SARS Coronavirus IgG ELISA Kit

Cat. No.:DEIA1035
Pkg.Size:96T

Intended use

For the qualitative determination of IgG class antibodies against SARS Coronavirus in Human serum or plasma. It is intended for diagnosing and monitoring of patients related to infection by SARS Coronavirus.

General Description

The SARS coronavirus, sometimes shortened to SARS-CoV, is the virus that causes severe acute respiratory syndrome (SARS). On April 16, 2003, following the outbreak of SARS in Asia and secondary cases elsewhere in the world, the World Health Organization (WHO) issued a press release stating that the coronavirus identified by a number of laboratories was the official cause of SARS. Samples of the virus are being held in laboratories in New York, San Francisco, Manila, Hong Kong, and Toronto. protein E is a kinesin-like motor protein that accumulates in the G2 phase of the cell cycle. Unlike other centromere-associated proteins, it is not present during interphase and first appears at the centromere region of chromosomes during prometaphase. CENPE is proposed to be one of the motors responsible for mammalian chromosome movement and/or spindle elongation.

Principle Of The Test

This kit employs solid phase, indirect ELISA assay for detection of IgG antibodies to SARS Coronavirus in two-step incubation procedure. The isolated SARS coronavirus was used to infect normal Vero E6 cells. After culture, the purified whole virus lysate was pre-coated on the polystyrene microwell strips. During the first incubation step, SARS Coronavirus IgG specific antibodies, if present, will be bound to the solid phase pre-coated antigen complexes. The wells are washed to remove unbound serum proteins, and horseradish peroxidase (HRP) labelled anti-human IgG antibodies (anti-IgG) conjugate are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-IgG complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. If the color is displayed, it means that the SARS coronavirus IgG antibody is present in the serum sample, which is a positive reaction, otherwise it is a negative reaction.

Reagents And Materials Provided

Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with SARS Coronavirus antigens
Positive control, human serum: 0.2 mL
Negative control, human serum: 0.2 mL
HRP-conjugated anti-human IgG antibodies: 13 mL
Sample Diluent: 13 mL
Wash Buffer (50×): 30 mL
Chromogen Solution A, Urea peroxide solution: 8 mL
Chromogen Solution B, TMB solution, avoid light: 8 mL
Stop Solution, 2M sulfuric acid: 8 mL
Cover film: 2 pieces
Plastic bag: 1 pieces
Inner pad: 1 pieces

Materials Required But Not Supplied

45-1 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-938-8221
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37 °C incubator.

Storage

Store at 2-8°C for frequent use. Do not freeze. The expiration date is 12 months, please use before the "Expiration Date" marked on the kit. The unused negative and positive reference materials should be sealed and stored at 2-8 ° after opening.

Specimen Collection And Handling

Samples: The collection and testing of patient serum samples must be performed in accordance with standards published by the Ministry of Health. Serum or plasma requires samples to be clear, free of turbidity, and free of yellow constant.
Sample storage requirements: Blood samples should be separated immediately after collection; samples can be stored in a container containing EDTA-K2 or heparin sodium at 2-8°C for a short time or stored below -20°C for 6 months, and repeated freeze-thaw should be avoided. Frozen specimens should be thawed and mixed before testing.
Sample safety: All samples are regarded as potentially infectious items, and the operations are performed in accordance with relevant national standards.

Reagent Preparation

All reagents should be equilibrated at room temperature (18-25 °) for 30 minutes before use. Make sure that the surface moisture is dry before use.
1. Coated snap-off Strips: Ready-to-use. The outer foil bag must be opened after the coating microplate is equilibrated to room temperature to prevent the slat from absorbing water vapor in the air. Please immediately return the remaining strips to the aluminum foil bag (or plastic bag) containing the desiccant and seal it.
2. Negative and positive control: Ready-to-use, mix thoroughly before use.
3. HRP-conjugated anti-human IgG antibodies: Ready-to-use, mix thoroughly before use.
4. Washing Solution (50x conc.): The bottle contains 30 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+49; e.g. 1 ml Washing Solution + 49 ml fresh and germ free redistilled water. The diluted buffer will keep for 1 week if stored at 2-8 °C. If crystals appear in the concentrated solution, it should be heated to 37 ° before dilution to fully dissolve and mix.
5. Chromogen Solution A/B: Ready-to-use. As the substrate is sensitive to light, the bottle cap should be capped immediately after use and stored as far as possible from light.
6. Stop solution: Ready-to-use.
7. Cover film: Ready-to-use. Cover film can be used only once to avoid cross-contamination.

Assay Steps

1. Add sample/control: Set 1 well of blank, 1 well of positive control, and 2 wells of negative controls for each test. No liquid is added to the blank wells; 50 µL of the negative control and positive control is directly added to the corresponding well without dilution; 100 µL of sample diluent is added to the remaining test wells, then add 10 µL sample to the test wells, after mixing well, seal the plate with the cover film and incubate at 37°C for 30 min.
2. Wash plate:
Manually washing: add 300 µL of 1x Wash Solution to each well, leave it to stand for 5-10 seconds, and discard it. After repeated washing 6 times, pat dry.
Automatic washing: add 300-350 µL of 1x Wash Solution to each well, stand 5-10s between each wash, after repeated washing 6 times, pat dry.

45-1 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-938-8221
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

3. Add 100 µL of HRP-conjugated anti-human IgG antibodies to each well and incubate at 37°C for 20 min.
4. Wash plate:
Manually washing: add 300 µL of 1x Wash Solution to each well, leave it to stand for 5-10 seconds, and discard it. After repeated washing 6 times, pat dry.
Automatic washing: add 300-350 µL of 1x Wash Solution to each well, stand 5-10s between each wash, after repeated washing 6 times, pat dry.
5. Color development: Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Mix gently, protected from light and incubates at 37°C for 10 min.
6. Stop reaction: Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 30 seconds.
7. Read result: Read optical density at 450 nm with a microtiter reader immediately.

Validation

Each test should satisfy the positive control OD ≥0.50 and the negative control OD≤0.10; otherwise, the test results are considered invalid.

Quanlity Control

Cut-off value(C.O.) = 0.13 + N
N = the mean absorbance value for the negative controls.
Important: If the mean OD value of the negative controls is lower than 0.05, take it as 0.05.

Result Interpretation

If the OD value of the tested sample is greater than the threshold value, it should be judged as positive for SARS coronavirus IgG antibody. If the OD value is less than the threshold value, it is judged as negative. It is recommended to re-test near the threshold value. If the test result is positive, it is judged as positive, otherwise it is judged as negative. Weakly positive samples for this product should be tested by other methods to exclude false positives.

Reproducibility

CV ≤ 15% (n = 10)

Limitations

1. Non-repeatable positive result may occur due to the general biological characteristics of the ELISA method. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the “indirect” model minimizes the unspecific reactions due to interference with unknown matters in sample and the anti-human IgG antibodies. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Positive results must be confirmed with another available method and must be interpreted together with the patient clinical information and other laboratory results like X-ray and microbiolog.
3. Common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay’s predictive values.
5. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antibodies concentrations.

45-1 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-938-8221
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com