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

IVD

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

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

PISHTAZ TEB DIAGNOSTICS

Intended Use

The Pishtaz Teb Q-SARS-CoV-2-anti-RBD IgG ELISA kit is designed for the quantitative detection of anti-RBD IgG in human serum samples. It is intended to detect levels of anti-RBD 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-RBD IgG ELISA kit is designed for the quantitative detection of anti-RBD 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 RBD antigen. Then, the anti-RBD 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-RBD 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-RBD IgG is directly proportional to the color intensity of the test sample.

CONT Materials provided with the kit

1. SARS-CoV-2 RBD antigen coated wells (1 plate, 96 wells): Microtiter wells coated with recombinant SARS-CoV-2 RBD 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): Contains 0, 5, 10, 25, 50, and 100 RU/ml of anti-RBD IgG which diluted in buffer containing protein as stabilizer and 0.05% Kathon CG as preservative, ready to use.
   Anti-RBD IgG in the standards (RU/ml) were calibrated to a human monoclonal antibody specific to RBD.
5. Low control serum (1 vial, 1.5 ml): Contains certain amount of anti-RBD 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-RBD IgG diluted in buffer containing protein as stabilizer and,

Pishtaz Teb Diagnostics
European authorized representative:
JTC Diagnosemittel UG Schulweg 8
D- 34516 Voehl /Germany
REF MA Q-CoV-2-RBD-IgG 96_01
IVD CE
Ver:02 May/2021

0.05 % of Kathon CG solution as preservatives, ready to use.
The concentration of anti-RBD 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

Pishtaz Teb Diagnostics
European authorized representative:
JTC Diagnosemittel UG Schulweg 8
D- 34516 Voehl /Germany
REF MA Q-CoV-2-RBD-IgG 96_01
IVD CE
Ver:02 May/2021

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

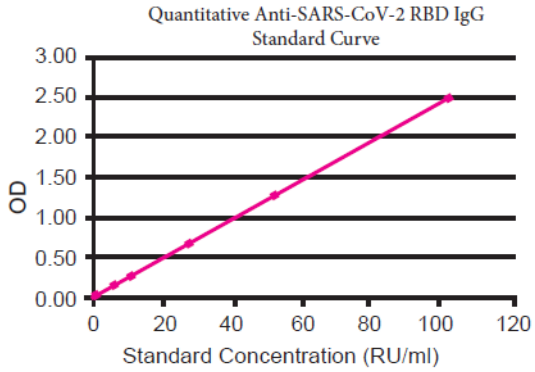
The assay is considered valid if:
The OD (450 nm) of standards 0 and 100 RU/ml less than 0.1 and higher than 1.5, respectively, are acceptable.

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-RBD IgG in RU/ml from the standard curve.

Example of Standard curve

Standards (RU/ml)	OD (450/630 nm)
0	0.011
5	0.150
10	0.234
25	0.587
50	1.274
100	2.40



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-RBD IgG antibody concentration in normal serum specimens collected before the COVID-19 pandemic, 5 RU/ml was considered as cut-off.

<5 RU/ml	Negative
≥5 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-RBD IgG by this assay is estimated to be 1.0 RU/ml.

2. Sensitivity

Sixty-nine (69) 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. Sixty-seven (67) samples showed positive results. Thus, the sensitivity for detection of anti-RBD IgG was 97.1%. The mean value of anti-RBD IgG in COVID-19 patients was 76.75 RU/ml (95% CI: 68.89-84.61 RU/ml).

3. Specificity

The total number of 118 negative serum samples collected two year prior to January 2019 that stored in -20 degree was tested with our kit. All samples showed negative results. The Pishtaz teb SARS-CoV-2 anti-RBD IgG ELISA kit had specificity of 100%. The mean value of anti-RBD IgG in normal sera was <1 RU/ml (95% CI: 1.2-1.7 RU/ml).

Serum samples containing over 100 RU/ml of anti-RBD IgG can be diluted and then obtained value should be multiplied by the dilution factor.

4. Accuracy

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=2.5x+40 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-RBD IgG samples. The results are presented as below:

NIBSC code	NIBSC: Anti-RBD IgG (BAU/ml)	Pisthaz teb Kit: Anti-RBD IgG (BAU/ml)	Pisthaz teb Kit: Anti-RBD IgG (RU/ml)
20/140	45	58	7.2
20/142	Negative	0	<0
201/144	66	61.5	8.6
20/148	205	237	79

Based on reference material WHO-NIBSC: 20/136 cut-off value 53 BAU/ml was determined for Pishtaz teb Quanti-SARS-CoV-2 anti-RBD IgG ELISA Kit. In this regard, serum with anti-RBD IgG higher than 53 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	2.42	0.2	8.26
2	20	12.1	0.85	7.02
3	20	83.9	4.5	5.36

Table 2: Inter-assay

No.	No. of tests*	Means RU/ml	SD RU/ml	CV %
1	20	2.6	0.23	8.84
2	20	12.3	0.91	7.39
3	20	85.7	4.9	5.71

*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-RBD 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).

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	2.36 18.6 63.8	2.32 18.9 62.1	-1.69 1.61 -2.66
Triglyceride	3000 mg/dL	2.36 18.6 63.8	2.4 17.9 64.9	1.69 -3.76 1.72
Bilirubin	20 ng/dL	2.36 18.6 63.8	2.37 19.1 65.1	0.42 2.68 2.03

References

1. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. J Med Virol. 2020;92(4):424–32.

2. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol [Internet]. 2020;0–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32104917.

3. Li M, Jin R, Peng Y, Wang C, Ren W, Lv F, et al. Generation of antibodies against COVID-19 virus for development of diagnostic tools. medRxiv. 2020;(1):2020.02.20.20025999.

4. Xia N, Wang G, Gong W. Serological test is an efficient supplement of RNA detection for confirmation of SARS - CoV - 2 infection. Preprints.2020;(March):1–6.

5. OKBA NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients medRxiv [Internet]. 2020;2020.03.18.20038059. Available from: https://www.medrxiv.org/content/10.1101/2020.03.18.20038059v1

6. Woo PCY, Lau SKP, Wong BHL, Tsoi HW, Fung AMY, Chan KH, et al. Detection of Specific Antibodies to Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein for Serodiagnosis of SARS Coronavirus Pneumonia. J ClinMicrobiol. 2004;42(5):2306–9.

7. Zhang W, Du RH, Li B, Zheng XS, Yang X Lou, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. 2020;9(1):386–9.

8. Liu L, Liu W, Wang S, Zheng S. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients.medRxiv. 2020;2020.03.06.20031856.

9. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. medRxiv [Internet]. 2020;2020.03.02.20030189. Available from: https://doi.org/10.1101/2020.03.02.20030189

10. To KK, Tak O, Tsang Y, Leung W, Tam AR, Wu T, et al. Articles Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis [Internet]. 2020;3099(20):1–10.

SARS-CoV-2 anti-RBD IgG Test Procedure

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

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

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°C.

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.

Chromogen -substrate solution	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature in dark.

Step 5

Stop Solution	100 µl	100 µl	100 µl

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

CE IVD

Pishtaz Teb Diagnostics
West 5th Golestan st. Baharestan Industrial Town,
Karaj, Alborz Province, Iran
Phone: +98 21 42 19 7000
www.Pishtazteb.com

EC REP **JTC Diagnosemittel UG**
Schulweg 8, D-34516 Voehl /Germany

ISO 10002 ISO 13485

Symbol	Symbol Title
	**Manufacturer**
	**CE marking Conformité Européene Notified Body Reference**
	**Use-by/Expiration Date**
	**Consult Instructions for Use**
	**Batch Code**
	**Temperature Limit**
	**Contains/Contents**
	**Catalog Number**
	**In Vitro Diagnostic Medical Device**
	**European authorized representative**