March 2021

QuantiFERON® ELISA Instructions for Use (Handbook)

Σ 2 x 96

Human IFN-γ ELISA test

For Research Use Only
Not for use in diagnostic procedures

REF	626410
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R2 MAT	1122892

Sample to Insight

QIAGEN

Contents

Intended Use

The QuantiFERON ELISA (QFN) is for research use only to detect human interferon gamma (IFN-γ) in plasma by enzyme-linked immunosorbent assay (ELISA).

The QuantiFERON ELISA can be used to detect IFN-γ in plasma generated from QuantiFERON Blood Collection Tubes.

The QFN ELISA is not for use in diagnostic procedures.

Principle of the Procedure

The concentration of IFN-γ in a human plasma sample reported in International Units per ml (IU/ml) is measured by QuantiFERON ELISA.

Time required to perform the assay

The time required to perform the QFN ELISA is estimated below. The time of testing multiple samples when batched is also indicated.

- ELISA — Approx. 3 hours for one ELISA plate (up to 88 samples)
  <1 hour labor
  Add 10–15 minutes for each extra plate

Materials Provided

Kit contents

QuantiFERON® 2 Plate Kit ELISA	**2 Plate kit ELISA**
Catalog no.	626410
Microplate Strips, 12 x 8 wells	2 sets 12 x 8-well Microplate Strips
IFN-γ Standard, lyophilized	1 x vial (8 IU/ml when reconstituted)
Green Diluent	1 x 30 ml vial
Conjugate 100x Concentrate, lyophilized	1 x 0.3 ml, when reconstituted
Wash Buffer 20x Concentrate	1 x 100 ml
Enzyme Substrate Solution	1 x 30 ml
Enzyme Stopping Solution*	1 x 15 ml
Language Sheet	1

* Contains sulfuric acid. See Warnings and Precautions.

Materials Required but not Provided

- Calibrated variable-volume pipets*
- Calibrated multichannel pipet* capable of delivering 50 µl and 100 µl with disposable tips
- Microplate shaker†
- Deionized or distilled water, 2 liters
- Microplate washer (automated washer recommended)
- Microplate reader† fitted with 450 nm filter and 620 nm to 650 nm reference filter
- Graduated cylinder (measuring cylinder)
- Low-lint absorbent towels
- Plate lid

* Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Safety information

For Research Use Only. Not for use in diagnostic procedures.

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.

Precautions

CAUTION 	Handle human plasma as if potentially infectious. (C1) Observe relevant blood and blood product handling guidelines. Dispose of samples and materials in contact with blood or blood products in accordance with federal, state, and local regulations.

Hazard statements

The following hazards and precautionary statements apply to components of the QuantiFERON ELISA.

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

- Do not use the ELISA kit if any reagent bottle shows signs of damage or leakage prior to use.
- Do not mix or use the Microplate Strips, IFN-γ Standard, Green Diluent, or Conjugate 100x Concentrate from different QFN ELISA batches. Other reagents (Wash Buffer 20x Concentrate, Enzyme Substrate Solution, and Enzyme Stopping Solution) can be interchanged among kits, providing the reagents are within their expiration periods and lot details are recorded.
- Discard unused reagents and biological samples in accordance with local and national safety and environmental regulations.
- Do not use the QFN ELISA after the expiration date.
- Make sure that laboratory equipment has been calibrated/validated for use.

Storage and Handling

ELISA kit reagents

- Store ELISA kit reagents at 2–8°C.
- Always protect Enzyme Substrate Solution from direct sunlight.

Reconstituted and unused ELISA reagents

For more information about how to constitute the ELISA reagents, see Procedure.

- The reconstituted kit standard may be kept for up to 3 months if stored at 2–8°C.
- Note the date on which the kit standard was reconstituted.
- Once reconstituted, unused Conjugate 100x Concentrate must be returned to storage at 2–8°C and must be used within 3 months.
- Note the date on which the conjugate was reconstituted.
- Working strength conjugate must be used within 6 hours of preparation (see Table 1).
- Working strength wash buffer may be stored at room temperature (22°C ± 5°C) for up to 2 weeks.

Procedure

Materials required

- QuantiFERON ELISA 2 Plate Kit*

Materials required but not provided

- See Materials required but not provided.

Important points before starting

- All plasma samples and reagents, except for Conjugate 100x Concentrate, must be brought to room temperature (22°C ± 5°C) before use. Allow at least 60 minutes for equilibration.

Procedure

1. Remove strips that are not required from the microplate frame, reseal in the foil pouch, and return to the refrigerator for storage until needed.

   Note: Allow at least one strip for the QFN standards and sufficient strips for the number of subjects being tested. After use, retain frame for use with remaining strips.

2. Reconstitute the lyophilized IFN-γ Standard with the volume of deionized or distilled water indicated on the label of the Standard vial. Mix gently to minimize frothing and ensure complete solubilization.

   Note: Reconstitution of the Standard with the stated volume will produce a solution with a concentration of 8.0 IU/ml. The Standard can be used to generate an 8-point Standard Curve or a 4-point Standard Curve. Each dilution within the standard curve should be run in at least duplicate.

* See Materials Provided.

Important: The reconstitution volume of the IFN-γ Standard differs between batches. Consult the label of the standard vial to ensure that you use the correct volume of deionized or distilled water.

Use the reconstituted Kit Standard to produce a dilution series of 8 IFN-γ concentrations (see Preparation of 8-point standard curve and Figure 1), or a dilution series of 4 IFN-γ (see Preparation of 4-point standard curve and Figure 2). If a dilution series other than that described is used, each laboratory should validate the alternative approach.

Note: Prepare fresh dilutions of the Kit Standard for each ELISA session (Figures 1 and 2)

- Preparation of 8-point standard curve
  - Add 300 µl of the Kit Standard to tube labeled as Standard 1.
  - Add 150 µl of Green Diluent to 7 tubes (labeled Standard 2 to Standard 8).
  - To perform serial dilutions, transfer 150 µl of each standard to the next tube. Mix each tube thoroughly before the next transfer.
  - The undiluted Kit Standard serves as the highest concentration.

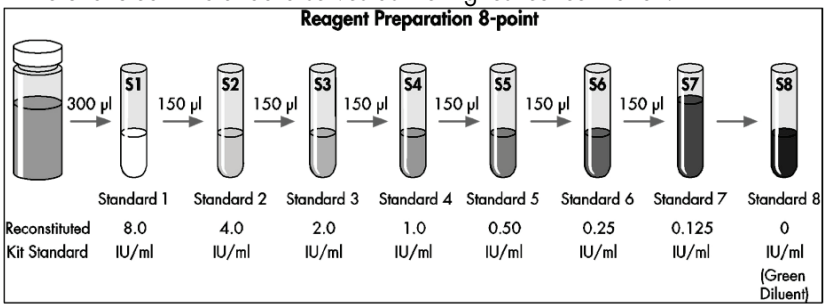


Figure 1. Preparation of 8-point standard curve

- Preparation of 4-point Standard Curve
  - Label 4 tubes as S1, S2, S3, and S4.
  - Add 150 µl of Green Diluent (GD) to S1, S2, S3, and S4.
  - Add 150 µl of the Kit Standard to S1. Mix thoroughly.
  - Transfer 50 µl from S1 to S2. Mix thoroughly.
  - Transfer 50 µl from S2 to S3. Mix thoroughly.
  - Green Diluent alone serves as the zero standard (S4).

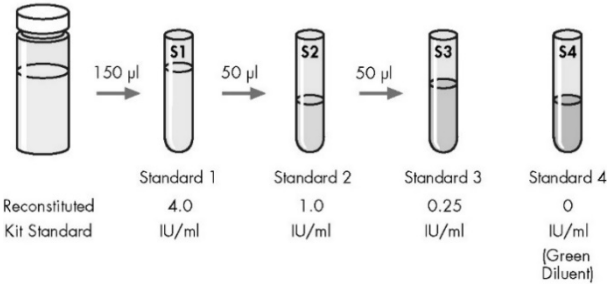


Figure 2. Preparation of 4-point standard curve

3. Reconstitute lyophilized Conjugate 100x Concentrate with 0.3 ml of deionized or distilled water. Mix gently to minimize frothing and ensure complete solubilization of the conjugate.

   Note: Working strength conjugate is prepared by diluting the required amount of reconstituted Conjugate 100x Concentrate in Green Diluent (Table 1). Return any unused Conjugate 100x Concentrate to 2–8°C immediately after use. Use only Green Diluent.

Table 1. Conjugate preparation

Number of strips	Volume of concentrate 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

4. For plasma samples subsequently stored or frozen, mix samples before addition to the ELISA well.
5. Add 50 µl of freshly prepared, working strength conjugate to the required ELISA wells using a multichannel pipet.
6. Add 50 µl of test plasma sample to appropriate wells using a multichannel pipet. Then, add 50 µl of each of standards 1 to 8, or 1 to 4; depending on the standard curve format used. Assay the standards in at least duplicate.
7. Cover each plate with a lid and mix the conjugate and plasma samples/standards thoroughly using a microplate shaker for 1 minute. Avoid splashing.
8. Incubate at room temperature (22°C ± 5°C) for 120 ± 5 minutes.

   Note: Plates should not be exposed to direct sunlight during incubation.

9. During incubation, dilute 1 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.

   Note: Wash wells with 400 µl of working strength wash buffer for at least 6 cycles in a microplate washer. An automated plate washer is recommended.

   Important: Thorough washing is very important to the performance of the ELISA. Make sure each well is completely filled with wash buffer for each wash cycle. It is recommended to soak wells for a period of at least 5 seconds between each cycle for best results.

   Add standard laboratory disinfectant to the effluent reservoir and follow established procedures for the decontamination of potentially infectious material.

10. Tap plates face down on absorbent, low-lint towel to remove residual wash buffer. Add 100 µl of Enzyme Substrate Solution to each well, cover each plate with a lid, and mix thoroughly using a microplate shaker.

11. Incubate at room temperature (22°C ± 5°C) for 30 minutes.

    Note: Plates should not be exposed to direct sunlight during incubation.

12. Following incubation, add 50 µl of Enzyme Stopping Solution to each well and mix thoroughly using a microplate shaker.

    Note: Enzyme Stopping Solution should be added to wells in the same order and at approximately the same speed as used when adding the Enzyme Substrate Solution in step 13.

13. Measure the Optical Density (OD) within 5 minutes of stopping the reaction using a microplate reader fitted with a 450 nm filter as well as a 620–650 nm reference filter. OD values are used to calculate results.

Interpretation of Results

QuantiFERON R&D Analysis Software is used to analyze raw data and calculate results. It is available at www.qiagen.com. Ensure that the most current version of the QuantiFERON R&D Analysis Software is used.

The software performs a quality control assessment of the ELISA, generates a standard curve, and provides a test result in IU/ml for each sample, as detailed in the Results section.

Generation of standard curve

As an alternative to using the QuantiFERON R&D Analysis Software, results can be determined using this method.

Determine the mean OD values of the kit standard replicates on each plate.

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.

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.

These calculations can be performed using software packages available with microplate readers and standard spreadsheet or statistical software (such as Microsoft® Excel® software). 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.

Quality control of 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:

8-point Standard

- The mean OD value for Standard 1 must be ≥1.200.
- The %CV for Standards 1 to 5 replicate OD values must be ≤15%.
- Replicate OD values for Standards 6 to 8 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.

4-point Standard

- The mean OD value for Standard 1 must be ≥0.600.
- The %CV for Standard 1 and Standard 2 replicate OD 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.

The QuantiFERON R&D Analysis Software calculates and reports these quality control parameters.

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.

Analytical performance

The QFN ELISA uses a recombinant human IFN-γ standard, which has been assayed against a reference IFN-γ preparation (NIH; Ref: Gxg 01-902-535). Results for test samples are reported in International Units (IU) relative to a standard curve prepared by testing dilution of the secondary 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 QFN ELISA is minimized by the addition of normal mouse serum to the Green Diluent and the use of F(ab')2monoclonal antibody fragments as the IFN-γ capture antibody coated to the microplate.

The QFN 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 6).

The limit of detection of the QFN ELISA is 0.065 IU/ml and there is no evidence of a high-dose hook (prozone) effect with concentrations of IFN-γ up to 10,000 IU/ml.

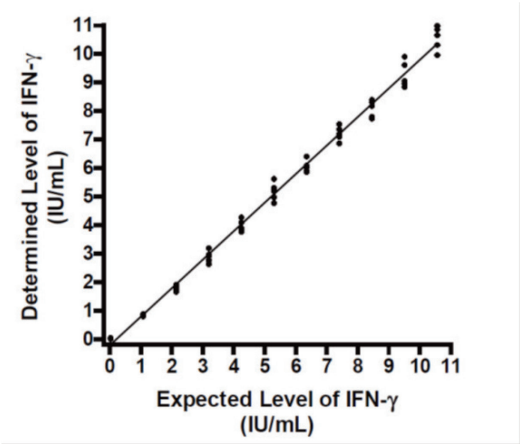


Figure 3. Linearity profile of QFN ELISA determined from testing 5 replicates of 11 plasma samples of known IFN-γ concentrations.

Technical Information

Clotted plasma samples

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

Troubleshooting Guide

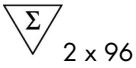
This troubleshooting guide may be helpful in solving any problems that may arise. For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/support (for contact information, visit www.qiagen.com)

		Comments and suggestions
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 when pipetting and mixing sample to minimize risk.
c)	Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 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 QFN 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.
b)	Pipetting error	Ensure pipets are calibrated and used according to manufacturer's instructions.
c)	Incubation temperature too low	Incubation of ELISA should be performed at room temperature (17–27°C).
d)	Incubation time too short	Incubate the plate with the conjugate, standards, and samples for 120 ± 5 minutes. Incubate the Enzyme Substrate Solution on the plate for 30 minutes.
e)	Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter between 620 and 650 nm.

		Comments and suggestions
f)	Reagents are too cold	All reagents, with the exception of the Conjugate 100x Concentrate, must be brought to room temperature prior to starting the ELISA. This takes approximately one hour.
g)	Kit/components have expired	Ensure that the kit is used before the expiry date. Ensure reconstituted standard and Conjugate 100x Concentrate are used within 3 months of their 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 depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
b)	Incubation temperature is too high	Incubation of the ELISA should be performed at room temperature (17–27°C).
c)	Kit/components have expired	Ensure that the 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.
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 depending on the washer being used. 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 package insert.
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 ELISA setup	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to starting the ELISA.

Symbols

The following table describes the symbols that may appear on the labeling or in this document:

Symbol	Description
	Sufficient for 2 x 96 sample preparations
	Legal manufacturer
	Batch code
	Catalog number
	Material number (i.e., component labeling)
	Use by date
	Temperature limitation
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

Contact Information

For technical assistance and more information, please call toll-free 800-362-7737, see our Technical Support Center at www.qiagen.com/contact or contact one of the QIAGEN Technical Service Departments (see back cover or visit www.qiagen.com).

Appendix A – Abbreviated 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 standard curve dilutions.
3. Reconstitute freeze-dried Conjugate 100x Concentrate with distilled or deionized water

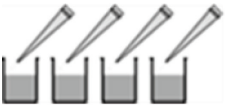
4. Prepare working strength conjugate in Green Diluent and add 50 µl to all wells.

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

6. Incubate for 120 ± 5 minutes at room temperature.

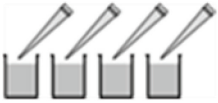
7. Wash wells at least 6 times with 400 µl/well of wash buffer.

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

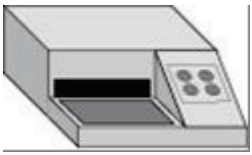


9. Incubate for 30 minutes at room temperature.

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



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



12. Analyze results.

Ordering Information

Product	Contents	Cat. no.
QuantiFERON ELISA	Contains Microtiter Plate, Conjugate (100x), IFN Gamma Standard, Green Diluent, Wash Buffer, Enzyme Substrate Solution, and Enzyme Stopping Solution	626410
Relative Products		
QuantiFERON SARS-CoV-2 Starter Pack	Contains QuantiFERON Starter Set (cat. no. 626115 SARS-CoV-2 Ag1 tube and SARS-CoV-2 Ag2 tube), and QuantiFERON Control Set (cat. no. 626015 Nil and Mitogen	626715
QuantiFERON Control Set	Contains Nil tube, and Mitogen Tube	626015
QuantiFERON Monitor Direct	Contains Monitor Direct Tube	626315

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	Changes
R1, November 2020	Initial release
R2, February 2021	Updated Ordering Information section

Limited License Agreement for QuantiFERON® ELISA

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