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biosensis® SARS-CoV-2 NP IgM ELISA Kit

Catalog Number: BEK-1001-1P/4P

For the qualitative determination of human anti-SARS-CoV-2 nucleocapsid protein (NP) antibodies (IgM class antibodies) in serum, or EDTA-, sodium citrate- or heparin-treated plasma specimens only when used as directed.

In line with FDA guidelines, test reports must include the following statements:

- This test has not been reviewed by the FDA.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
- Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

For research use only, not for use in clinical and diagnostic procedures.

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1. Intended Use

The SARS-CoV-2 NP IgM ELISA kit is a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the detection and qualitative measurement of IgM class antibodies against the nucleocapsid protein (NP) of SARS-CoV-2 virus in human blood.

This product is intended for use by professional persons only. For research use only. Not for use in clinical & diagnostic procedures.

2. Assay Background

In December 2019, a novel coronavirus, now officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been identified in Wuhan China, which caused the outbreak of a coronavirus-associated acute respiratory disease called coronavirus disease 19 (COVID-19). Signs and symptoms of COVID-19 may occur 2 to 14 days after infection, which include fever, cough, shortness of breath or difficulties in breathing, muscle pain and tiredness. In severe cases, the infection can further lead to pneumonia, severe acute respiratory syndrome (SARS), kidney failure and death.

Nucleocapsid protein (NP) is the most abundant protein on the helical nucleocapsid of coronaviruses, which envelopes the entire genomic RNA. NP also interacts with other viral structural proteins to play important roles during host cell entry and virus particle assembly and release. Anti-NP antibodies have been shown to be the earliest and the most predominant antibodies detectable in patient's blood samples after coronavirus infection.

3. Assay Principle

The Biosensis® SARS-CoV-2 NP IgM ELISA kit is a two-step incubation immunoassay kit. Recombinant nucleocapsid protein (NP) of SARS-CoV-2 pre-coated onto the polystyrene microwell strips can specifically recognize anti-NP antibodies in human serum or plasma specimens. After a 1 hour incubation, anti-NP antibodies are captured by immobilized NP protein while the unbound components are washed away. Afterwards, a detection solution containing HRP-conjugated anti-human IgM is added for another 1 hour incubation, wherein HRP-conjugated anti-human IgM binds to the IgM class antibodies previously bound to NP protein on the plate. After removal of nonspecific bindings, an HRP substrate solution containing 3,3′,5,5′-Tetramethylbenzidine (TMB) is added, resulting in the formation of a blue color. Color reaction is stopped by 2M H_2SO_4, transforming the blue color to yellow, which is quantified by an absorbance microplate reader at 450 nm. Microplate wells with increased color intensity indicate presence of higher amount of anti-NP antibodies in the tested samples.

4. Materials Provided, Storage and Stability

Reagent	Quantity per 1 Plate Kit
SARS-CoV-2 NP Coated ELISA Plate	12 strips of 8 wells (96 wells in total)
5X Assay Buffer	1 x 20 mL
10X Wash Buffer	1 x 40 mL
100X Detection Antibody Solution	1 x 120 µL
Substrate Solution	1 x 12 mL
Stop Solution	1 x 12 mL
Blank Control	1 x 1 mL

BEK-1001-4P contains reagents equivalent to four 1-Plate Kits.

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antigen-coated strips from the microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month. To assure maximum performance, protect the reagents from contamination with microorganism or chemicals during storage.

The unopened kit is stable for 6 months from date of receipt when stored at 2-8°C. Once opened, store reagents at 2-8°C for up to 1 month.

5. Equipment Required but Not Supplied

- Pipettes and pipette tips.
- 96-well plate or manual strip washer.
- Buffer and reagent reservoirs.
- Paper towels or absorbent paper.
- A microplate reader capable of reading absorbance at 450 nm.
- Distilled water or deionized water.

6. Before You Start….

- Read the entire protocol to familiarize yourself with the assay procedure
- Bring the microplate and all reagents and solutions to room temperature before starting the assay
- Remove only the required amount of TMB substrate from the fridge to prevent multiple warm-ups and cool downs; keep the TMB substrate in the dark
- Remove the number of strips required and return unused strips to the pack and reseal
- Centrifuge all kit components to collect reagents to the bottom of tubes before use
- The stop solution provided with this kit is an acid; wear protective equipment when handling

7. Reagent Preparation

1X Assay Buffer

Prepare 1X assay buffer by mixing the 5X assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5X assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1x assay buffer may be stored at 2- 8°C for up to one month.

1X Wash Buffer

Prepare 1X wash buffer by mixing the 10X wash buffer (40mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10X wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1X wash buffer may be stored at 2-8°C for up to one month.

1X Detection Antibody Solution

Spin down the 100X detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1X assay buffer. 100 μL of the 1X detection antibody solution is required per well. Prepare only as much 1X detection antibody solution as needed. Return the 100X detection antibody solution to 2-8°C immediately after the necessary volume is removed.

8. Sample Preparation

Test samples are suggested to be assayed immediately after separation of serum or plasma, or preferably stored frozen (-20^{0}C or below) in aliquots. Multiple freeze-thaw cycles should be avoided. Duplicate test is recommended.

Serum or plasma specimens with EDTA, sodium citrate or heparin anticoagulants can be tested. Highly lipemic, icteric, or hemolytic specimens are not recommended. Specimens with visible microbial contamination should not be used.

When required, vortex test serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 to 15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy or contain particles.

Serum or plasma sample generally require a 100-fold dilution in the 1×Assay buffer. A suggested dilution step is to add 10 μL of sample to 990 μL of 1X assay buffer. The dilution factor can be adjusted based on the titer of the antibodies in the samples

9. Assay Procedure

CRITICAL STEP: Please equilibrate all the reagents to room temperature (20-25°C) for at least 30 minutes before use. Shake reagent gently before use. Return to 2-8°C immediately after use. All assay incubation steps are performed at room temperature (20-25°C) without shaking.

Step 1	**Adding controls and specimen**: Add 100 µL of specimen and 100 µL of blank control into their respective wells. Duplicate test is recommended for both specimen and blank. **Note:** Use a separate disposal pipette tip for each specimen and blank to avoid cross-contamination. Mix by tapping the plate gently.
Step 2	**Incubation**: Cover the plate and incubate at room temperature for 1 hour.
Step 3	**Washing:** Discard the content and tap the plate on a clean paper towel to remove residual solution. Add 300 µL of 1X wash buffer to each well and incubate for 1 minute. Discard the wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for **a total 3 washes.**
Step 4	**Adding HRP-conjugated detection antibody:** Add 100 µL of 1X detection solution to each well.
Step 5	**Incubation**: Cover the plate and incubate at room temperature for 1 hour.
Step 6	**Washing:** Discard the content and tap the plate on a clean paper towel to remove residual solution. Add 300 µL of 1X wash buffer to each well and incubate for 1 minute. Discard the wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for **a total 4 washes.**
Step 7	**Detection/Signal generation:** Add 100 µL of substrate solution to each well, incubate at room temperature for 15 minutes. Protect from light.
Step 8	**Stop reaction:** Add 100 µL of stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
Step 9	**Measurement:** Measure absorbance of each well at 450 nm immediately. Note: read the absorbance within 10 minutes after stopping the reaction.

10. Requirements for Reporting Results

In line with FDA guidelines, test reports must include the following statements:

- This test has not been reviewed by the FDA.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
- Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

11. Quality Control

The Absorbance value of the Blank well should be < 0.10 at 450 nm. Negative control samples should read below 0.20 but each laboratory should ascertain their own values based upon their controls.

The test results are valid if the Quality Control criteria are fulfilled. Note each laboratory needs to establish its own appropriate quality standards and control system with quality control material similar to or identical with the patient specimen being analyzed.

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed.

12. Typical Results

Samples	OD450
Negative Control	0.113
Negative Control	0.098
Negative Control	0.121
Serum from healthy subjects	0.127
Serum from healthy subjects	0.154
Serum from healthy subjects	0.097
Serum from COVID-19 patients	1.052
Serum from COVID-19 patients	0.380
Serum from COVID-19 patients	0.242

13. Validation

Clinical validation study of Biosensis® SARS-CoV-2 NP IgM ELISA was conducted in 2020 in Shenzhen, China. Samples were collected from COVID-19 confirmed cases with clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period. The kit showed higher positive detection rate in specimens from patients with delayed onset. Therefore, the interpretation of the test results should consider the specimen's collection time.

It is highly recommended that each laboratory establishes its own normal and pathological reference range for anti-NP IgM level. Furthermore, it is also recommended that each laboratory includes its own panel of control samples in the assay.

14. Performance Characteristics

Inter Assay Precision	
Samples	CV
1	7.16%
2	5.35%
3	6.19%
Intra Assay Precision	
Samples	CV
1	4.93%
2	6.77%
3	5.89%

15. Precautions and Safety

ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.

2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.

3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (20-25°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.

4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.

5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.

6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.

7. Avoid long-time interruptions of assay steps. Assure same working conditions for all wells.

8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.

9. When adding specimens, do not touch the well's bottom with the pipette tip.

10. When measuring with a plate reader, determine the absorbance at 450 nm.

11. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

12. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

13. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.

14. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.

15. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) are available upon request.

16. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: The stop solution, the substrate solution, and the wash buffer.

17. The stop solution (2M H_2SO_4) is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.

18. ProClin™ 300 (0.1%) is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

19. Test reports must include the following statements:

- This test has not been reviewed by the FDA.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
- Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
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16. Summary of Assay Procedure

Add 100 µL of sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1X detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation and result interpretation