

Instruction for use

EIA COVID-19 RBD IgM

REF CoRM96



Kit for professional use

IVD **CE**

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1 Intended Use

Enzyme immunoassay for the detection of IgM antibodies to RBD, Spike S1 protein subunit, SARS-CoV-2 virus (COVID-19) in human serum or plasma.

2 Introduction

Coronaviruses, which were discovered in the 1960s, belong to the family of enveloped RNA viruses. They fall in the group of zoonotic infections that cause diseases of the respiratory and digestive tracts in humans and animals (birds, mammals). Coronaviruses cause diverse clinical pictures, from common cold to severe respiratory syndromes (MERS, SARS and COVID-19). The majority of known coronaviruses circulate among animals. Alpha- and Beta-coronaviruses can infect only mammals whereas Gamma- and Delta-coronaviruses infect both birds and mammals. Alpha- and Beta-coronaviruses occur in humans. A total of 7 types of human coronaviruses are known so far – 229E, NL63, OC43, HKU1, MERS, SARS, SARS – 2.

Transmission: The infection can be transmitted from an infected person 1–3 days before the onset of the disease. The new coronavirus is a respiratory virus. It is primarily transmitted to an individual through a close contact with an infected person, during which infectious droplets spread to the environment, especially when the infected person talks, coughs and/or sneezes. Things freshly contaminated with secretions of an infected person can also contribute to the transmission. The virus has been successfully isolated from samples taken from the lower respiratory tract (bronchoalveolar lavage). Viral RNA has been detected in nasopharyngeal and throat swabs, serum, blood, rectal swabs, saliva, urine and faeces.

The virus has been found in airway samples 1–2 days before the onset of symptoms and up to 8 days after the onset in case of a mild disease, longer in case of a more severe disease development. Susceptibility seems to be general. Existing experience suggests that the infection is as likely in children as in adults but with milder clinical manifestations. Immunity to COVID-19, if any, has not been established so far. Reported mortality ranges from 2% to 3%. Due to the several-day-long interval between the first symptoms and the onset of the antibody response (the "window period"), serological tests play only a supporting role and, as stressed by the WHO, the results of such tests should always be verified by direct detection of the virus to diagnose an acute COVID-19 disease. Determination of the level of antibodies present after the disease is also an option.

SARS-CoV-2 virus (COVID-19) contains four structural proteins: spike (S), nucleocapsid (N), envelope (E) and membrane (M) protein. The most commonly used antigens in diagnosis include:

Nucleocapsid protein (NP) encapsulates viral genomic RNA and forms a major component of the viral structure. NP is a highly antigenic epitope and is associated with several virus-host interactions.

The receptor-binding domain (RBD), a subunit of the Spike S1 protein, specifically binds to the angiotensin-converting enzyme 2 (ACE2) of the host cell. The binding of RBD to ACE2 is highly associated with the formation of neutralizing antibodies.

3 Test Principle

The kit is intended for detection of specific IgM antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgM conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgM antibodies in the sample.

Antigen used

Recombinant antigen Receptor-binding domain (RBD), a subunit of the Spike S1 protein.

4 Materials Provided

MICROPLATE	Microtitre Plate	1 pc
	coated with antigen, 12 x 8 wells in bag with desiccant	
CONTROL - CAL1	Negative Control (Calibrator 1) 5 U/ml	1 x 2 ml
	Solution containing no specific human antibodies, ready to use	
CUTOFF CAL2	CUT-OFF (Calibrator 2) 20 U/ml	1 x 3 ml
	Solution containing specific human antibodies in cut-off concentration, ready to use	
CONTROL + CAL3	Positive Control (Calibrator 3) 80 U/ml	1 x 2 ml
	Solution containing specific human antibodies, ready to use	
CAL4	Calibrator 4 (320 U/ml)	1 x 2 ml
	Solution containing specific human antibodies, ready to use	
CONJUGATE	Conjugate	1 x 15 ml
	Solution containing peroxidase labelled animal immunoglobulin to human IgM, ready to use	
DILUENT 2	Sample Diluent 2	1 x 105 ml
	Buffer with protein stabilisers, ready to use	
SUBSTRATE 2	TMB-Complete 2	1 x 15 ml
	Chromogenic substrate solution containing TMB/H ₂ O ₂ , ready to use	
WASH 20x	Wash Solution	1 x 75 ml
	20x concentrated buffer	
STOP	Stop Solution	1 x 15 ml
	Acid solution, ready to use	
	Instructions for use	1 pc

5 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

6 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum and citrate plasma. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

7 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls and the Calibrators are supplied ready to use, do not dilute further!

The Conjugate is supplied ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

8 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

9 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

Quantitative evaluation in Units U/ml

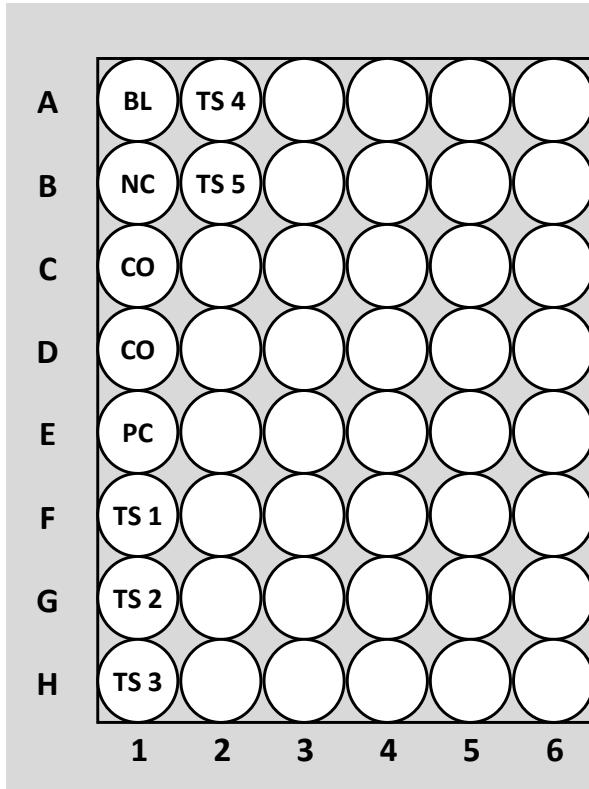
- Leave A1 well empty (blank).
 - Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
 - Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
 - Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
 - Pipette 100 µl of the Calibrator 4 into 2 wells.
 - Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.
2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
 3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
 4. Pipette 100 µl of the Conjugate into all wells except A1 well.
 5. Cover the microplate with the lid and incubate it at 37°C for 30 minutes.

6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

10 Working Schedule

Semiquantitative evaluation

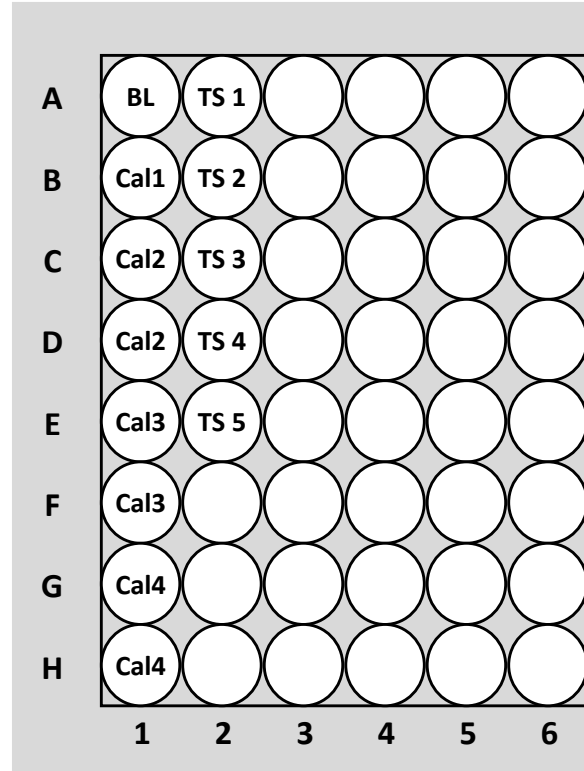
Index of Positivity (IP)



BL	Blank (empty well)			
NC	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>-</td><td>CAL1</td></tr></table>	CONTROL	-	CAL1
CONTROL	-	CAL1		
CO	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CUTOFF</td><td>CAL2</td></tr></table>	CUTOFF	CAL2	
CUTOFF	CAL2			
PC	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>+</td><td>CAL3</td></tr></table>	CONTROL	+	CAL3
CONTROL	+	CAL3		
TS 1-x	100 µl diluted tested sample			

Quantitative evaluation

Units U/ml



BL	Blank (empty well)			
Cal1	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>-</td><td>CAL1</td></tr></table>	CONTROL	-	CAL1
CONTROL	-	CAL1		
Cal2	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CUTOFF</td><td>CAL2</td></tr></table>	CUTOFF	CAL2	
CUTOFF	CAL2			
Cal3	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CONTROL</td><td>+</td><td>CAL3</td></tr></table>	CONTROL	+	CAL3
CONTROL	+	CAL3		
Cal4	100 µl <table border="1" style="display: inline-table; vertical-align: middle;"><tr><td>CAL4</td></tr></table>	CAL4		
CAL4				
TS 1-x	100 µl diluted tested sample			

11 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.150.

$$\text{BLANK} < 0.150$$

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT-OFF (Calibrator 2).

$$\boxed{\text{CONTROL}} \mid \boxed{-} \mid \boxed{\text{CAL1}} < 0.5 \times \boxed{\text{CUTOFF}} \mid \boxed{\text{CAL2}}$$

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.200 – 1.000.

$$0.200 < \boxed{\text{CUTOFF}} \mid \boxed{\text{CAL2}} < 1.000$$

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT-OFF (Calibrator 2).

$$\boxed{\text{CONTROL}} \mid \boxed{+} \mid \boxed{\text{CAL3}} > 1.5 \times \boxed{\text{CUTOFF}} \mid \boxed{\text{CAL2}}$$

The absorbance of the Calibrator 4 is higher than the absorbance of the Positive Control (Calibrator 3).

$$\boxed{\text{CAL4}} > \boxed{\text{CONTROL}} \mid \boxed{+} \mid \boxed{\text{CAL3}}$$

12 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$\text{IP} = \frac{\text{Absorbance of serum, plasma}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in Table 1.

Table 1 Interpretation of test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Quantitative evaluation in Units (U/ml)

Construct a calibration curve by plotting the concentration (X) of the calibrators in U/ml against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (U/ml) in samples from the calibration curve. Interpretation of the quantitative test results is described in Table 2.

Table 2 Quantitative interpretation in Units (U/ml)

Antibody level (U/ml)	Evaluation
lower than 18	negative
18 to 22	borderline
higher than 22	positive

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

13 Test Performance

13.1 Specificity and Sensitivity

The diagnostic specificity was determined in the panel of negative sera, the number of tested sera was 88. The diagnostic sensitivity was determined in the panel of positive sera, the number of tested sera was 44. The specificity and sensitivity are based on the results found.

Specificity: IgM 95.12%

Sensitivity: IgM 97.5%

13.2 Precision and reproducibility

Intra-assay reproducibility was determined by testing samples of different levels of antibody reactivity for at least 16 times in one test. The coefficient of variation (CV) of the reactive IgM samples was **5.758%**

Inter-assay reproducibility was determined by testing samples of different levels of antibody reactivity in 40 different test runs. The CV of the reactive IgM samples was **6.619%**.

14 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

15 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

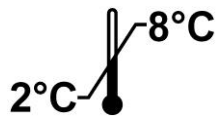
If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

16 References

1. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. *Trends Immunol.* 2020, 41(12), 1100-1115.
2. Fu Y, Pan Y, Li Z, Li Y. The Utility of Specific Antibodies Against SARS-CoV-2 in Laboratory Diagnosis. *Front Microbiol.* 2021, 11(603058), 1-10.
3. Long QX, Liu BZ, Deng HJ et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med.* 2020, 26, 845-848.
4. Liu Z, Xu W, Xia S et al. RBD-Fc-based COVID-19 vaccine candidate induces highly potent SARS-CoV-2 neutralizing antibody response. *Signal Transduct Target Ther.* 2020, 5 (282), 1-10.

17 IFU Symbols



Temperature limitation



Keep dry



Expiry date



Lot number



Manufactured by



Consult instructions



Catalogue number



Number of tests














In vitro diagnostic medical device

Notes

Notes

Notes

Summary of EIA COVID-19 RBD IgM Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml)
2		Pipette Controls and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 min
4		Aspirate and wash the wells 5×
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5×
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 15 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm