INgezim® COVID 19 DR

50.CoV.K0

Dual recognition enzyme-linked immunosorbent assay for detecting SARS-CoV-2 antibodies in human serum and plasma samples.

Marked product

CE

Manufactured by INGENASA

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Table of contents

1. INTRODUCTION

In December 2019, a new coronavirus (CoV) emerged in China that caused an acute respiratory illness named coronavirus 19 (COVID-19) (1). The virus was identified as a betacoronavirus related to the severe acute respiratory syndrome coronavirus (SARS-CoV) and was therefore named SARS-CoV-2 (2). In a period of less than two decades, this is the third coronavirus known to have crossed the species barrier and caused severe respiratory infections in humans, after SARS-CoV in 2003 and Middle East Respiratory Syndrome (MERS) in 2012. However, the spread of this virus is unprecedented compared to the previous two. Due to the rapid increase in the number of cases and the uncontrolled spread around the world, the WHO has declared SARS-CoV-2 a pandemic. As of 14 March, 2020, the virus has infected more than 3,778,012 people, 6.9% of whom have died (3). Validated serological assays are crucial for patient contact tracing, identifying viral reservoir hosts, and epidemiological studies. Epidemiological studies can help to elucidate the disease burden, especially the rate of asymptomatic infections, and provide better morbidity and mortality estimates. In addition, these epidemiological studies can help to reveal the extent of the spread of the virus within particular households, communities and settings; which could help inform control measures. Serological tests are also needed to evaluate the results of vaccine trials and the development of therapeutic antibodies. Among the four structural proteins of the coronavirus, the main immunogens are S and the nucleocapsid (N).

SARS-CoV-2 infection is transmitted predominantly through the droplets produced by coughing or sneezing, and by close contact with infected patients. The incubation period is 5 to 6 days (and up to 14 days at most).

Clinical symptoms include fever, coughing, respiratory problems and fatigue. In most patients, the symptoms are mild with irregular pulmonary infiltrates. The initial symptom of COVID-19 that allowed cases to be detected was pneumonia. It has subsequently been confirmed that the course of the disease varies widely with a spectrum of symptoms ranging from asymptomatic cases to severe pneumonia with pulmonary failure and death. However, it has now been confirmed that about 80% of people who become infected suffer from mild or moderate symptoms.

Although it has been observed that severe symptoms may also occur in younger patients and people with no pre-existing disease, the groups at greatest risk are the elderly (from around 50-60 years of age), smokers and people with certain cardiovascular or pulmonary diseases, patients with chronic liver disease, diabetes mellitus sufferers, cancer patients, and individuals who have a weakened immune system. Currently, there is no specific treatment or vaccine available for SARS-CoV-2 infection.

2. APPLICATION

INgezim® COVID19 DR is designed to semi-quantitatively determine total SARS-CoV-2 virus N-protein-specific antibodies in human serum or plasma (citrate or heparin) samples, to assist in the diagnosis of COVID 19 disease; as a complementary tool to the direct detection of the pathogen. In addition, serology can help to gather epidemiological information on the disease prevalence. This highly sensitive test is particularly recommended for the early detection of SARS-CoV2-specific antibodies as it favours the detection of IgM.

3. PRINCIPLE OF THE ASSAY

The kit has been developed using the SARS-CoV-2 N protein as an antigen (recombinant origin). The technical basis of the kit is a so-called dual recognition enzyme-linked immunosorbent assay (ELISA), the rationale for which is described briefly below.

The SARS-CoV-2 antigen, in this case recombinant protein N, is fixed on a polystyrene plate. If serum samples are added that contain specific antibodies, these will bind to the antigen. By adding the specific conjugate based on the use of N-protein conjugated to peroxidase, if the samples contain antibodies for this protein, many of them will be able to capture the N protein-peroxidase while remaining bound to the protein N fixed in the wells. This bond will be detected after adding a suitable substrate that develops colour in the presence of peroxidase. The assay is capable of detecting any type of antibody specific to the SARS-CoV-2 N protein (IgA, IgG, IgM) without differentiating between them. The geometry of the assay allows the signal to be dramatically amplified in the presence of IgM due to its ability to capture more than one conjugated N-protein molecule, thus promoting the early detection of antibodies.

4. CONTENTS OF THE KIT

Component	1 plate kit (1x8x12 wells)		5 plate kit (5x8x12 wells)	
	Units	Volume.	Units	Volume.
96-well plates divided into 12 antigen strips with recombinant SARS-CoV-2 N protein; in a sealed plastic bag.	1	-	5	-
COVID-19 Positive Control serum vials. Red lid.	1	500 µl	1	500 µl
Bottles of ready-to-use diluent (DE13) containing salts and detergents.	1	60ml	1	60ml
Bottles of Conjugate (concentrated x10).	1	3ml	1	3ml
Bottles of 25x Concentrated Washing Solution containing phosphate buffer and salt (concentrated x25). Blue cap.	1	60 ml	1	125 ml
Bottles containing substrate (TMB) at working dilution. Brown pot.	1	15ml	1	30 ml
Bottles of Stop Solution at working dilution. Red cap.	1	15 ml	1	60 ml

For potentially dangerous agents, check the safety data sheets available on request.

5. CONSERVATION OF THE KIT

All components must be stored in refrigerated conditions (between +2ºC and +8ºC), to ensure that they remain stable until the indicated expiry date.

6. INFORMATION ON HOW TO DO THE WASHINGS

Washing can be performed using an automatic plate washer, a washing bottle or a multi-channel micropipette that allows 300 µl to be dispensed per well. In all cases, washing should involve a certain amount of pressure.

After incubation, perform the washings according to the following instructions:

- Remove the contents of the plate by turning it over sharply to prevent the exchange of fluids between wells.
- Distribute about 300 µl of washing solution per well.
- Gently shake the plate avoiding the exchange of material between wells.
- Turn the plate over sharply to empty out its contents.
- Repeat the process as many times as indicated in the procedure.
- Before removing the contents of the final washing, make sure the reagent is ready to be used immediately. The plate must not be allowed to dry.
- After the final washing, shake the plate upside down onto absorbent filter paper.

7. REAGENT PREPARATION

- Plates:

  The plates, which are divisible into strips, are coated with SARS-CoV-2 recombinant N protein. Immediately after taking out the strips needed for the test, the rest should be put back into the bag and stored between +2ºC and +8ºC.

- Washing solution:

  Dilute one part of 25x concentrated washing solution, supplied with the kit, in 24 parts of distilled water. (40 ml of 25x concentrate plus 960 ml of distilled water). Once diluted, the solution remains stable between +2ºC and +8ºC for up to 3 months, provided that it is kept in suitable conditions.

- Control serums:

  Treat like the samples: Make a 1/5 dilution in diluent DE13. This dilution can be performed directly in the well by first adding the diluent and then the control. (40µl diluent and 10µl control).

- Preparation of the conjugate:

  Make a 1/10 dilution in diluent DE13. Dilute one part of concentrated 10X conjugate in 9 parts of the diluent provided. Prepare only the amount needed for each test and discard the remainder.

8. SAMPLE PREPARATION

Serum or plasma samples can be used. If the test is performed within 5 days after extraction, the samples may be kept at 2-8°C; if not, they should be aliquoted and frozen at between -20°C and -70°C. Once defrosted, mix well before use. Do not repeat freeze-thaw cycles. It is not recommended the use of heat-inactivated samples. Hyperlipidic, haemolysed or microbiologically contaminated and turbid samples may affect test performance and should not be used (See "interferences" in section 11).

Sample dilution: Make a 1/5 dilution in diluent DE13. This dilution can be performed directly in the well by first adding the diluent and then the sample (40µl of diluent and 10µl of serum). Shake gently to correctly homogenise the mixture. If there is a large number of samples, multi-channel pipettes are essential for this process.

9. PROCEDURE

Please read the instructions for use carefully before performing the test. The reliability of the result depends on strict compliance with the instructions for use provided. Please refer to section 6 with regard to the washing process. Before starting the test, the plan for distributing and identifying all the samples and standards/controls should be carefully established (duplicates are recommended). Select the required number of microtiter strips and insert them into the holder.

- Perform all steps of the assay in the order indicated and without any delays.
- A clean, disposable tip should be used to dispense each standard/control and sample.
- The assay can be performed without an incubator if the room temperature is 23 ± 2ºC. However, the use of an incubator set at this temperature is recommended.
- Before starting the assay, bring all kit components to room temperature.

1. First add the samples, as described in section 8.
2. Next, add the positive control and the diluent to at least two wells (50 µl/well). The diluent will be used to make the blank (which will be needed to calculate the cut-off point).
   For greater certainty, it is advisable to evaluate samples and controls in duplicate.
3. Cover the plate and shake to ensure correct homogenisation.
4. Incubate for 30 minutes at room temperature (23 ± 2ºC).
5. Wash 4 times according to the procedure described above.
6. Add 50 µl of 1x conjugate prepared according to the above instructions.
7. Incubate for 30 minutes at room temperature (23 ± 2ºC).
8. Wash 4 times according to the procedure described above.
9. Add 50 µl of substrate. Incubate for 15 minutes at room temperature. (Count the time from when the solution is added to the first well).
10. Add 50 µl of stop solution to each well. ATTENTION: The stop solution must be dispensed in the same order as the substrate solution was added.
11. Read the 450nm absorbance values within 20 minutes of adding the stop solution.

10. READING AND INTERPRETING THE RESULTS

If the samples have been tested in duplicate, the arithmetic mean of the two OD values obtained should be used. Likewise, the arithmetic mean of the values obtained in the two wells for the positive control and the two wells for the negative control should be taken.

VALIDATION OF THE RESULTS

Positive Control > 0.5
Positive Control / Blank > 2

CUT-OFF POINT CALCULATION

Positive cut-off point = S/P = 6
Negative cut-off point = S/P = 4

INTERPRETATION OF THE RESULTS

To interpret the results, the S/P of each sample is calculated:

$$S/P = ((OD\ sample - OD\ blank) / (OD\ positive\ control - OD\ blank)) \times 10$$

The samples are considered POSITIVE (presenting SARS-CoV-2 antibodies), when their S/P is equal to or higher than the positive cut-off point.

The samples are considered NEGATIVE (not presenting SARS-CoV-2 antibodies), when their S/P is equal to or lower than the negative cut-off point.

The samples will be considered DOUBTFUL when their S/P is between the two cut-off points. In these cases, it is recommended that a new sample, obtained 2 weeks later, is tested.

The diagnosis of an infectious disease should not be established on the basis of a single test result. An accurate diagnosis must take into account the patient's clinical history, symptomatology and serological data.

In immunocompromised patients and newborns, serological data has only limited value.

11. OPERATING SPECIFICATIONS

The results obtained with the studied samples are those described here. These results do not imply guaranteed specifications. The test operating data was obtained from human serum samples.

For further information on the particular operating specifications, please contact INGENASA.

Precision:

	No. replicates	mean	C.V.
intraplate	96	0.98	5.96
interplate	152	0.94	9,43

Diagnostic specificity

Diagnostic specificity is defined as the ability of the test to determine that a sample is negative when antibodies specific to SARS-CoV-2 are absent.

Diagnostic specificity 99.2% (95% confidence interval: 97.3%-99.9% on the 261 samples used in the validation study)

Diagnostic sensitivity

Diagnostic sensitivity is defined as the ability of the assay to determine that a sample is positive when antibodies specific to SARS-CoV-2 are present. As there is no standard reference technique, the term "correspondence" or "relative sensitivity" will be used. The INgezim® COVID 19 DR test has been compared with 4 other serological tests available on the market.

Correspondence with serological assays is 98.3% for positive detection (95% confidence interval: 94.5%-99.8% on the 116 samples used in the validation study)

Analytical sensitivity

In 85% of cases, on the samples used for validation, the assay was able to detect antibodies between days 7 and 16 after the onset of symptoms, and in 100% of cases from day 17.

Interference

Hyperlipidic, haemolysed or icteric samples with concentrations greater than 3mg/ml haemoglobin, 1.5mg/ml triglycerides and 0.05mg/ml bilirubin, or microbiologically contaminated and turbid samples may affect test performance and should not be used.

Cross-reactivity

Human serum samples positive for other respiratory coronaviruses (229E, NL63, OC43 and HKU1) have been used. The results indicate that there is no cross-reactivity with antibodies specific to these agents. Neither have interferences been found for antibodies for other respiratory viruses such as Influenza or RSV.

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freezing/thawing may affect the results. Hyperlipidic, haemolysed or microbiologically contaminated and turbid samples may affect test performance and should not be used.

The test only indicates the presence/absence of SARS-CoV-2-specific antibodies in the sample and should not be used as the sole criterion for diagnosing a SARS-CoV-2 infection.

The results should be checked against clinical data and other available information.

13. PRECAUTIONS AND WARNINGS

- In accordance with Article 1, Paragraph 2b of European Directive 98/79/EC, the use of *in-vitro* diagnostic medical devices is envisaged by the manufacturer to ensure the suitability, performance and safety of these products. Consequently, the testing procedure, information, precautions and warnings in the instructions for use must be followed rigorously. The use of test kits with analysers and similar equipment must be validated. No changes to the test design, composition or procedure are permitted, nor is any use in combination with other products not approved by the manufacturer; the user is solely responsible for any such changes. The manufacturer is not responsible for false results nor incidents arising as a result of these. The manufacturer is not responsible for any results obtained by visual analysis of patient samples.
- Only for *in-vitro* diagnosis.
- The product should only be used by health professionals (official or approved laboratories). The ELISA assay is only designed for use by qualified personnel who are familiar with good laboratory practice.
- All components of human or animal origin should be treated and managed as potentially infectious.
- Read the instructions for use carefully.
- Keep reagents at room temperature prior to use.
- Do not mix reagents or instructions from different kits.
- Do not mix reagents, instructions or plates from different batches.
- Avoid reagent contamination.
- Do not use the kits after the expiry date or mix components from different batches.
- Do not use reagents from other manufacturers when using this kit.
- To avoid contamination, do not interchange caps from different reagents.
- Close reagent bottles/vials immediately after use to avoid contamination and evaporation.
- After opening the reagents for the first time and storing them, check them for contamination before starting a new test.
- To avoid cross-contamination and falsely high results, pipette patient samples and dispense reagents into the wells accurately and without splashing.
- Do not eat, drink or smoke while handling the reagents and/or samples.
- Do not pipette the reagents by mouth.
- Use a new pipette tip for each sample to be tested.
- Systematically include one positive control and one negative control each time the kit is used.
- The stop solution must be handled with care as it is a strong acid. In case of contact with the skin, rinse immediately with plenty of water.
- The substrate is very sensitive to both light and contamination. For this reason it is recommended that the necessary amount is removed from the pot by decanting or with a sterile pipette, and any excess substrate should never be returned to the pot.

14. SAFETY NOTE

TMB contains NMP (N-Methyl-2- pyrrolidone). For this reason, apply safety protocols H360D (may harm the foetus), P280 (wear gloves, protective clothing and eye protection) and P308+P313 (if exposed or if symptoms appear, seek medical help or advice).

All this information can be found on the available safety data sheet.

15. WASTE CONSIDERATIONS

Waste from chemicals and preparations is generally considered to be hazardous waste. The disposal of this type of waste is regulated by national and regional laws and regulations. Contact your local authorities or waste management companies for advice on how to dispose of hazardous waste.

16. TECHNICAL ASSISTANCE AND ORDERS

For more information on how this product works, or if you have a complaint, please contact:

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For orders please contact:

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17. REFERENCES

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18. PROCEDURE DIAGRAM

Preparation of the assay

Reagent temperature adjustment

Prepare reagents and samples as described.

Establish the distribution and identification plan for all samples and standards/controls.

Select the required number of microtiter strips and insert these into the holder.

Assay procedure

1. Add 50µl of samples diluted 1/5 in diluent
2. Add 50µl of positive control diluted 1/5 in diluent into two wells
3. Add 50µl of diluent to two wells (blank)
4. Incubate for 30 min at room temperature (23 ± 2ºC)
5. Wash 4 times with washing solution 1x
6. Add 50µl of Conjugate, previously diluted 1/10 in diluent
7. Incubate for 30 min at room temperature (23 ± 2ºC)
8. Wash 4 times with washing solution 1x
9. Add 50 µl of substrate (TMB)
10. Incubate in the dark for 15 min at room temperature (23 ± 2ºC)
11. Add 50µl of Stop Solution
12. Photometric measurement at 450 nm