Quanti SARS-CoV-2 Anti-Spike IgG ELISA Kit

IVD

96 Tests Kit
Enzyme Immunoassay for the
Quantitative detection of anti-Spike IgG in Human Serum
(For In Vitro Diagnostic Use Only)

REF   Catalogue No. PT-QCoV-2-anti-Spike IgG-96

PISHTAZ TEB DIAGNOSTICS

Intended Use

The Pishtaz Teb Q-SARS-CoV-2-anti-Spike IgG ELISA kit is designed for the quantitative detection of anti-Spike IgG in human serum samples. It is intended to detect levels of anti-spike IgG in COVID-19 recovered patients or potentially in vaccinated persons with SARS-CoV-2 Vaccines. The components of the kit are for in vitro diagnostic use only and should not apply solely in clinical diagnosis

Introduction

The SARS-CoV-2 virus was discovered in 2019 in Wuhan, China which is the cause of the COVID-19 disease in humans. This virus is a single-stranded-RNA coronavirus. Many proteins (antigens) can be found within the structure of the virus, including spike(S), membrane (M), envelope (E), and nucleocapsids (N). The spike protein (S) contains a receptor-binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells and viral replication.
The Pishtaz Teb Q-SARS-CoV-2-anti-Spike IgG ELISA kit is designed for the quantitative detection of anti-Spike IgG that could be applicable as a tool to detect these antibodies in COVID-19 recovered and vaccinated persons.

Test Principle

The test principle is based on indirect solid phase enzyme-linked immune sorbent assay. In this technique, microwells are coated with certain amounts of SARS-CoV-2 Spike antigen. Then, the anti-Spike antibodies in sample are allowed to react with the solid phase antigens. After incubation and washing, the anti-human IgG HRP conjugate will be added. In case of presence of anti-Spike IgG, conjugated anti-human IgG will bind them. After second wash step a solution of chromogen-substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of anti-Spike IgG is directly proportional to the color intensity of the test sample.

CONT Materials provided with the kit

1. SARS-CoV-2 Spike antigen coated wells (1 plate, 96 wells): Microtiter wells coated with recombinant SARS-CoV-2 Spike protein.
2. Sample diluent (2 vials, 50 ml): Contains phosphate buffered solution with protein as stabilizer and Kathon CG as preservative, ready to use.
3. Enzyme conjugate (1 vial, 12 ml): anti-human IgG labeled with HRP in buffer containing protein as stabilizer and Kathon CG as preservative, ready to use.
4. Standards set (6 vials, 1.5 ml): Contain 0, 5, 10, 25, 50, and 100 RU/ml of anti-Spike IgG which diluted in buffer containing protein as stabilizer and 0.05% Kathon CG as preservative, ready to use.
   Anti-Spike IgG in the standards (RU/ml) were calibrated to a human monoclonal antibody specific to Spike-RBD.
5. Low control serum (1 vial, 1.5 ml): Contains certain amount of anti-Spike IgG diluted in buffer containing protein as stabilizer and 0.05 % of Kathon CG solution as preservative, ready to use.
6. High control serum (1 vial, 1.5 ml): Contains certain amount of anti-Spike IgG diluted in

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buffer containing protein as stabilizer and, 0.05 % of Kathon CG solution as preservatives, ready to use.

The concentration of anti-Spike IgG in the controls specify on the vials.

7. Chromogen substrate reagent (1 vial, 12 ml): Contains tetramethyl benzidine and hydrogen peroxide, ready to use solution.

8. Wash buffer (1 vials, 50 ml): Contains phosphate buffered salt solution with 0.05 % Tween 20 as detergent, pH = 6, concentrated (10X).

9. Stop solution (1 vial, 12 ml): 1 molar hydrochloric acid solution, pH < 1.

10. Cardboard sealer.

Materials/equipment required but not provided

- ELISA reader with 450 & 630 nm(reference) filters
- Precision pipettes
- Disposable pipette tips.
- Distilled water.
- Test tubes for dilution.
- Vortex mixer or equivalent
- Absorbent paper or paper towel.
- Graph paper.

General Notices for Users

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents in this kit are for in vitro diagnostic use only.
3. Only experienced laboratory personnel should use this test.
4. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8ºC upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.

3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.
6. Formation of crystals in the concentrated wash buffer is not a problem. Before the preparation of the ready to use buffer, put the vial in 37°C environment to dissolve the crystals.

Specimen Collection and Preparation

The kit is for use with serum. Serum should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20ºC. Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28ºC) before use.
2. All stages of the procedure must be done in an order and a continuous way.
3. A separate disposable micro-pipette tip must be used for each specimen.
4. Working wash solution: dilute concentrated wash solution 1:10 with distilled water before use.
5. Dilute specimens 1:101 with sample diluent (i.e. 10 µl of the specimen with 1000 µl of sample diluent).
6. Kit control sera and standards are ready to use.
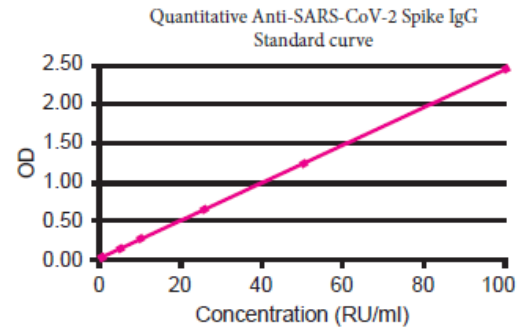
Assay Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 100 µl of standard, control serum and specimen in appropriate wells in duplicate.
3. Gently mix for 15 seconds and cover the microtiter wells with cardboard sealer firmly. Leave wells for 30 minutes at 37ºC.
4. Remove the sealer and take out wells contents by flicking the microplate into a waste container. Rinse and flick the

10	0.23
25	0.58
50	1.25
100	2.50

microtiter wells 5 times (each time with 300 μl of working wash solution).

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add 100 μl of anti-human IgG-HRP conjugate into the wells. Seal the plate with cardboard sealer again. Leave wells for 30 minutes at 37ºC.
7. Repeat step 4 and 5.
8. Dispense 100 μl of chromogen/ substrate solution into the microplate wells.
9. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
10. Stop the reaction by adding 100 μl of stop solution to the microplate wells.
11. Measure absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).

The validity of the Assay

The assay is considered valid if:
1. The OD (450 nm) of standards 0 and 100 RU/ml less than 0.1 and higher than 1.5, respectively, are acceptable.

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in RU/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample; determine the corresponding concentration of anti-Spike IgG in RU/ml from the standard curve.

Example of Standard curve

Standards (RU/ml)	OD (450/630 nm)
0	0.015
5	0.12

Quantitative Anti-SARS-CoV-2 Spike IgG Standard curve

Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Expected Values

It is important for each laboratory to establish the normal range limits. The following reference range from healthy persons should be considered as a guideline only. Based on mean and 3 standard deviation of SARS-CoV-2 anti-Spike IgG antibody concentration in normal serum specimens collected before the COVID-19 pandemic, 8 RU/ml was considered as cut-off.

<8 RU/ml	Negative
≥8 RU/ml	Positive

Performance Characteristics

1. Minimum Detection Limit

According to optical density of Standard 0 RU/ml and 3 standard deviations, the minimum detection limit of anti-Spike IgG by this assay is estimated to be 0.6 RU/ml.

2. Sensitivity

One hundred nine (109) positive serum samples were collected from COVID-19 confirmed cases who had clinical finding, lung HR-CT positive sign and a positive RT-PCR test. One hundred seven

(107) samples showed positive results. Thus, the sensitivity for detection of anti-Spike IgG was 98.16%. The mean value of anti-Spike IgG in COVID-19 patients was 84.2 RU/ml (95% CI: 78.6-98.7 RU/ml).

3. Specificity

The total number of 102 negative serum samples collected two year prior to January 2019 that stored in -20 degree was tested with our kit. One hundred one samples showed negative results. The Pishtaz teb SARS-CoV-2 anti-Spike IgG ELISA kit had specificity of 99.1%. The mean value of anti-Spike IgG in normal sera was 1.48 RU/ml (95% CI: 1.2-1.7 RU/ml).

Dilute serum samples contain over 100 RU/ml of anti-Spike IgG and the obtained value should be multiplied with dilution factor.

4. Accuracy

The Pishtaz Teb Diagnostics kit was compared with commercially valid quantitative SARS-CoV-2 anti-Spike IgG ELISA kit. In this study, 40 serum specimens of COVID-19 patients and 32 normal serum samples collected two year prior to COVID-19 pandemic were tested in parallel. The total agreement between two kits was 98.6%. In other words, the comparative study on the samples indicates 99.6% correlation between the Pishtaz Teb Diagnostics kit and the commercial competitor kit.

Serial dilution of reference material WHO-NIBSC 20/136 were tested by Pishtaz teb kit. The value higher than 1 RU/ml could convert to Binding Antibody Unit per milliliter (BAU/ml) unit by $y=3x+20$ formula. X is the obtained value in RU/ml and y is the concentration in BAU/ml.

Furthermore, the accuracy of the kit was also checked using WHO-NIBSC 20/140, 20/142, 20/144, and 20/148 in detecting negative and positive anti-Spike IgG samples. The results are presented as below:

NIBSC code	NIBSC: Anti-spike IgG (BAU/ml)	Pisthaz teb Kit: Anti-spike IgG (BAU/ml)	Pisthaz teb Kit: Anti-spike IgG (RU/ml)
20/140	46	44.6	8.2
20/142	Negative	0	<0
201/144	50	48.5	9.5
20/148	241	236	72

Based on reference material WHO-NIBSC: 20/136 cut-off value 44 BAU/ml was determined for Pishtaz teb Quanti-SARS-CoV-2 anti-Spike IgG ELISA Kit. In this regard, serum with anti-Spike IgG higher than 44 BAU/ml is positive.

5. Test Precision

Intra, as well as Inter-assay precision, carried out by 3 different sera, and its results were shown in tables 1 and 2:

Table 1: Intra-assay

No.	No. of tests*	Means RU/ml	SD RU/ml	CV %
1	20	5.1	0.36	7.05
2	20	27.4	1.7	6.2
3	20	53.6	2.7	5.04

Table 2: Inter-assay

No.	No. of tests*	Means RU/ml	SD RU/ml	CV %
1	20	5.5	0.43	7.81
2	20	26.7	1.8	6.74
3	20	55.9	3.1	5.54

*Each test has been run in duplicate

6. Cross reactivity

No cross reactivity was seen with sera positive for anti-SARS-CoV-2 neucleocapsid which were negative for anti-spike antibody (n=5). There is also no cross reactivity with serum positive for anti-HCV (n-=10), anti-HBV (n=10) and anti-HIV (n=10).

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7. Interference

In order to determine the interferences with the kit, the following amounts of hemoglobin, triglyceride and bilirubin were added to three specimens and the neutralizing antibody concentration was measured before and after the involvement of the substances mentioned above and the results are shown in the table below.

Interferent analyte	The concentration of the interferent analyte	The value of the specimen before adding the interferent (RU/ml)	The value of the specimen after adding the interferent (RU/ml)	The change of the results (%)
Hemoglobin	1 mg/ml	7.6 53.6 10.3	7.3 53.8 10.6	-3.94 0.37 2.91
Triglyceride	3000 mg/dL	7.6 53.6 10.3	7.8 54.1 10.1	2.63 0.93 -1.94
Bilirubin	20 ng/dL	7.6 53.6 10.3	7.4 55.1 10.6	-2.63 2.79 2.91

References

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	Chromogen-substrate solution	100 µl	100 µl	100 µl

SARS-CoV-2 anti-Spike IgG Test Procedure

Incubate wells for 15 minutes at room temperature in dark.

Dilute specimens 1:101 with sample diluent.

Step 1

Reagent	**Standard**	**Control Serum**	**Sample**
Standard	100 µl	None	None
Control Serum	None	100 µl	None
Sample	None	None	100 µl

Step 5

	Stop Solution	100 µl	100 µl	100 µl

Gently mix for 15 seconds and cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at 37℃.

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).

Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Step 3

Anti-human IgG-HRP conjugate	100 µl	100 µl	100 µl

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at 37℃.

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Step 4

Remove the microplate wells cover and remove contents by flicking of the wells into waste container wells 5 times according to test manual.

QS ISO 10002   QS FDA QSR 21 CFR 820 cGMP   QS ISO 13485

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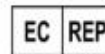
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	Batch Code
	Temperature Limit
	Contains/Contents
	Catalog Number
	In Vitro Diagnostic Medical Device
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