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User's Manual

SARS-CoV-2 IgG Agile ELISA Kit

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Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ELISA agile SARS-CoV-2 IgG test is qualitative and quantitative immunoassays for the detection of human antibodies in serum or plasma directed against SARS-CoV-2.

General Description

The beta coronavirus SARS-CoV-2, which causes the disease COVID-19, has been responsible for a global pandemic since early 2020. Common symptoms of COVID-19 are similar to those of a cold or flu, such as fever, cough, difficulty breathing or pneumonia in both lungs. In severe cases, the infection can lead to death.

During the first week after the onset of symptoms, qRT-PCR is used as a reliable method for detecting SARS-Cov-2 infection, and, as the infection progresses, the combination of qRT-PCT and antibody tests is often used. The use of antibody detection is useful in the later stages of infection when the virus is no longer detectable and can also be used to identify individuals who have developed immunity after infection and/or who may be potential plasma donors for therapeutic purposes.

For the ELISA agile SARS-CoV-2 IgG test, the whole spike protein is used exclusively for specific antibody detection and for correlation with protective antibodies that mainly target the spike protein.

Principles of Testing

The test strips of the ELISA agile microtiter plates are coated with specific antigens of the pathogen of interest. Diluted samples are incubated in the coated wells. Specific antibodies present in positive samples bind to the antigens and are detected with alkaline phosphataselabeled secondary antibodies. This enzyme catalyzes the conversion of the colorless substrate p-nitrophenyl phosphate into the colored product p-nitrophenol. The signal intensity of the reaction product is proportional to the antibody concentration in the sample and is measured photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with eight antigen coated single wells (altogether 96), 1 frame. The coating material is inactivated. 12. Unopened / After opening: at 2-8°C in closed aluminum bag with desiccant. See expiry date 6 months

2. Standard serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti HCV Ab; Preservative: <0.1% sodium azide; coloring: Amaranth O. 2 × 1 ml. Unopened / After opening: at 2-8°C. See expiry date 6 months

3. Negative control serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Lissamin Green V. 1 ml. Unopened / After opening: at 2-8°C. See expiry date 6 months

4. Anti-human IgG conjugate (ready-to-use), Anti-human IgG polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; Preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane.14ml. Unopened / After opening: at 2-8°C. See expiry date 6 months

5. Washing solution concentrate (sufficient for 1000ml), Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH

7. **4; Preservative: <0.1% sodium azide. 33.3ml. Unopened / after opening at 2-8°C, See expiry date. Working dilution at 2-8°C, 2 weeks. Working dilution at room temperature, 1 week**

6. Dilution buffer (ready-to-use), Protein-containing phosphate buffer with Tween 20; Preservative: <0.1% sodium azide; coloring: 0.01g/l Bromphenol blue. 2 × 55ml. Unopened / After opening at 2-8°C. See expiry date 6 months

7. Stopping solution (ready-to-use), <0.1N sodium hydroxide, 40mM EDTA. 15ml. Unopened / After opening at 2-8°C. See expiry date 6 months

8. Substrate (ready-to-use), Para-nitrophenylphosphate in solvent-free buffer; Preservative: <0.1% sodium azide. 14ml. Unopened / After opening at 2-8°C. See expiry date 6 months.

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g., 650nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water
7. Click-Clips
8. Optional: ELISA control

Specimen Collection And Preparation

1. Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples.

2. Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

ELISA agile SARS-CoV-2 IgA

$V_1 + V_2 = 1:100$	e.g. add each to	5ul 500ul	sample dilution buffer

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

3. Sample Storage

Samples should not be stored more than 7 days at 2-8°C. Extended storage is possible at ≤-20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

Reagent Preparation

Bring all reagents to room temperature before use.

1. Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

2. Negative Control Sera / Standard Sera (ready-to-use)

Negative control and standard sera are ready-to-use. For each test run (independent of the number of microtiter test strips to be used) negative control and standard sera must be included. Standard sera should be set up in duplicate.

3. Anti-human IgG Conjugate (ready-to-use)

4. Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with distilled H_2O to a final volume of V_2. Bottles used for the working solution should be cleaned regularly. Discard cloudy solutions.

Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

5. Dilution Buffer for Samples (ready-to-use)

Discard cloudy solutions.

6. Substrate (ready-to-use)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product! Avoid contamination.

7. Stopping Solution (ready-to-use)

Assay Procedure

1. Place the required number of wells in the frame and prepare a protocol sheet.
2. Add each 100ul of diluted sample or ready-to-use negative control / standard sera into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

   A1 Substrate blank

   B1 Negative control serum

   C1 Standard serum

   D1 Standard serum

   E1 Sample 1 . . .

   F1 Sample 2 . . .
3. Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber.

4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300ul washing solution

   aspirate or shake out the washing solution

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtiter plate on a paper towel

5. Addition of conjugate, Add 100ul of the ready-to-use IgG conjugate to the appropriate wells (except substrate blank).
6. Conjugate incubation for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate, Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber. Ensure incubation is in the dark.
10. Stopping the reaction, Add 100ul of stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction, Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

Quality Control

For the periodic verification of the test method, and in order to fulfill the requirements of laboratory internal quality management systems, we recommend using ELISA controls to determine precision and accuracy of ELISA agile test runs. ELISA controls are separately available, and the usage is described in specific instruction manuals. ELISA controls are not available in all countries, and the customer should consult the local distributor.

Calculation

1. Qualitative Evaluation

For the ELISA agile test evaluation, a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit so that the obtained OD values may be assigned to the corresponding antibody activities. The substrate blank must be subtracted from all OD values prior to evaluation. Mean OD value of the standard serum STD tested in duplicate has to be used.

Method 1:

In the first line of the evaluation table, several ranges of OD values for the standard serum are depicted covering the whole standard validity range. According to the measured mean OD value of the standard serum, the corresponding column can be chosen. That column contains the information of the upper and lower cut-off OD values to allow evaluation of the test sample. OD values below the lower cut-off are evaluated as negative for antibody and values above the upper-cut off are evaluated as positive for antibody. Implementation of the correction factor F is not necessary in the context of the evaluation table.

Method 2:

To fix the cut-off ranges, multiply the mean value of the measured standard OD with numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 X MW (STD) with upper cut-off

OD = 0.352 X MS(STD) with lower cut-off

2. Quantitative Evaluation

The mathematical curve fitting for antibody quantification with ELISA agile immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve and are indicated on the quality control certificate of each individual ELISA agile test. The correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ STD\ OD\ value}$$

3. Borderline Range

The borderline ranges are specified on the quality control certificate and indicate the range of borderline test results. Values below this range indicate a negative result; values above the borderline range indicate a positive result.

4. Limits of Quantification

The limits of quantification are specified on the quality control certificate. If a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

5. Criteria of Validity

The substrate blank must be <0.25 OD.

The negative control must be negative.

The mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot-specific quality control certificate.

The variation of OD values of the standard serum must not be higher than 20%.

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

Specificity

No cross-reactivity with sera positive for Epstein-Barr Virus VCA IgG, Adenovirus IgG, Influenza A Virus IgG, rheumatoid factor (RF), anti-nuclear antibodies (ANA), and other coronaviruses has been observed. Potential cross-reactivities not tested cannot be ruled out.

Interferences

To determine the influence of interfering substances, sera with different reactivities were analyzed with ELISA agile SARS-CoV-2 IgA. No interferences have been detected for sera with concentrations up to 2.00g/L hemoglobin, 11.50g/L lipemia/triglyceride 0.201g/L bilirubin (conjugated and unconjugated), 1.6mg/ml EDTA, 16 IU/ml heparin, or 0.106 mol/L citrate.

Precautions

The ELISA agile is designed for use by qualified personnel who are familiar with good laboratory practice.

All kit reagents and samples should be handled carefully using established good laboratory practice.

The test kit contains dilutions of human sera. Although all sera used have been tested and found negative for anti-HIV Ab, HBs-Ag (Hepatitis B Virus Surface Antigen) and anti-HCV Ab, they should be considered potentially infectious.

The instructions for use must be strictly followed.

Only use ELISA agile reagents when using ELISA agile immunoassays.

They must not be exchanged with reagents of other manufacturers or components of the ELISA classic line. Standard and control sera and conjugate of the ELISA agile are lot-specific.

The test reagents should be protected from strong light during storage and incubation.

The ELISA agile is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.