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QuantiFERON[®] SARS-CoV-2 Analysis Software User Manual

For use with QuantiFERON[®] SARS-CoV-2 ELISA Kit

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1 Introduction

This guide contains all the information required to download the QuantiFERON® SARS-CoV-2 (QFN SARS) Analysis Software. The QuantiFERON SARS-CoV-2 Analysis Software is a PC-based program for analyzing QFN SARS ELISA raw data. The software may be downloaded from www.qiagen.com. Alternatively, contact your local QIAGEN representative or authorized QuantiFERON SARS-CoV-2 distributor.

1.1 About this user manual

This user manual provides information about the QFN SARS Analysis Software in the following sections:

- Introduction
- General Description
- Installation Procedures
- Operating Procedures
- Maintenance
- Troubleshooting

1.2 General information

1.2.1 Technical assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QFN SARS Analysis Software or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance, contact QIAGEN Technical Services.

Website: support.qiagen.com

When contacting QIAGEN Technical Services about errors, please have the following information ready:

- QFN SARS Analysis Software version
- Error code (if applicable)
- Timepoint when the error occurred for the first time
- Frequency of error occurrence (i.e., intermittent or persistent error)
- Copy of log files

1.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time. In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.3 Intended use of the QuantiFERON SARS-CoV-2 Software

The QFN SARS Analysis Software is for optional use with QuantiFERON ELISA.

For in vitro diagnostic use.

Note: The QFN SARS assay and software is to be used by trained personnel in a professional laboratory environment.

1.3.1 Requirements for the QFN SARS Analysis Software

The table below shows the system requirements for QFN SARS Analysis Software:

Table 1. System requirements

Description	Minimum requirement
Operating system	Microsoft® Windows 10
Processor	Intel Pentium processor, or equivalent 1-GHz processor or higher, dependent on operating system
Main memory	1 GB ram or higher
Hard disk space	Windows, with at least: <ul style="list-style-type: none">● 5 MB available hard disk space
Monitor	1024 x 768 pixels or higher

1.4 Software specifications

QuantifERON SARS-CoV-2 Analysis Software, version 1.1.0 (including all sub-versions).

3 General Description

3.1 Software features

The QFN SARS Analysis Software is a PC-based program for analyzing QFN SARS ELISA raw data.

The following features are available in the QFN SARS Analysis Software version 1.1.0:

- Record assay-related information
- Automatically import or manually enter raw data
- Highlight standards and samples to create an Analysis Format
- Save Analysis Format for use with future assays
- Assign subject's identification to each sample
- Obtain quality control analysis of the standard curve
- Export data and results to other applications

4 Installation Procedures

4.1 Software installation from website

The most recent version of QuantiFERON SARS CoV-2 (QFN SARS) Analysis software is available for download at www.qiagen.com. Navigate to the QuantiFERON SARS-CoV-2 product webpage at www.qiagen.com.

To ensure proper installation of the software, the following requirements must be met:

- The system used to install the software has sufficient capacity to store the files created by the application.
- An antivirus (malware protection) software shall be installed on the computer where the user installs the application.

4.1.1 Downloading the QFN SARS Analysis Software

Note: To download the software, ensure that you are logged in to your QIAGEN account.

1. In the QuantiFERON SARS-CoV-2 product page, go to Resources > Operating Software.
2. Click QuantiFERON SARS-CoV-2 Analysis Software to start the download.
3. After the download is complete, save the downloaded zip file to your preferred location within the computer.

4.1.2 Installing the QFN SARS Analysis Software

To install the software:

1. Navigate to the folder where the zip file is stored.
2. Extract the contents of the zip file in the same location.
3. Double-click `Qiagen.QuantiFERON-SARS-COV-2`.

Note: The User Access Control dialog box must show QIAGEN GmbH as the verified publisher. If this is not shown, cancel the software installation and contact QIAGEN Technical Support.

4.2 Getting started

To open the QFN SARS Analysis Software, double-click the QFN SARS shortcut or directly from the downloaded file.

4.3 Software upgrade

You may need to update the QFN SARS Analysis Software in case a new version becomes available. Software updates for QFN SARS Analysis Software are available for download in the QuantiFERON SARS-CoV-2 product webpage at www.qiagen.com. Go to Resources > Operating Software to download the latest QFN SARS Analysis Software version. Save the downloaded file in your preferred location.

For more information about updating the software, see *Maintenance*.

5 Operating Procedures

5.1 Starting the QFN SARS Analysis Software

From your Home screen, double-click the QFN SARS icon on your desktop to open the QFN SARS Analysis Software.

The program will open to the first of four screens that sequentially progresses through the calculations.

- Run Details
 - Enter general test details such as Run Date, Run Number, Kit Batch Number, and Operator.
- Raw Data
 - Enter Optical Density (OD) values and apply a format that defines the standards and samples.
- Standards Results
 - View standard curve results, which indicate the validity of the ELISA.
- Subject Results
 - View test results for each sample. Save, print, and export data and results.

For more information about these screens, see Screens in the QFN SARS Analysis Software.

5.2 Screens in the QFN SARS Analysis Software

5.2.1 Run Details screen

Perform these steps in the Run Details screen:

1. Use the drop-down calendar to enter the run date.
2. Enter the kit batch number.
Note: The kit batch number is shown on the outer box label of the QFN SARS ELISA.
3. Enter the run number.
4. Enter the Operator.
5. Click the Raw Data tab or click Next in the lower right corner to move to the next screen.

5.2.2 Raw Data screen

The QFN SARS Analysis Software uses optical density (OD) values as the basis for all calculations. The user does not need to perform any calculations prior to using the software. Simply enter the raw data from the plate reader into the software.

There are two methods of data entry: automatic data entry and manual data entry.

Automatic data entry

Copy the raw data (OD values) to be analyzed from the ELISA plate reader program. Some plate reader programs require the data to be exported first into a spreadsheet.

Select Paste Raw Data. The data will be entered into the program's data cells.

Data from plates with less than 12 strips can be analyzed; however, each strip of data pasted must contain 8 values (including empty cells, if necessary). Data cells for standards cannot be blank or contain text. If such situation arises, the analysis software will report this as an Invalid ELISA.

Due to the logarithmic calculations performed by the software, negative OD values cannot be analyzed. Negative OD values are not normally obtained for the QFN SARS ELISA and may indicate the need to service the plate reader.

Manual data entry

Select Manual Data Entry. Click a cell to enter data manually.

When all data have been entered, click Complete on the Manual Data Entry toolbar to proceed.

Important: It is critical to compare the original raw OD data/format with data on the report, as errors in manual data entry (or copy/paste errors) can cause incorrect report results.

5.2.3 Analysis Format

Before data can be analyzed, users must apply a format to select the cells that contain samples and those that are standards. This format is assigned using the manual format option.

Manual format

The Manual Formatting Toolbar is used to manually assign both standards and subject samples to the data's format. By default, the toolbar opens in "Standards" mode with the standards ready to be assigned in a vertical orientation. The settings can be changed by selecting the appropriate radio buttons.

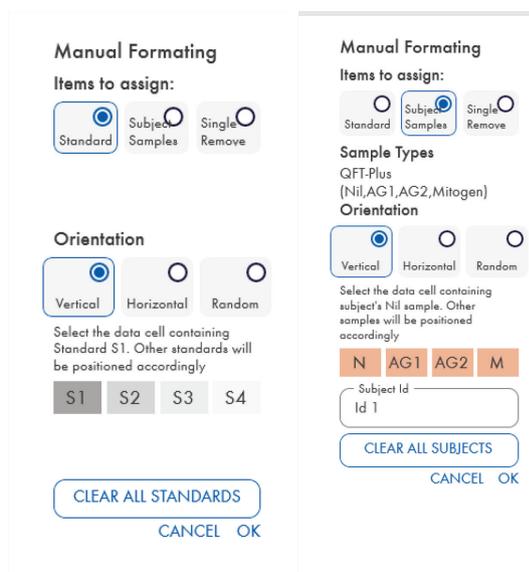


Figure 1. Manual Formatting Toolbar in "Standards" mode (left) for the Standard Curve and "Subject Samples" mode (right).

5.2.4 Standards screen

Standard S1 is the highest standard, containing 4.0 IU/ml of interferon-gamma (IFN- γ). Standard S4 is the lowest standard, containing 0 IU/ml of IFN- γ .

Once the set of standards, S1 to S4, has been assigned, the toolbar resets, ready to automatically assign another set of standards.

The standard orientation can be adjusted at any time, allowing replicates of standards to have different orientations in one format.

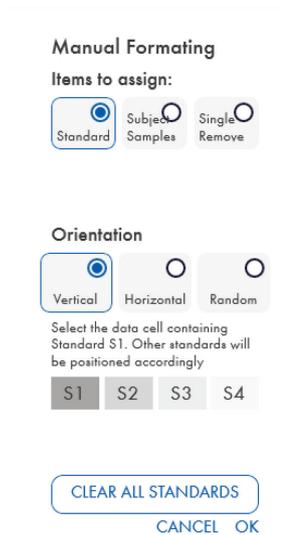


Figure 2. Manual Formatting Toolbar in “Standards” mode for the Standard Curve.

- To assign a set of standards (S1, S2, S3, S4), within “Standards” mode, select Vertical or Horizontal in the Standards Orientation list. Then, click the cell in the Raw Data screen that contains the data for standard S1. The selected cell is designated as S1, and the other standards will be appropriately positioned in adjacent cells in order.
- To assign a set of standards in a random manner, select Random in the Standards Orientation list. Then, click the appropriate cells within the Raw Data screen to manually position each of the standards S1 to S4 in order.
- To delete a single set of standards, click Single Remove and select the cells you want to delete.
- Alternatively, select Standards and click Clear All Standards on the Manual Formatting Toolbar to delete all standards.

5.2.5 Subject (patient) samples

- To assign subject samples to the data, select Subject Samples in the Manual Formatting Toolbar.

For Subject Samples mode, the subject’s name/ID can be entered into the Subject ID field on the toolbar prior to assigning a sample to the data. Alternatively, subject naming can be performed in the Raw Data screen: Subject names, page 15.

If a subject's name/ID is not entered, the first selected cell will be automatically designated as ID1. Each following sample selected will be designated sequentially as ID2, ID3... unless designated with a different Sample ID.

15To delete a single set of subjects, click **Single Remove** and select the cells you want to delete.

Alternatively, select **Subject Samples** and click **Clear All Subjects** on the **Manual Formatting Toolbar** to delete all subjects.

After the standards and subject samples have been assigned, click **OK**. Upon completing a format, it can be saved as a file and reloaded for analysis of future data, allowing the user to create just a few format files for all of their analysis needs. See **Saving and loading files**, page 19 for more information.

To delete all standards and subject samples, right-click any colored block and select **Clear Format** from the context menu.

Non-format information, such as run details and subject (patient) names, is not retained as part of the saved format file. These details are, however, retained as part of all saved result files.

5.2.6 Raw Data screen: Subject names

Subject names can be up to 15 characters in length. For this reason, they are not displayed on the Raw Data screen. Instead, the stored subject names can be viewed by clicking the cell.

Subject names can be changed at any stage. To change a subject name, click a cell and enter the new name in the ID field of the **Change ID** dialog box that appears.

To assign subject samples in a random manner, each of the samples must be positioned manually by clicking the appropriate cells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1N 0.007	3N 0.006	5N 0.019	7N 0.3	9N 0.3	11N 0.3	13N 0.3	15N 0.3	17N 1.285	S1 1.183		
B	1Ag1 0.224	3Ag1 0.175	5Ag1 0.060	7Ag1 3.3					17Ag1 OVRFLW	S2 0.375		
C	1Ag2 0.226	3Ag2 0.175	5Ag2 0.055	7Ag2 3.0					17Ag2 OVRFLW	S3 0.094		
D	1M 3.826	3M 3.707	5M 1.499	7M OVRFLW					17M 3.693	S4 0.004		
E	2N 0.365	4N 0.143	6N 0.006	8N 3.520	10N 1.971	12N 0.173	14N 3.647	16N 2.511	18N 0.210	S1 1.245		
F	2Ag1 3.412	4Ag1 2.355	6Ag1 1.012	8Ag1 OVRFLW	10Ag1 OVRFLW	12Ag1 OVRFLW	14Ag1 OVRFLW	16Ag1 OVRFLW	18Ag1 OVRFLW	S2 0.360		
G	2Ag2 3.575	4Ag2 2.511	6Ag2 1.253	8Ag2 OVRFLW	10Ag2 OVRFLW	12Ag2 OVRFLW	14Ag2 OVRFLW	16Ag2 OVRFLW	18Ag2 3.955	S3 0.089		
H	2M 3.827	4M 3.883	6M 3.867	8M OVRFLW	10M 3.901	12M OVRFLW	14M OVRFLW	16M 3.892	18M 3.964	S4 0.006		

Change Id

Id:

OK Cancel

Figure 3. Viewing Subject ID by clicking the cell.

After the format is generated, click Calculate. The standard curve for the assay will be automatically analyzed, and the Standards Results screen will be displayed. For the Calculate function to be enabled, at least two blocks of Standards and one Subject Samples block must be assigned.

5.2.7 Standards results screen: Quality control of standard curve

The accuracy of test results is dependent on the accuracy of the standard curve. The software automatically performs quality control (QC) analysis of the standard curve prior to interpreting test sample results.

The Standards Results screen provides information that is directly related to the acceptance criteria of the ELISA:

- Mean of the replicate standards
- Coefficient of variation (%CV) of the replicate standards
- Correlation coefficient of OD values and known IFN- γ concentrations (Conc)

The results of the QC acceptance criteria for the Standard Curve are shown as PASS or FAIL. For more information about the acceptance criteria, see the *QuantiFERON SARS-CoV-2 ELISA Instructions for Use*.

The following information is displayed:

- A graph of the Standard Curve, including linear regression line
- Intercept and slope of the linear regression

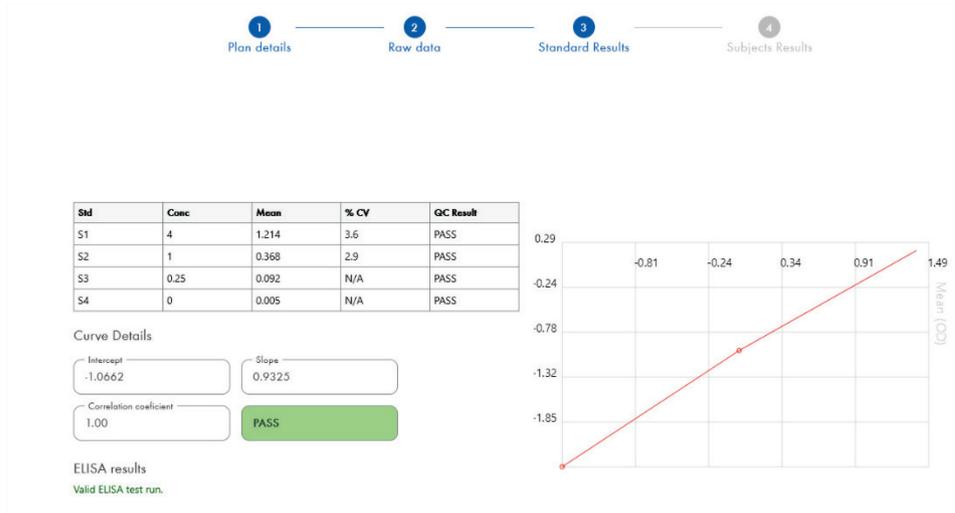


Figure 4. Standards Results screen.

A message indicating whether the ELISA is “Valid” or “Invalid” based on the QC criteria is provided in the ELISA Results section (bottom left corner of the screen). This message is also displayed on all printed and PDF reports.

Important: If any of the QC criteria are not met, the ELISA test run is “Invalid” and must be repeated.

In the event that the Mean value of the zero standard (zero IFN- γ) is greater than 0.150 OD units, a message is displayed suggesting that the ELISA plate washing procedures be investigated. This message is also displayed on all printed and PDF reports.

To proceed to the next screen, click the Subject Results tab.

5.2.8 Standard curve

The standard curve is used to calculate a value (IU/ml) for each patient’s sample.

The result “Data Missing” is reported if any of a patient’s plasma sample display the value N/S (No Sample).

Samples that have results beyond the linear range of the assay are reported as “>10 IU/ml” and are flagged using the ¶ or # symbols. The ¶ symbol indicates that the result is outside the linear range of the assay. The # symbol indicates that a value outside the plate reader range was used to

determine the result – non-numerical characters include “OUT” or “***”. In the case of non-numerical entries, an OD of 4.000 is used to calculate the IU/ml result.

5.3 Data export

Results and/or data can be exported through Windows Clipboard or structured text file to external spreadsheet applications, such as Microsoft® Excel®.

To export results the following steps should be followed:

1. Click the Export Results button.

The Export Type dialog box appears.

2. Select your preferred export type and click OK.

- Clipboard: If you select this option the data will be copied to clipboard and can be pasted into a spreadsheet.
- File: If you select this option, another dialog box appears enabling you to save the results as a file on your computer. Click Save to proceed. The data are then saved as a .txt file.

Similarly, the Data Export tab enables you to export the assay details, raw data, and QC results to either the Windows Clipboard or a text file. The process for exporting data using the Export Data button is the same procedure described to export results.

Note: The optional step of exporting data is not required to obtain QFN SARS ELISA results. It may be utilized by the user for pooling and trending data. Take care when pasting data into spreadsheet programs, due to the possibility of the spreadsheet’s default formatting affecting the presentation of the data.

5.4 Reports

The Print tab displays a print screen that is divided into two sections. The upper section shows the available printing options, while the lower section shows a summary report of the ELISA details and results.

Important: It is critical to compare the original raw OD data/format with data on the report, as errors in manual data entry or copy/paste errors can cause incorrect results.

- Select one of the following options in the Report Type list to print a particular report:

- All Subjects (Group Report): This option prints the results for all subjects on one page. The Raw OD values used to generate the Standard Curve are highlighted (bold and underlined) in this report.
 - All Subjects (Individual Report): This option prints the results for each subject on a separate page.
 - Single Subject Report: This option prints the results for one subject, as selected from the list.
- To generate an additional report page that includes the plate layout and standard curve, select Print Standard Curve and Plate Formatting.
 - To close the print screen and return to the main software, click Close Print Window.
 - Alternatively, click Save As PDF to save reports as PDF files. For more information, see Saving and loading files, page 19.
 - After the desired type of summary report is selected, click Print to print the report.

Note: The upper range of the QuantiFERON ELISA is 10 IU/ml. Therefore, samples determined to have an IFN- γ concentration greater than this range are reported as >10 IU/ml.

5.5 Saving and loading files

5.5.1 Saving files

Upon opening the QFN SARS Analysis Software for the first time, the software creates the folder path My Documents\QuantiFERON or Documents\QuantiFERON depending on your Windows operating system. By default, all files are saved to subfolders within this folder and are given default file names (Table 2).

Table 2. File names and extensions

File type	File extension	Sub-folder name	Default file name
Format	.qff	Format	OperatorDate
Results	.qdf	Save	Date_RunNumber
PDF results	.pdf	PDF	Date_RunNumber

File types

- **Format files:** Select Save Format to save a completed format to file which can be reloaded for use with future analysis.

Note: The Run Details information is not retained within a saved format file.

- Results files: Select Save File to save a copy of the results to file which can be reloaded for further analysis.

Note: The Run Details information is retained within a saved result file.

- PDF files: Select Save As PDF to save the results report in PDF format for electronic viewing by others. It is recommended that PDF files be used for record-keeping purposes.

Note: PDF files contain all of the information available in the printed report.

5.5.2 Loading files

- Select Load Format to reload the format files within the QFN SARS Analysis Software.
- Results can be reloaded by selecting Load File at any time.
- After reloading a results file, click Calculate to regenerate results.

5.6 End of analysis

The software enables you to work on one run at a time (single session mode). Use the New Test feature to work on a second run without restarting the software.

- Click New Test to clear all entered information. This enables new assay data to be analyzed.
- Click Close to close the program.

For your convenience, the information previously entered into the Run Date, Kit Batch Details, and Operator fields on the Run Details screen is retained as default until the software is closed.

Note: These details can be modified as required.

6 Maintenance

6.1 Upgrading the QFN SARS Analysis Software

You may need to update the QFN SARS Analysis Software in case a new version becomes available.

6.1.1 Uninstalling the existing QFN SARS Analysis Software version

Note: Before you upgrade the software, ensure that you have uninstalled the existing version.

To uninstall the existing software version:

1. From your Home screen, go to Control Panel > Programs and Features.
2. In the Uninstall or change a program window, select the file for the existing QFN SARS Analysis Software version installed in your computer.
3. Click Uninstall.
4. Wait until the uninstallation status becomes completed.
5. In case the application icon stays in the Start menu, right click the icon and select Uninstall.

6.1.2 Downloading the QFN SARS Analysis Software version update

Software updates for QFN SARS Analysis Software are available for download at the QuantiFERON SARS-CoV-2 product webpage at www.qiagen.com.

Note: You will be notified when a software upgrade is needed.

To download the software version update:

1. In the QuantiFERON SARS-CoV-2 product webpage, go to Resources > Operating Software.
Note: You must be logged in to your QIAGEN account to download the software.
2. Click QuantiFERON SARS-CoV-2 Analysis Software to start the download.
3. After the download is complete, save the downloaded zip file to your preferred location within your computer.

6.1.3 Installing the QFN SARS Analysis Software version update

Note: Before you install the software version update, ensure that you have uninstalled the existing version of the QFN SARS Analysis Software in your computer. For more information, see [Uninstalling the existing QFN SARS Analysis Software version](#).

1. Navigate to the folder where the downloaded zip file is located.
2. Extract the contents of the zip file to the same location.
3. Double-click the installer file contained in the zip file.

7 Troubleshooting

This section provides information about what to do if an error occurs when using the QFN SARS Analysis Software.

If further assistance is required, contact QIAGEN Technical Services using the contact information below:

Website: support.qiagen.com

When contacting QIAGEN Technical Services about an error with the QFN SARS Analysis Software, note the steps leading up to the error and any information appearing in any dialog boxes. This information will help the QIAGEN Technical Services solve the problem.

When contacting QIAGEN Technical Services about errors, please have the following information ready:

- QFN SARS Analysis version
- Timepoint when the error occurred for the first time
- Frequency of error occurrence (i.e., intermittent or persistent error)
- Detailed description of the error situation
- Photo of the error, if possible
- Copy of log files

This information will help you and your QIAGEN Technical Service Specialist to deal most efficiently with your issue.

Note: Information about the latest software and protocol versions can be found at www.qiagen.com. In some cases, updates may be available for addressing specific problems.

7.1 Error and warning messages

Table 3. Error messages

Error	Description	Frequency
This field is required.	The specified field has to have a value.	This error is displayed under the empty mandatory fields from Run details (Kit batch number, Run number, Operator)
Wrong data on clipboard	Invalid data on clipboard. Data not pasted.	This error is displayed as a popup when the user tries to paste raw data that are invalid. This typically occurs due to the user importing an incorrect decimal separator for the language convention used.
Wrong data on clipboard (for negative values)	Negative values on clipboard. Data not pasted.	This error is displayed as a popup when the user tries to paste raw data that contain negative values.
Invalid data	The data provided cannot be calculated. Please review values entered.	This error is displayed as a popup when the user tries to conduct the calculation using a loaded test which contains invalid data (negative values, etc.)
Invalid cell	Only one standard/sample block per data cell. Please select a different cell.	This error is displayed as a popup when the user tries to apply a manual format to a cell already formatted.
ELISA results warning	WARNING: QC criteria not met (refer to Package Insert; 'Quality Control of Test'). ELISA is invalid and should be repeated.	This error is displayed under the ELISA results field if the QC criteria failed after performing calculation.
Warning regarding plate washing	NOTE: The mean OD value from the zero standard should be less than 0.150. Plate washing procedures should be investigated.	This error is displayed under the ELISA results field if the criteria failed after performing calculation.

8 Ordering Information

Product	Contents	Cat. no.
QuantifERON SARS-CoV-2 Analysis Software	N/A	1125128
Relative Products		
QuantifERON ELISA Kit	Contains Microplate strips, IFN- γ Standard, Green Diluent, Conjugate (100x), Wash Buffer, Enzyme Substrate Solution, and Enzyme Stopping Solution	626420

For up-to-date licensing 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.

9 Document Revision History

Date	Changes
R1, November 2021	Initial release
R2, March 2022	Updated Error and warning messages section to add information about Wrong data on clipboard error
R3, April 2022	Updated QuantiFERON SARS-CoV-2 Analysis Software version from 1.0.0 to 1.1.0

Limited License Agreement for QuantiFERON® SARS-CoV-2 Analysis Software

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this Instructions for Use and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this Instructions for Use, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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