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QuantiFERON® SARS-CoV-2 ELISA Kit Instructions for Use

Σ 2 x 96

Version 1

IVD

For In Vitro Diagnostic Use

For use with QuantiFERON® SARS-CoV-2 Blood Collection Tubes

CE

REF 626420

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Sample to Insight

QIAGEN

Contents

Intended Use

The QuantiFERON SARS-CoV-2 assay is an in vitro diagnostic test designed for the qualitative detection of interferon-γ (IFN-γ) produced by CD4+ and CD8+ T cells in response to stimulation by a SARS-CoV-2 peptide cocktail in heparinized whole blood. The amount of IFN-γ produced is measured using enzyme-linked immunosorbent assay (ELISA).

The QuantiFERON SARS-CoV-2 assay is intended to aid in assessing the cell-mediated immune (CMI) response in individuals without a history of SARS-CoV-2 infection and who have received COVID-19 vaccination using vaccines targeting the viral spike (S) protein of the SARS-CoV-2 virus.

The QuantiFERON SARS-CoV-2 assay should be used in conjunction with other laboratory testing and epidemiological/clinical evaluation to assess an individual's immune response due to COVID-19 vaccination.

It may take several days after vaccination to develop T cell immune responses, although the duration of time that T cell immune responses are present is not well characterized in vaccinated individuals.

Non-reactive results do not preclude active SARS-CoV-2 infection or determine the effectiveness of COVID-19 vaccines. If active infection is suspected, confirm by using another molecular or antigen test for SARS-CoV-2. The results from the assay should always be used in combination with clinical examination, patient medical history, and other findings.

For in vitro diagnostic use.

Intended User

This kit is intended for professional use.

The product is to be used only by personnel specifically instructed and trained in molecular biology techniques and familiar with this technology.

Description and Principle

Summary and explanation

QuantiFERON SARS-CoV-2 (QFN SARS) is a qualitative assay that uses specialized blood collection tubes, containing peptide antigens that stimulate immune cells using SARS-CoV-2 specific proteins. Incubation of the blood occurs in the tubes for 16 to 24 hours, after which, plasma is harvested and tested for the presence of IFN-γ produced in response to the peptide antigens. Specific T cell mediated responses to SARS-CoV-2 infection have been reported post vaccination with different types of vaccines targeting spike protein [1–34].

First, whole blood is collected into each of the QuantiFERON SARS-CoV-2 Blood Collection Tubes, which include a Nil tube, Ag1 tube, Ag2 tube, and a Mitogen tube. Alternatively, blood may be collected in a single blood collection tube that contains lithium or sodium heparin as the anticoagulant, and then transferred to QuantiFERON SARS-CoV-2 Blood Collection Tubes.

The QuantiFERON SARS-CoV-2 Blood Collection Tubes are shaken to mix antigen with the blood and should be incubated at 37°C ± 1°C as soon as possible, and within 16 hours of collection. Following a 16 to 24 hour incubation period, the tubes are centrifuged, the plasma is processed, and the amount of IFN-γ (IU/ml) is measured by ELISA. The QuantiFERON SARS-CoV-2 ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535). Results for test samples are reported in International Units per ml (IU/ml) relative to a standard curve prepared by testing dilutions of the standard supplied with the kit.

Heterophile (e.g., human anti-mouse) antibodies in serum or plasma of certain individuals are known to cause interference with immunoassays. The effect of heterophile antibodies in the QuantiFERON SARS-CoV-2 ELISA is minimized by the addition of normal mouse serum to the

Green Diluent and the use of F(ab')2 monoclonal antibody fragments as the IFN-γ capture antibody coated to the microplate wells.

The plasma sample from the Mitogen tube serves as an IFN-γ positive control for each specimen tested. The Nil tube adjusts for background (e.g., elevated levels of circulating IFN-γ or presence of heterophile antibodies). The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the Ag1, Ag2, and Mitogen tubes.

Materials Provided

Kit contents

ELISA components	**2-plate kit**
Catalog no.	626420
Microplate strips (12 x 8 wells) coated with murine anti-human IFN-γ monoclonal antibody	2 sets of 12 x 8 Microplate Strips
IFN-γ Standard, lyophilized (contains recombinant human IFN-γ, bovine casein, 0.01% w/v Thimerosal)	1 x vial (8 IU/ml when reconstituted)
Green Diluent (contains bovine casein, normal mouse serum, 0.01% w/v Thimerosal)	1 x 30 ml
Conjugate 100x Concentrate, lyophilized (murine anti-human IFN-γ HRP, contains 0.01% Thimerosal)	1 x 0.3 ml (when reconstituted)
Wash Buffer 20x Concentrate (pH 7.2, contains 0.05% v/v ProClin® 300)	1 x 100 ml
Enzyme Substrate Solution (contains H_2O_2, 3,3′,5,5′ Tetramethylbenzidine)	1 x 30 ml
Enzyme Stopping Solution (contains 0.5 M H_2SO_4)*	1 x 15 ml
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* Contains sulfuric acid

Components of the kit

Controls and calibrators

The QFN SARS ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH Ref: Gxg01-902-535).

Platform and software

QFN SARS Analysis Software is optional for use and can be used to analyze raw data and calculate results. It is available for download at www.qiagen.com.

Materials Required but Not Provided

Additional reagents

- Deionized or distilled water, 2 liters

Equipment*

- 37 ± 1°C incubator (with or without CO_2)
- Calibrated variable volume pipets for delivery of 10 µl to 1000 µl with disposable tips
- Calibrated multichannel pipet capable of delivering 50 µl and 100 µl with disposable tips
- Microplate shaker capable of speeds from 500 to 1000 rpm
- Microplate washer (for safety in handling plasma samples, an automated plate washer is recommended)
- Microplate reader fitted with 450 nm filter and 620 nm to 650 nm reference filter
- Variable speed vortex
- Centrifuge capable of centrifuging the blood collection tubes at least to 3000 RCF (g)
- Graduated cylinder, 1 liter or 2 liters
- Plate lid
- Low-lint absorbent towels

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

For customers in the European Union, please be aware that you are required to report serious incidents that have occurred in relation to the device to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view and print the SDS for each QIAGEN kit and kit component.

- All chemicals and biological materials are potentially hazardous. Specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard sample and assay waste according to your local safety procedures.
- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.
- The QFN SARS assay should be used in conjunction with other laboratory testing and epidemiological/clinical evaluation to assess an individual's immune response due to COVID-19 vaccination.
- A non-reactive QFN SARS result does not preclude the possibility of SARS-CoV-2 infection or determine the effectiveness of COVID-19 vaccines. False non-reactive results can be due to incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay, or other individual immunological variables including those related to any comorbidities. Heterophile antibodies or non-specific IFN-γ production from other inflammatory conditions may mask specific responses to SARS-CoV-2 peptides.

- A reactive QFN SARS result should not be the sole or definitive basis for determining COVID-19 vaccine effectiveness. Incorrect performance of the assay may cause false reactive QFN SARS results.
- A false reactive QFN SARS result can be caused by incorrect blood sample collection or improper handling of the specimen affecting lymphocyte function. Please refer to "Procedure: Performing the ELISA" section, page 18, for correct handling of the blood specimens. Delay in incubation may cause false non-reactive or indeterminate results, and other technical parameters may affect ability to detect a significant IFN-γ response.
- A low response to Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a non-reactive response to the SARS CoV-2 proteins. This pattern may occur with insufficient lymphocytes, reduced lymphocyte activity due to improper specimen handling, filling/mixing of the Mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ. Elevated levels of IFN-γ in the Nil sample may occur with the presence of heterophile antibodies, or to intrinsic IFN-γ secretion.

Precautions

CAUTION 	Handle human blood as if potentially infectious. Observe relevant blood handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

QuantiFERON Enzyme Stopping Solution

Contains: sulfuric acid. Warning! May be corrosive to metals. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Enzyme Substrate Solution

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Green Diluent

Contains: tartrazine. Warning! May cause an allergic skin reaction. Wear protective gloves/ protective clothing/ eye protection/ face protection.

QuantiFERON Wash Buffer 20x Concentrate

Harmful to aquatic life with long lasting effects. Avoid release to the environment.

Further information

Safety Data Sheets: www.qiagen.com/safety

- Thimerosal is used as a preservative in some QFN SARS reagents. It may be toxic upon ingestion, inhalation, or skin contact.
- Deviations from the *QuantiFERON ELISA Kit Instructions for Use* may yield erroneous results. Please read the instructions carefully before use.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.
- Important: Inspect vials prior to use. Do not use Conjugate or IFN-γ Standard vials that show signs of damage or if the rubber seal has been compromised. Do not handle broken vials. Take the appropriate safety precautions to dispose of vials safely. It is recommended to use a vial de-crimper to open the Conjugate or IFN-γ Standard vials to minimize risk of injury from the metal crimp cap.
- Do not mix or use the Microplate strips, IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QFN SARS kit batches. Other reagents (Wash Buffer 20x Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be

interchanged between kits providing the reagents are within their expiration periods and lot details recorded.

- Discard unused reagents and biological samples in accordance with local, state, and federal regulations.
- Do not use the QFN SARS ELISA kit after the expiration date.
- Correct laboratory procedures should be adhered to at all times.
- Ensure that laboratory equipment such as plate washers and readers have been calibrated/validated for use.

Reagent Storage and Handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

In-use stability

- Store ELISA kit at 2–8°C (36–46°F).
- Always protect Enzyme Substrate Solution from direct sunlight.

Reconstituted and unused reagents

- For instructions on how to reconstitute the reagents, refer to "Procedure: Performing the ELISA", page 18.
- The reconstituted kit standard may be kept for up to 3 months if stored at 2–8°C.

  Note the date the kit standard was reconstituted.
- The reconstituted Conjugate 100X Concentrate must be returned to storage at 2–8°C and must also be used within 3 months.

  Note the date the conjugate was reconstituted.
- Working strength conjugate must be used within 6 hours of preparation.
- Working strength wash buffer may be stored at room temperature for up to 2 weeks.

Specimen Storage and Handling

Refer to the *QuantiFERON SARS-CoV-2 (QFN SARS) Blood Collection Tubes Instructions for Use* (1124422) for details on the blood collection workflow for the QFN SARS test.

Procedure: Performing the ELISA

Protocol: IFN-γ ELISA

Important points

- Refer to Kit contents, page 9 and Materials Required but Not Provided, page 11 for materials required to perform ELISA.

Setting up (Time required for performing the assay)

In order to obtain valid results from the QFN SARS assay, the operator needs to perform specific tasks within set times. Prior to use of the assay, it is recommended that the operator plan each stage of the assay carefully to allow adequate time to perform each stage. The time required is estimated below; the time of testing multiple samples when batched is also indicated.

- Approximately 3 hours for one ELISA plate
- <1 hour labor
- Add 10 to 15 minutes for each extra plate

Procedure

1. All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C [71.6°F ± 9°F]) before use. Allow at least 60 minutes for equilibration.
2. Remove ELISA plate strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until required.
3. Allow at least 1 strip for the QFN SARS standards and sufficient strips for the number of subjects being tested (refer to Figure 2 for recommended plate format). After use, retain frame and lid for use with remaining strips.

3a. Reconstitute the IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the vial. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved. Reconstitution of the IFN-γ standard to the correct volume will produce a solution with a concentration of 8.0 IU/ml.

3b. Using the reconstituted standard, prepare a dilution series of 4 IFN-γ concentrations (refer to Figure 1).

3c. A standard curve should be generated with the following IFN-γ concentrations:

- S1 (Standard 1) contains 4.0 IU/ml
- S2 (Standard 2) contains 1.0 IU/ml
- S3 (Standard 3) contains 0.25 IU/ml
- S4 (Standard 4) contains 0 IU/ml (Green Diluent [GD] alone).

3d. The standards must be assayed at least in duplicate.

3e. Prepare fresh dilutions of the kit standard for each ELISA session.

	Procedure
A	Label 4 tubes: S1, S2, S3, S4
B	Add 150 µl of GD to S1, S2, S3, S4
C	Add 150 µl of the kit standard to S1 and mix thoroughly
D	Transfer 50 µl from S1 to S2 and mix thoroughly
E	Transfer 50 µl from S2 to S3 and mix thoroughly
F	GD alone serves as the zero standard (S4)

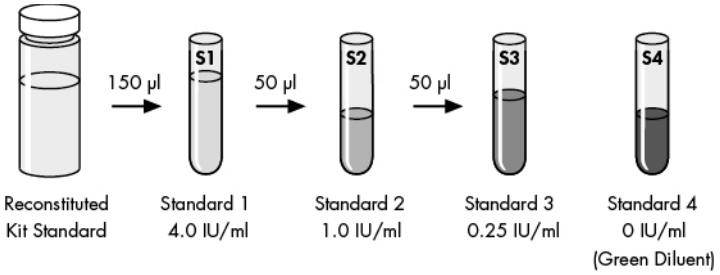

Figure 1. Preparation of standard curve dilution series.

4. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure that the entire content of the vial is completely dissolved.

   4a. Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1).

   4b. Working strength conjugate should be used within 6 hours of preparation.

   4c. Return any unused Conjugate 100x Concentrate to 2–8°C immediately after use.

Table 1. Conjugate preparation (working strength)

Number of strips	Volume of conjugate (100x concentrate)	Volume of Green Diluent
2	10 µl	1.0 ml
3	15 µl	1.5 ml
4	20 µl	2.0 ml
5	25 µl	2.5 ml
6	30 µl	3.0 ml
7	35 µl	3.5 ml
8	40 µl	4.0 ml
9	45 µl	4.5 ml
10	50 µl	5.0 ml
11	55 µl	5.5 ml
12	60 µl	6.0 ml

5. For plasma samples harvested from blood collection tubes and subsequently stored (refrigerated or frozen), thoroughly mix the stored sample before addition to the ELISA well. Plasma samples can be stored in centrifuged QFN SARS Blood Collection Tubes for up to 28 days at 2–8°C, or harvested plasma samples can be stored for up to 28

days at 2–8°C. Harvested plasma samples can also be stored below –20°C (preferably less than –70°C) for up to 24 months.

Plasma samples can be loaded/used directly from centrifuged blood collection tubes for measurement in the QFN SARS ELISA plate.

Important: If plasma samples are to be transferred directly from the centrifuged QFN SARS Blood Collection Tubes, any mixing of the plasma should be avoided. At all times, take care not to disturb material on the surface of the gel.

6. Add 50 µl of freshly prepared working strength conjugate to each ELISA plate well.
7. Add 50 µl of test plasma sample to appropriate wells (refer to recommended ELISA plate layout in Figure 2).
8. Finally, add 50 µl each of the Standards 1 to 4 to the appropriate plate wells (refer to recommended ELISA plate layout in Figure 2). The standards should be assayed in at least duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 N	3 N	5 N	7 N	9 N	S1	S1	13 N	15 N	17 N	19 N	21 N
B	1 Ag1	3 Ag1	5 Ag1	7 Ag1	9 Ag1	S2	S2	13 Ag1	15 Ag1	17 Ag1	19 Ag1	21 Ag1
C	1 Ag2	3 Ag2	5 Ag2	7 Ag2	9 Ag2	S3	S3	13 Ag2	15 Ag2	17 Ag2	19 Ag2	21 Ag2
D	1 M	3 M	5 M	7 M	9 M	S4	S4	13 M	15 M	17 M	19 M	21 M
E	2 N	4 N	6 N	8 N	10 N	11 N	12 N	14 N	16 N	18 N	20 N	22 N
F	2 Ag1	4 Ag1	6 Ag1	8 Ag1	10 Ag1	11 Ag1	12 Ag1	14 Ag1	16 Ag1	18 Ag1	20 Ag1	22 Ag1
G	2 Ag2	4 Ag2	6 Ag2	8 Ag2	10 Ag2	11 Ag2	12 Ag2	14 Ag2	16 Ag2	18 Ag2	20 Ag2	22 Ag2
H	2 M	4 M	6 M	8 M	10 M	11 M	12 M	14 M	16 M	18 M	20 M	22 M

Figure 2. **Recommended ELISA plate layout**. S1 (Standard 1), S2 (Standard 2), S3 (Standard 3), S4 (Standard 4). 1N (Sample 1. Nil Control plasma), 1 Ag1 (Sample 1. Ag1 plasma), 1 Ag2 (Sample 1. Ag2 plasma), 1M (Sample 1. Mitogen plasma).

9. Cover ELISA plate and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute at 500 to 1000 rpm. Avoid splashing.

10. Cover ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 120 ± 5 minutes. The ELISA plate should not be exposed to direct sunlight during incubation. Deviation from the specified temperature range can lead to erroneous results.
11. During the ELISA plate incubation prepare working strength wash buffer. Dilute one part Wash Buffer 20x Concentrate with 19 parts deionized or distilled water and mix thoroughly. Sufficient Wash Buffer 20x Concentrate has been provided to prepare 2 liters of working strength wash buffer.
12. When ELISA plate incubation is complete, wash ELISA plate wells with 400 µl of working strength wash buffer. Perform wash step at least 6 times. An automated plate washer is recommended for safety reasons when handling plasma samples.

    Thorough washing is very important to the performance of the assay. Make sure each well is completely filled with wash buffer to the top of the well for each wash cycle. A soak period of at least 5 seconds between each cycle is recommended.

    Standard laboratory disinfectant should be added to the effluent reservoir, and established procedures followed for the decontamination of potentially infectious material.
13. Tap ELISA plate face down on an absorbent (low-lint) towel to remove residual wash buffer. Add 100 µl of Enzyme Substrate Solution to each plate well, cover the plate, and mix thoroughly for 1 minute at 500 to 1000 rpm using a microplate shaker.
14. Cover the ELISA plate and incubate at room temperature (22°C ± 5°C [71.6°F ± 9°F]) for 30 minutes. The ELISA plate should not be exposed to direct sunlight during incubation.
15. Following the 30-minute incubation, add 50 µl of Enzyme Stopping Solution to each plate well in the same order as the substrate was added and mix thoroughly at 500 to 1000 rpm using a microplate shaker.
16. Measure the Optical Density (OD) of ELISA plate wells within 5 minutes of stopping the reaction using a microplate reader fitted with a 450 nm filter and with a 620 nm to 650 nm reference filter. OD values are used to calculate results.

Results (Calculations)

QFN SARS Analysis Software can be used to analyze raw data and calculate results. It is available at www.qiagen.com. Please make sure that the latest version of the QFN SARS Analysis Software is used.

The software performs a Quality Control assessment of the assay, generates a standard curve, and provides a test result for each subject, as detailed in "Interpretation of Results", page 27. The software reports all concentrations greater than 10 IU/ml as ">10" as such values fall beyond the validated linear range of the ELISA.

As an alternative to using the QFN SARS Analysis Software, results can be determined according to the following method.

Generation of standard curve and sample values

If QFN SARS Analysis Software is not used

Determination of the standard curve and determination of sample IU/ml values require a spreadsheet program, such as Microsoft® Excel®, if the QFN SARS Analysis software is not used.

Using a spreadsheet program

1. Determine the mean OD values of the kit standard replicates on each plate.
2. Construct a $\log_{(e)}$–$\log_{(e)}$ standard curve by plotting the $\log_{(e)}$ of the mean OD (y axis) against the $\log_{(e)}$ of the IFN-γ concentration of the standards in IU/ml (x axis), omitting the zero standard from these calculations. Calculate the line of best fit for the standard curve by regression analysis.
3. Use the standard curve to determine the IFN-γ concentration (IU/ml) for each of the test plasma samples, using the OD value of each sample.

4. These calculations can be performed using software packages available with microplate readers, and standard spreadsheet or statistical software (such as Microsoft Excel). It is recommended that these packages be used to calculate the regression analysis, the coefficient of variation (%CV) for the standards, and the correlation coefficient (*r*) of the standard curve.

Sample calculation

If the following OD readings were obtained for the standards, the calculations using – log(e) – would follow those in Table 2.

Table 2. Standard curve

Standard	IU/ml	OD values a and b	Mean OD	%CV	$Log_{(e)}$ IU/ml	$Log_{(e)}$ Mean (OD)
Standard 1	4	1.089, 1.136	1.113	3.0	1.386	0.107
Standard 2	1	0.357, 0.395	0.376	7.1	0.000	–0.978
Standard 3	0.25	0.114, 0.136	0.125	NA	–1.386	–2.079
Standard 4	0	0.034, 0.037	0.036	NA	NA	NA

The equation of the curve is y = 0.7885(X) – 0.9837, where "m" = 0.7885 and "c" = –0.9837. These values are used in the equation X = (Y–c)/m to solve for X. Based on the standard curve, the calculated correlation coefficient (*r*) = 1.000. NA: Not applicable.

Using the criteria specified in "Quality control of the test", page 25, the validity of the assay is determined.

The standard curve (Table 2) is used to convert the Antigen OD responses to International Units (IU/ml).

Table 3. Sample calculation

Antigen	OD value	$Log_{(e)}$ OD value	X	e^X (IU/ml)	Antigen –Nil (IU/ml)
Nil	0.037	–3.297	–2.934	0.05	–
Ag1	1.161	0.149	1.437	4.21	4.15
Ag2	1.356	0.305	1.634	5.12	5.07
Mitogen	1.783	0.578	1.981	7.25	7.20

IFN-γ values (in IU/ml) for Ag1, Ag2, and Mitogen are corrected for background by subtracting the IU/ml value obtained for the respective Nil control. These corrected values are used for interpretation of the test results.

Quality control of the test

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the ELISA to be valid:

- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate values must be ≤15%.
- Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
- The correlation coefficient (*r*) calculated from the mean absorbance values of the standards must be ≥0.98.
- If the above criteria are not met, the run is invalid and must be repeated.
- The mean OD value for the Zero Standard (Green Diluent) should be ≤0.150. If the mean OD value is > 0.150, the plate washing procedure should be investigated.

The QFN SARS Analysis Software calculates and reports these quality control parameters.

Each laboratory should determine appropriate types of control materials and frequency of testing in accordance with local, state, federal, or other applicable accrediting organizations. External quality assessment and alternative validation procedures should be considered.

Note: Plasmas spiked with recombinant IFN-γ have shown reductions of up to 50% in concentration when stored at either 2–8°C and –20°C. Recombinant IFN-γ is not recommended for establishing control standards in plasma samples.

Interpretation of Results

QFN SARS results are interpreted using the following criteria (Table 4).

Important: The QFN SARS assay should be used in conjunction with other laboratory testing and epidemiological/clinical evaluation to assess an individual's immune response due to COVID-19 vaccination.

Table 4. Interpretation of QFN SARS test results

Nil (IU/ml)	Ag1 Antigen minus Nil (IU/ml)	Ag2 Antigen minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFN SARS result	Report/Interpretation
≤8.0	≥0.15 and ≥25% of Nil	Any	Any	Reactive	*SARS-CoV-2 response detected*
	Any	≥0.15 and ≥25% of Nil			
	<0.15 or ≥0.15 and <25% of Nil	<0.15 or ≥0.15 and <25% of Nil	≥0.50	Non-reactive	*SARS-CoV-2 response NOT detected*
	<0.15 or ≥0.15 and <25% of Nil	<0.15 or ≥0.15 and <25% of Nil	<0.50	Indeterminate‡	*SARS-CoV-2 response and Mitogen cannot be detected*
>8.0§	Any				

*Responses to the Mitogen positive control (and occasionally the Ag Antigen responses) can be outside the range of the microplate reader. This has no impact on test results. Values >10 IU/ml are reported by the QFN SARS software as >10 IU/ml.

‡ Refer to "Troubleshooting Guide", page 50 possible causes.

§ In clinical studies, less than 0.25% of subjects had IFN-γ levels of >8.0 IU/ml for the Nil value.

Limitations

Results from QFN SARS testing must be used in conjunction with each individual's epidemiological history, current medical status, and other diagnostic evaluations.

Individuals with Nil values greater than 8 IU/ml are classed as "Indeterminate" because a 25% higher response to Ag Antigens may be outside the assay measurement range.

- A non-reactive result must be considered with the individual's medical and historical data relevant to probability of immune response to vaccination, particularly for individuals with impaired immune function.
- The QFN SARS assay should be used in conjunction with other laboratory testing and epidemiological/clinical evaluation to assess an individual's immune response due to COVID-19 vaccination.

Unreliable or indeterminate results may occur due to:

- Deviations from the procedure described in the Instructions for Use
- Incorrect transport/handling of blood specimen
- Elevated levels of circulating IFN-γ or presence of heterophile antibodies
- Exceeding validated blood times from blood specimen draw to incubation. Refer to the *QFN SARS Blood Collection Tubes Instructions for Use* (1124422).

Assay Performance Characteristics

Analytical performance

Assay cut-off

The QFN SARS assay cut-off was determined using data from twenty (20) subjects who tested SARS-CoV-2 non-reactive with a RT-PCR test or serology test and twenty (20) donors that were fully vaccinated (between 2–16 weeks post full vaccination status) with an FDA EUA authorized vaccine. The sensitivity and specificity data along with the exact two-sided 95% confidence intervals (CIs) were analyzed and demonstrated that the optimal ELISA cut-off was 0.15 IU/mL (see Table 5).

Table 5. QFN SARS Cut-off Values (IU/mL) with Corresponding Sensitivity and Specificity with Exact Two-Sided 95% CI

	Sensitivity			Specificity		
Cut-off value	**Value**	**Lower 95% CI**	**Upper 95% CI**	**Value**	**Lower 95% CI**	**Upper 95% CI**
0.1	1.000	0.940	1.000	0.933	0.838	0.982
0.15	0.983	0.911	1.000	1.000	0.940	1.000
0.2	0.900	0.795	0.962	1.000	0.940	1.000
0.25	0.733	0.603	0.839	1.000	0.940	1.000
0.3	0.717	0.586	0.825	1.000	0.940	1.000
0.35	0.650	0.516	0.769	1.000	0.940	1.000
0.4	0.600	0.465	0.724	1.000	0.940	1.000
0.45	0.567	0.432	0.694	1.000	0.940	1.000
0.5	0.467	0.337	0.600	1.000	0.940	1.000
0.55	0.433	0.306	0.568	1.000	0.940	1.000
0.6	0.400	0.276	0.535	1.000	0.940	1.000
0.65	0.333	0.217	0.467	1.000	0.940	1.000
0.7	0.317	0.203	0.450	1.000	0.940	1.000
0.75	0.300	0.188	0.432	1.000	0.940	1.000
0.8	0.300	0.188	0.432	1.000	0.940	1.000

Linearity

The QFN SARS ELISA has been demonstrated to be linear by placing 5 replicates of 11 plasma pools of known IFN-γ concentrations randomly on the ELISA plate. The linear regression line has a slope of 1.002 ± 0.011 and a correlation coefficient of 0.99 (Figure 3).

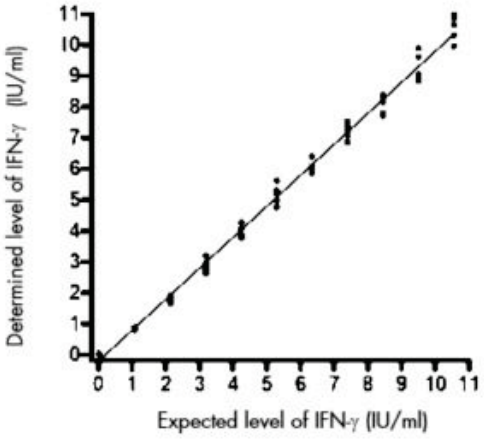

Figure 3. **Illustration of Linearity Study Regression Analysis.**

Reproducibility

A multi-laboratory reproducibility study was conducted to evaluate performance of the QFN SARS assay across laboratories with multiple operators. This study was conducted at three laboratories within QIAGEN. A total of three (3) SARS-CoV-2 reactive and three (3) SARS-CoV-2 non-reactive study subjects (determined by RT-PCR test or serology test) were enrolled.

Blood collected in four (4) lithium heparin blood collection tubes were obtained from each study subject. The lithium heparin blood collection tubes were then transferred to one of the testing laboratories where the blood was aliquoted into three (3) sets of QFN SARS Blood Collection Tubes (QFN SARS Ag1, Ag2, Mitogen, and Nil). One set each of QFN SARS Blood Collection Tubes (BCTs) were transferred to each of the testing laboratories and then tested in

accordance with the QFN SARS assay procedure. Each subject was tested with ten (10) replicates (five (5) replicates for Ag1 and five (5) replicates for Ag2) in each laboratory. At each laboratory one (1) operator ran the QFN SARS test independently. Each operator was blinded to the results obtained by the other operators and blinded to the RT-PCR or serology test results of the study subject.

There were 30 results generated at each of the three (3) testing laboratories, resulting in a total of 90 data points. A summary of the reproducibility study results is provided in Table 6.

Table 6. Reproducibility study results summary –N = 30 patient samples

Laboratory 1 – 1 Operator	**Laboratory 2 – 1 Operator**	**Laboratory 3 – 1 Operator**
25/30 = 83%	30/30 = 100%	30/30 = 100%
Agreement of Qualitative Results	Agreement of Qualitative Results	Agreement of Qualitative Results

The overall percent agreement across all reactive and non-reactive samples to the expected qualitative results (reactive subject returning a reactive result and non-reactive subject returning a non-reactive result based on the subject reference method result) was 94.4% (85/90) across all three (3) laboratories.

Inter-lot repeatability

A study was conducted to determine the inter-lot variability of QFN SARS Blood Collection Tubes. A total of two (2) SARS-CoV-2 reactive and three (3) SARS-CoV-2 non-reactive (determined by RT-PCR test or serology test) study subjects were tested. Three (3) separate lots each of the QFN SARS Ag1 and Ag2 Blood Collection Tubes were included in this study. Five (5) replicates per donor per blood collection tube lot were tested. A summary of the inter-lot precision results is provided in Table 7.

Table 7. Inter-lot Precision study results summary - Overall Percent Agreement for the QFN SARS Ag1 and Ag2 Blood Collection Tubes; N = 25

QFN SARS BCT	BCT Lot number	Number of qualitative calls in agreement / Total calls	Proportion	Lower confidence limit	Upper confidence limit
Ag1	1	25/25	100.00%	86.28%	100.00%
	2	25/25	100.00%	86.28%	100.00%
	3	25/25	100.00%	86.28%	100.00%
Ag2	1	25/25	100.00%	86.28%	100.00%
	2	25/25	100.00%	86.28%	100.00%
	3	25/25	100.00%	86.28%	100.00%

The overall percent agreement across all reactive and non-reactive samples to the expected results (reactive subject returning a reactive result and non-reactive subject returning a non-reactive result based on the subject reference method result) was 100% across all three (3) lots of QFN SARS Ag1 and Ag2 BCTs.

Limit of Blank (LoB)

The Limit of Blank (LoB) was evaluated for the QFN SARS assay. Two (2) replicates each of fourteen (14) individual normal human plasma samples (as the blanks) were tested with two (2) lots of the QFN SARS ELISA by three (3) operators on three (3) testing days, one (1) operator per testing day for a total of 84 replicates from each ELISA kit lot.

The LoB values (IU/mL) for the two (2) ELISA kit lots were calculated separately as shown in Table 8.

Table 8. LoB Values (IU/mL) for the two (2) QFN SARS ELISA Kit lots

QFN SARS ELISA Kit	LoB estimated (IU/ml)
Kit 1	0.030
Kit 2	0.040

The larger LoB value, 0.040 IU/mL, across both QFN SARS ELISA kit lots, was reported as the final LoB value.

Limit of Detection (LoD)

The Limit of Detection (LoD) was evaluated for the QFN SARS assay. A human plasma pool was generated by combining fourteen (14) individual plasma samples. Each of the three (3) operators prepared a IFN-γ reference standard stock at 1.0 IU/mL diluted in buffer. A dilution series of eight (8) concentrations were made in plasma. The study was conducted over three (3) days, by three (3) alternating operators using two (2) QFN SARS ELISA kit lots. For each testing day, five (5) replicates of each concentration within each set of the serial dilution series were tested for a total of 45 replicates for each dilution of IFN-γ concentration for each QFN SARS ELISA kit lot.

The LoD value for each of the QFN SARS ELISA kit lots tested were calculated separately as shown in Table 9. The LoD was estimated using a Probit regression model. The LoD was based on the estimated concentration (IU/mL) that gave a 95% estimated probability of obtaining a hit-rate greater than 0.04 IU/mL (determined by the LoB).

Table 9. Estimated LoD Values (IU/mL) for the two (2) QFN SARS ELISA Kit Lots

QFN SARS ELISA Kit	Probability	Concentration estimate (IU/ml)	Lower 95% Confidence Limit for Estimate	Upper 95% Confidence Limit for Estimate
Kit 1	0.95	0.063	0.060	0.067
Kit2	0.95	0.065	0.060	0.073

The larger LoD value calculated across both QFN SARS ELISA kit lots, 0.065 IU/mL, was reported as the final LoD value.

Interfering substances

A study was conducted to determine the effects of potential interfering substances on the performance of the QFN SARS ELISA detection of IFN-γ. The interferents included in this testing were: triglycerides (total), hemoglobin, protein (total serum), bilirubin (conjugated), bilirubin (unconjugated), abacavir sulfate, cyclosporine, and prednisolone. Five (5) plasma pools with known concentrations of IFN-γ were prepared using different interferent concentrations. The base pool IFN-γ level was previously prepared with a pre-determined amount of IFN-γ present (approximately 0.21, 0.45 and 1.4 IU/mL). This pool was then used to prepare the interferent pools. Five different levels of interferent concentrations were tested and were based on reference intervals, pathological values, therapeutic ranges, and toxic ranges or as recommended by vendor or general clinical levels. Six (6) replicates were tested for each interferent sample concentration level.

For each sample concentration, a T-test was performed, comparing the difference in mean log10 (IU/mL) of the high interferent level (10) compared to the control (i.e. interferent-free level). The estimated difference in mean response, along with the corresponding two-sided 95% confidence limits and p-value are reported in the table.

Table 10. Log10 IU/mL: T-Test Summary Table for Differences in Means between Control and High Interferent Level for each Interferent and IFN-γ Concentration Level

Interferent	Interferent level	Sample concentration (IU/ml)	Mean Difference	Lower 95% CI	Upper 95% CI	P-value
Triglycerides	High	1.4	0.053	–0.004	0.110	0.063
		0.45	0.039	–0.021	0.058	<.001
		0.21	0.034	–0.002	0.071	0.061
Hemoglobin	High	1.4	–0.001	–0.042	0.040	0.967
		0.45	0.016	–0.007	0.040	0.152
		0.21	0.014	–0.030	0.059	0.489
Protein	High	1.4	–0.030	–0.071	0.011	0.136
		0.45	0.000	–0.046	0.046	0.992
		0.21	–0.045	–0.103	0.012	0.109
Bilirubin Conjugated	High	1.4	0.001	–0.046	0.048	0.961
		0.45	0.012	–0.043	0.067	0.639
		0.21	0.015	–0.044	0.074	0.586
Bilirubin Unconjugated	High	1.4	0.015	–0.011	0.042	0.231
		0.45	0.015	–0.023	0.052	0.411
		0.21	0.012	–0.033	0.057	0.566
Abacavir	High	1.4	0.013	–0.015	0.040	0.322
		0.45	0.015	–0.014	0.044	0.283
		0.21	0.008	–0.034	0.050	0.677

Table continued on next page

Table continued from previous page

Table 10. Log10 IU/mL: T-Test Summary Table for Differences in Means between Control and High Interferent Level for each Interferent and IFN-γ Concentration Level

Interferent	Interferent level	Sample concentration (IU/ml)	Mean Difference	Lower 95% CI	Upper 95% CI	P-value
Cyclosporine	High	1.4	0.002	–0.019	0.024	0.816
		0.45	0.007	–0.030	0.043	0.682
		0.21	0.015	–0.007	0.038	0.155
Prednisolone	High	1.4	0.007	–0.016	0.030	0.518
		0.45	–0.001	–0.034	0.033	0.964
		0.21	0.021	–0.025	0.068	0.334

The results showed no statistically significant differences between the highest interferent level tested and the control (interferent-free level) except for the triglyceride 0.45 IU/mL concentration level. The mean difference for this value was determined to be within ± 2 standard deviations of the mean control level measurement, demonstrating that the observed difference is within the expected variability of the assay and that clinically relevant levels of triglycerides are not expected to interfere with the QFN SARS ELISA.

Clinical Performance

The clinical performance of the QFN SARS assay was evaluated in a prospective, observational study conducted from June to October 2021 using subjects without a history of SARS-CoV-2 infection who had received COVID-19 vaccination with vaccines targeting the viral S protein of SARS-CoV-2, as well as those without a history of SARS-CoV-2 infection who had not received COVID-19 vaccination.

Consenting subjects were evaluated against study inclusion and exclusion criteria, and only subjects meeting all inclusion criteria but none of the exclusion criteria were enrolled and underwent blood collection for QFN SARS.

A summary of the population enrolled is below:

- Group 1: Included subjects without a history of natural SARS-CoV-2 infection, had not received COVID-19 vaccination at time of blood collection for QFN SARS, had never tested positive for SARS-CoV-2 infection, had reported a non-reactive serology test result, and had no signs or symptoms of COVID-19 within a 4-week period before enrollment.
- Group 2: Included subjects without a history of SARS-CoV-2 infection, had received COVID-19 vaccination that targeted the S protein of SARS-CoV-2 at time of blood collection for QFN SARS, and had never tested positive for SARS-CoV-2 infection.
- None of subjects were transplant recipients (solid organ or cell) and/or on any treatment for cancer at the time of study participation.

A total of 218 subjects were enrolled into Group 1, while 171 subjects were enrolled into Group 2. After QFN SARS blood collection, four subjects in Group 1 were found to be not eligible due to a reactive serology test result obtained using a sample collected at the same visit where blood collection for QFN SARS occurred and were subsequently excluded from analysis.

Samples were collected, the QFN SARS blood collection tubes were processed, and plasma was stored at ≤ –20ºC until ready for testing with the QFN SARS ELISA. All QFN SARS ELISA plate runs were valid, and no indeterminate results were obtained, resulting in 214 and 171 evaluable samples in Groups 1 and 2, respectively.

Demographics

The number of samples collected in each country and percent of total for each study group are presented in Table 11.

Table 11. Summary of Country of Sample Collection

Country of sample collection	Group 1		Group 2	
	N	%	N	%
Netherlands	214	100.00%	153	89.47%
US	0	0.00%	18	10.53%

A summary of subject age, including mean, median, minimum, and maximum age, and age standard deviation (SD), is shown in Table 12.

Table 12. Summary of Subject Age (Years)

N	Mean	Median	SD	Minimum	Maximum
385	40.47	37.00	14.168	18.00	80.00

A summary of subject gender is provided in Table 13.

Table 13. Summary of Subject Gender

Gender	N	%
Female	234	60.78%
Male	151	39.22%

Specificity

The clinical agreement comparing QFN SARS results against the reference method results is shown in Table 14.

Table 14. Clinical Agreement: QFN SARS Result vs. Reference Method

		Reference method result		
		Group 1 (- vax, -infection)	**Group 2** (+ vax, -infection)	**Total**
QFN SARS result	**Non-reactive**	199	34	233
	Reactive	15	137	152
	Total	214	171	385

For unvaccinated subjects (Group 1), 199 of 214 tested non-reactive using QFN SARS, while the remaining 15 tested reactive. For vaccinated subjects (Group 2), 137 of 171 tested reactive using QFN SARS, while the remaining 34 tested non-reactive. None of the 15 and 34 discordant samples in Groups 1 and 2, respectively, received additional testing with a discordant method.

The negative percent agreement (NPA) (specificity) was calculated for unvaccinated subjects (Group 1), along with the two-sided 95% exact confidence interval (CI), and is presented in Table 15.

Table 15. Negative Percent Agreement (Specificity)

Group #	NPA (specificity)	95% CI
Group 1 (-vax, -infection)	92.99% (199 / 214)	88.70–96.02%

Sensitivity

The positive percent agreement (PPA) (sensitivity) was calculated for vaccinated subjects (Group 2), along with the two-sided 95% exact CI, and is presented in Table 16.

Table 16. Positive Percent Agreement (Sensitivity)

Group #	PPA (sensitivity)	95% CI
Group 2 (+vax, -infection)	80.12% (137 / 171)	73.34–85.82%

Positive Percent Agreement by Age

For vaccinated subjects (Group 2), positive percent agreement was stratified by age < 60 and ≥ 60 years and is presented in Table 17.

Table 17. Positive Percent Agreement by < 60 and ≥ 60 Years of Age

Age range (years)	PPA (Sensitivity)	95% CI
< 60	85.33% (128/150)	78.78–90.64%
≥ 60	42.86% (9/21)	21.82–65.98%

Positive Percent Agreement by COVID-19 Vaccine

For vaccinated subjects (Group 2), positive percent agreement was stratified by COVID-19 vaccine received and is presented in Table 18.

Table 18. Positive Percent Agreement by COVID-19 Vaccine

Vaccine	PPA (Sensitivity)	95% CI
Astra Zeneca	62.50% (5/8)	24.49–91.48%
Janssen (Johnson & Johnson)	86.67% (13/15)	59.54–98.34%
Moderna	77.27% (17/22)	54.63–92.18%
Pfizer - BioNTech	80.95% (102/126)	73.00–87.40%

Factors Associated with Non-reactive Results in Vaccinated Subjects

To determine if increasing age, time from completion of COVID-19 vaccination, vaccine received, and gender associates with non-reactive results in vaccinated subjects (Group 2), univariate logistic regression analysis was performed. The association between each factor and non-reactive results was calculated in terms of odds ratio (OR), and the results are presented in Table 19.

Table 19. Association Between Factors and Non-reactive Results in Vaccinated Subjects

Factor		OR (95% CI)	p-value
Age (years)		1.08 (1.05–1.12)	< 0.001
Time from Vaccination to QFN SARS Blood Collection (days)		1.02 (1.01–1.03)	< 0.001
Vaccine	**Pfizer – BioNTech**	1	–
	Astra Zeneca	2.55 (0.57–11.42)	0.221
	Janssen (Johnson & Johnson)	0.65 (0.14–3.09)	0.592
	Moderna	1.25 (0.42–3.72)	0.689
Gender	**Female**	1	–
	Male	1.25 (0.59–2.65)	0.565

The only factors significantly associated with non-reactive results in vaccinated subjects were age and time from vaccination.

Because the study was conducted in countries where the COVID-19 vaccines were made available to older individuals first, age may have impacted the association between time from vaccination and non-reactive results. Table 20 shows regression analysis with age as a covariate.

Table 20. Association Between Factors and Non-reactive Results Controlled for Age

Factor	OR (95% CI)	p-value
Age (years)	1.07 (1.03–1.11)	< 0.001
Time from Vaccination to QFN SARS Blood Collection (days)	1.01 (1.00–1.02)	0.214

When age is controlled, the association between time from vaccination and non-reactive results is no longer significant, however age remained significantly associated.

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Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
ELISA troubleshooting	
Nonspecific color development	
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b) Cross-contamination of ELISA wells	Take care while pipetting and mixing sample to minimize risk.
c) Kit/components have expired	Ensure kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.
e) Mixing of plasma in in QFN SARS Blood Collection Tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.
Low optical density readings for standards	
a) Standard dilution error	Ensure dilutions of the Kit Standard are prepared correctly as per this Instructions for Use.

	Comments and suggestions
b) Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c) Incubation temperature too low	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
d) Incubation time too short	Incubation of the plate with the conjugate, standards, and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution should be incubated on the plate for 30 minutes.
e) Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter of from 620 to 650 nm.
f) Reagents are too cold	All reagents, with the exception of the Conjugate 100X Concentrate, must be brought to room temperature prior to commencing the assay. This takes approximately 1 hour.
g) Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure that the reconstituted Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
High background	
a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required. A soak time of at least 5 seconds between cycles should be used.
b) Incubation temperature too high	Incubation of the ELISA should be performed at room temperature (22°C ± 5°C).
c) Kit/components have expired	Ensure that the kit is used within the expiry date. Ensure reconstituted standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.
d) Enzyme Substrate Solution is contaminated	Discard substrate if blue coloration exists. Ensure clean reagent reservoirs are used.

Nonlinear standard curve and duplicate variability

a) Incomplete washing of the plate	Wash the plate at least 6 times with 400 µl/well of wash buffer. More than 6 washing cycles may be required. A soak time of at least 5 seconds between cycles should be used.
b) Standard dilution error	Ensure dilutions of the standard are prepared correctly as per this Instructions for Use.
c) Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
d) Inconsistent pipetting technique or interruption during assay setup	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number (i.e., component labeling)
COMP	Components
CONT	Contains
NUM	Number
GTIN	Global Trade Item Number
EC REP	Authorized representative
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Consult instructions for use
	Keep away from sunlight
	Warning/caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Appendix A: Technical Information

Indeterminate results

Indeterminate results are uncommon and may relate to the immune status of the individual being tested, but may also be related to a number of technical factors (e.g., inappropriate handling/storage of blood collection tubes, incomplete ELISA plate washing) if the above instructions for use are not followed.

If technical issues are suspected with the reagent storage, blood collection, or handling of the blood samples, repeat the entire QFN SARS test with new blood specimens. Repeating the ELISA testing of stimulated plasmas can be performed if inadequate washing or other procedural deviation with the ELISA test is suspected. Physicians may choose to redraw a specimen or perform other procedures as appropriate.

Clotted plasma samples

Should fibrin clots occur with long-term storage of plasma samples, centrifuge samples to sediment clotted material and facilitate pipetting of plasma.

Lipemic plasma samples

Care should be exercised when pipetting lipemic samples as fatty deposits can block pipet tips.

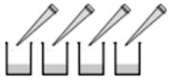
Appendix B: Abbreviated ELISA Test Procedure

1. Equilibrate ELISA components, with the exception of the Conjugate 100x Concentrate, to room temperature for at least 60 minutes.

2. Reconstitute the Kit Standard to 8.0 IU/ml with distilled or deionized water. Prepare four (4) standard dilutions.

3. Reconstitute freeze-dried Conjugate 100x Concentrate with distilled or deionized water.

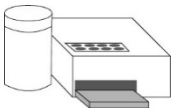
4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.



5. Add 50 µl of test plasma samples and 50 µl standards to appropriate wells. Mix using shaker.

6. Incubate for 120 minutes at room temperature.

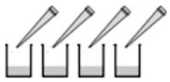
7. Wash wells at least 6 times with 400 µl/well of wash buffer.



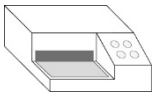
8. Add 100 µl Enzyme Substrate Solution to wells. Mix using shaker.

9. Incubate for 30 minutes at room temperature.

10. Add 50 µl Enzyme Stopping Solution to all wells. Mix using shaker.



11. Read results at 450 nm with a 620 to 650 nm reference filter.



12. Analyze results.

Ordering Information

Product	Contents	Cat. no.
QuantiFERON SARS-CoV-2 (QFN SARS) ELISA Kit	2-plate ELISA kit	626420
Related products		
QuantiFERON SARS-CoV-2 Blood Collection Tubes	200 tubes (50 each Nil, Ag1, Ag2 and Mitogen)	626725

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Description
R1, October 2021	Initial release
R2, November 2021	Updated Performance Characteristics and Clinical Performance sections
R3, April 2022	Updated Analytical Performance Characteristics section for Interfering Substances

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