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

12.3 Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the plate layout.

12.4 Bichromatic measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.

13. Results

Validation Criteria:

In order for an assay to be considered valid, the following criteria must be met:

Substrate Blank:	Absorbance value	< 0.100
Negative Control:	Absorbance value	< 0.200 and < Cut-off
Cut-off Control:	Absorbance value	0.150 – 1.300
Positive Control:	Absorbance value	> Cut-off

If these criteria are not met, the test is not valid and must be repeated.

Cut-off:

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

Results in Arbitrary Units:

$$\frac{Sample\ (mean) absorbance\ x10}{Cut\ off} = \text{Units}$$

Example: (1.591 x 10)/0.43 = 37 Units

Result Interpretation:

Cut-off	10 Units	---
Positive	>11 Units	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9-11 Units	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	< 9 Units	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.

Δ Note: Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

Δ Note: In immunocompromised patients and newborns serological data only have restricted value.

14. Limitations

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

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