QuantiFERON®-TB Gold Plus Analysis Software (v2.71) Instructional Guide

For installation, setup, and use of the QuantiFERON-TB Gold Plus Analysis Software
QuantiFERON-TB Gold Plus Analysis Software is for use with QuantiFERON-TB Gold Plus ELISA and blood tubes. For comprehensive instructions for use, please refer to the QuantiFERON-TB Gold Plus ELISA Package Insert, available in up to 27 different languages, at www.QuantiFERON.com.

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.

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1 Introduction

This guide contains all the information required to download QuantiFERON-TB Gold Plus (QFT®-Plus) Analysis Software, Version 2.71. QuantiFERON-TB Gold Plus Analysis Software is a PC-based program for calculating QuantiFERON-TB Gold Plus (QFT-Plus) test results. The software may be downloaded from the www.QuantiFERON.com website. Alternatively, contact your authorized QuantiFERON distributor to obtain a copy via email.

Customers will be advised by QIAGEN or their QuantiFERON distributor as new editions of the software are made available.


Table 1. Release information

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1.1 General information

1.1.1 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 QuantiFERON-TB Gold Plus 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, call one of the QIAGEN Technical Services Departments (see back cover).

For up-to-date information about QuantiFERON-TB Gold Plus, visit www.QuantiFERON.com.

1.1.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change the specifications of products at any time.

1.1.3 Version management

This document is the QuantiFERON-TB Gold Plus Analysis software (v2.71) Instructional Guide version 1.0.
1.2 Intended use of QuantiFERON-TB Gold Plus Software

QuantiFERON-TB Gold Plus Analysis Software is for optional use with the QuantiFERON-TB Gold Plus ELISA.

1.3 Requirements for QuantiFERON-TB Gold Plus Software users

System requirements:
Intel® Pentium® processor, or equivalent
Microsoft® Windows® 7 or 8
1-GHz processor or higher, dependent on operating system
1 GB RAM or higher
5 MB available hard disk space
Minimum screen resolution set to 800 x 600 pixels, but higher resolution is recommended

1.4 Software specifications

QuantiFERON-TB Gold Plus Analysis Software
Version 2.71
2 Installation

2.1 Software installation

2.1.1 Software installation from website

The most recent version of QuantiFERON-TB Gold Plus Analysis software is available for download at www.QuantiFERON.com under Technical Resources. In order to download the software you must enter your contact information, read and accept the terms of the End User License Agreement, and submit. The download screen will then appear and the software *.exe file can be saved to an appropriate location on the computer’s hard drive. First download the *.exe file from the website and save “QFT Plus v.2.71.exe” to a location on your hard drive. In addition, you may create a shortcut on your desktop. Start the software by double clicking on “QFT Plus v2.71.exe” or shortcut. During the very first startup, the software will create a folder “QuantiFERON” and subfolders on your personal directory (e.g., “My Documents\QuantiFERON”, depending on your computer operating).
3 Software Features

QuantiFERON-TB Gold Plus Analysis Software is a PC-based program for calculating QuantiFERON-TB Gold Plus (QFT-Plus) ELISA results.

Software features:

- Record test-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 tests
- Assign subject's identity to each sample
- Quality control analysis of standard curve
- Export data and results to other applications
- Selection of reporting options
4 Getting Started

4.1 Starting the QFT-Plus v2.71 software

Double click on the QFT-Plus v2.71 Software shortcut, or directly on the *.exe file, to open the QuantiFERON-TB Gold Plus Analysis Software.

The program will open to the first of 4 screens that sequentially progress through the calculations. These 4 screens are:

1. Run Details
   Enter general test details such as the Run date, Run number, Kit batch number, and Operator.

2. Raw Data
   Enter Optical Density (OD) values and apply a format that defines the standards and samples.

3. Standards Results
   View Standard Curve results, which indicate the validity of the ELISA.

4. Subject Results
   View test results for each sample. Save, print, and export data and results.

The four screens are described in more detail below and on the following pages.

4.1.1 Run details screen

![Image of language selection screen]

Figure 1. Language selection screen.
Enter the following information in the fields provided:

- **Run Date** (drop-down calendar)
- **Run Number**
- **Kit Batch Number** (shown on QuantiFERON-TB Gold Plus ELISA outer box label)
- **Operator**

Select the “Raw Data” tab to advance to the next screen.

### 4.2 Raw data screen

![Raw data screen](image)

**Figure 3.** Raw data screen.

**Figure 2.** Run details screen.
4.3 Data entry
The QuantiFERON-TB Gold Plus 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.

4.3.1 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 first be exported into a spreadsheet.

Select the “Paste Raw Data” button — the data will be entered into the program’s data cells.

Figure 4. Raw data screen with “Paste Raw Data” highlighted.
Figure 5. Raw data screen after pasting raw data.

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 a 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 QuantiFERON-TB Gold Plus ELISA, and may indicate the need to service the plate reader.

4.3.2 Manual data entry

Select the “Manual Data Entry” button. Click on a cell to enter data manually. Press “Enter” — or click on another cell — to store the value.

When all data have been entered, select the “Complete” button on the “Manual Data Entry” toolbar to proceed.
Select the “Manual Data Entry” button.

Select the “Complete” button on the “Manual Data Entry” toolbar.

Use ↑/↓ arrows — or the mouse — to navigate between cells.

Note: Manual entry is more prone to errors, and the user should take additional care to ensure data entry accuracy.

Figure 6. Raw Data screen while manually entering raw data.

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.
4.4 Analysis format

Before data can be analyzed, users must apply a format to nominate which cells are samples and which are standards.

There are two methods for assigning a format.

4.4.1 Default format

Select the “Default Format” button to automatically assign the relevant QuantiFERON-recommended testing layout to the data. The standards and samples will be set out in the same configuration as outlined in the QuantiFERON-TB Gold Plus ELISA Package Insert.
The format can be applied either before or after data entry. This allows formats to be prepared prior to obtaining the ELISA results.

Depending on the number of strips of data entered, the Default Format option may or may not be available, due to the location/orientation of samples and standards for each QuantiFERON-TB Gold Plus ELISA method.

1. Once the Default Format has been applied it can be edited by selecting the “Manual Format” button and following the instructions outlined below.
4.4.2 Manual format

1 The “Manual Formatting Toolbar” is used to manually assign both standards and subject samples to the data’s format.

2 By default, the toolbar opens in Standards mode with standards ready to be assigned in a vertical operation. These settings can be changed by selecting the appropriate radio buttons.

Figure 9. Manual Formatting Toolbar, in “Standards mode” and “Subject Samples mode”.
4.5 Standards

1. To assign a set of standards (S1, S2, S3, S4) — either vertically or horizontally — click on the cell that contains the data for standard S1. The chosen cell will be 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, each of the standards S1 to S4 must be positioned manually by clicking on the appropriate cells, in order.

2. To delete a single set of standards, right-click on the colored block and select “Delete Block” from the menu.

3. Alternatively, to delete all standards, select the “Clear All Standards” button on the Manual Formatting Toolbar.

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 entire 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 the one format.
4.6 Subject samples

4. In order to assign subject samples to the data, select the “Subject Samples” radio button on the Manual Formatting Toolbar.

5. To assign subject samples — either vertically or horizontally — click on the cell that contains the data for the subject’s Nil sample. The chosen cell will be designated as Nil, and the other samples will be appropriately positioned in adjacent cells, in order.

To assign subject samples in a random manner, each of the samples must be positioned manually by clicking on the appropriate cells.
6 Prior to assigning a sample to the data, the subject’s name/ID can be entered into the “Subject ID” field on the toolbar.

Alternatively, subject naming can be performed according to the instructions in the next section.

To delete a single subject sample, right-click on the colored block and select “Delete Block” from the pop-up menu.

7 Alternatively, to delete all subject samples, select the “Clear All Samples” button on the Manual Formatting Toolbar.

8 Once the standards and subject samples have been applied, finish by selecting the “Complete” button. 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.

Refer to Saving/Loading Files section for further details.

By default, Subject Sample mode opens with Nil, TB1, TB2, and Mitogen samples ready to be assigned in a vertical orientation. Settings can be changed by selecting the appropriate radio (round) buttons.

Once the entire subject sample has been assigned, the toolbar is automatically ready to assign another sample of the same type. Subsequent subject samples are colored differently in order to assist recognition of individual subjects.

The Sample Type and Sample Orientation can be adjusted at any time in order to create a format containing a mixture of different QuantiFERON-TB Gold Plus sample layouts.

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

Non-format information — such as run details and subject names — is not retained as part of the saved format file. These details are, however, retained as part of all saved result files.
5 Raw Data Screen

5.1 Subject names

As subject names can be up to 15 characters in length, they are not displayed on the Raw Data screen. Instead the stored subject names can be viewed via the “View Names” button.

In order for the “Calculate” button to be enabled at least 2 blocks of Standards and one Subject Sample block must be assigned.

9 Subject names can be changed at any stage by left-clicking on the colored block for each subject and typing the new name in the “Change Subject ID” dialog box.

10 Alternatively, multiple Subject Names (IDs) can be changed more easily by selecting the “View Names” button. If all subject names are to begin with an identical prefix (e.g., A009), then these characters can be entered into the “ID Prefix” field. Afterwards, left click on each subject’s name in the list to add the remainder of the name manually.

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

11 Once the format has been generated, select the “Calculate” button. The standard curve for the assay will be automatically analyzed, and the Standards Results screen will be displayed.
6 Standards Results Screen

The accuracy of test results is dependent on the accuracy of the standard curve. The software automatically performs Quality Control analysis of the standard curve prior to interpreting test sample results.

6.1 Quality control analysis of standard curve

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 Quality Control acceptance criteria for the Standard Curve are shown as PASS or FAIL.

2 The following information is also displayed:

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

For further details of the acceptance criteria, refer to QuantiFERON-TB Gold Plus ELISA Package Insert.
3 A statement indicating whether the ELISA is Valid or Invalid — based on the QC criteria — is shown at the bottom of the screen. This statement is also displayed on all printed and PDF reports. 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 statement is displayed suggesting that ELISA plate washing procedures be investigated. This statement is also displayed on all printed and PDF reports.

4 Select the “Subject Results” tab to proceed to the next screen.

6.2 Standard curve

The Standard Curve is used to calculate a value (IU/mL of IFN-γ) for each subject’s samples. The software subtracts the value of the Nil plasma sample from each of the other samples; based on these values, the result for each subject is reported.
1 In the unlikely event that a subject’s result is reported as positive and their Mitogen minus Nil result is less than 0.5 IU/ml, the software will flag the result as a possible sample mix-up using the “*” symbol. This warning helps to limit the possibility of a false-positive result due to a mix-up of the TB antigen and Mitogen samples.

2 The result Data Missing is reported if any of a subject’s plasma samples display the value N/S (No Sample).

3 Samples that have results beyond the linear range of the assay are reported as “>10 IU/ml” and are flagged using the “¶” or “#” symbols. “¶” indicates that the result is outside the linear range of the assay. “#” 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.
7 Data Export

If desired by the user, the user can export results and/or data via Windows Clipboard or structured text file to external spreadsheet applications, such as Microsoft Excel® software.

To export the results, select the “Results Export” button. Export Type is in default set to “Export to Clipboard”. The user can then choose to export the assay details to either the Windows Clipboard or a text file. After you click the “OK” button, a “Results copied to Windows Clipboard” pop-up window appears. After clicking the “OK” button, data can be pasted into a spreadsheet. After clicking the “Results Export” button, a prompt window appears. “Export Type” can also be manually set to “Export to File”. After clicking OK, a window allows you to save the export into a file on your computer. After clicking “Save”, data are saved as a text file.

Similarly, selecting the Data Export button offers the user the choice of exporting the assay details, raw data and QC results to either the Windows Clipboard or a text file.

Note: This optional step is not required to obtain QuantiFERON-TB Gold Plus results. It may be employed by the user for the purpose of 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.
8 Reports

Selecting the print button will display a printing screen that is divided into two sections. The upper section displays the various printing options available, while the lower section displays 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 report results.

The Report Type options allow the user to print various reports as follows:

- “All Subjects (Group Report)” prints the results for all subjects on one page.
- “All Subjects (Individual Report)” prints the results for each subject on a separate page.
- “Single Subject Report” prints the results for one subject, as selected from the drop-down box.

On “All Subjects (Group Report)”, the Raw OD values used to generate the Standard Curve are highlighted (bold and underlined).
The upper range of the QuantiFERON-TB Gold Plus ELISA is 10 IU/ml. Therefore, samples determined to have an IFN-γ concentration greater than this range are reported as >10 IU/ml.

Although values above 10 IU/ml are reported as >10 IU/ml, the calculations for subtracting the Nil control value are based on the original value. Therefore it is possible for a subject’s TB1, TB2, or Mitogen value to be reported as “>10 IU/ml”, yet their “minus Nil” value be less than 10 IU/ml.
9 Saving/Loading Files

9.1 Saving files

Upon opening the QFT-Plus Analysis Software for the first time, the software creates a folder called “My Documents\QuantiFERON” or “Documents\QuantiFERON”, depending on your Windows operating system. By default, all files are saved to sub-folders within this folder, and are given default file names as per the following table.

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<th>File Type</th>
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<tr>
<td>PDF Results</td>
<td>.pdf</td>
<td>PDF</td>
<td>Date_RunNumber</td>
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</tbody>
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- Format files. Select the “Save Format” button to save a completed format to file, which can be reloaded for use with future analysis.
  
  Run Details information is not retained within a saved format file.

- Results files. Select the “Save File” button to save a copy of the Results to file, which can be reloaded for further analysis.
  
  Run Details information is retained within a saved result file.

- PDF files. Select the “Save As PDF” button 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.
  
  PDF files contain all of the information available in the printed report.

9.2 Loading files

- Format files can be reloaded within the QFT-Plus Analysis Software by selecting the “Load Format” button.
  
- Results can be reloaded by selecting the “Load File” button at any time.
  
- After reloading a results file, the “Calculate” button must be selected in order to re-generate results.
10 End of Analysis

- The software allows the user to work on one run simultaneously (single session mode).
- Using the “New Test” button, the user can work on a second run sequentially without having to start the software.
- Selecting the “New Test” button clears all entered information, enabling new assay data to be analyzed.
- Selecting the “Close” button will close the program.

  For 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. These details can be modified as required.
11 Frequently Asked Questions

Q. Why do I need to use the QuantiFERON-TB Gold Plus Analysis Software? Can I use my own spreadsheet to calculate results instead?

A. You can use your own spreadsheet to calculate QuantiFERON-TB Gold Plus test results. However, the calculations required to obtain the correct IFN-γ values are logarithm based. Therefore, it is essential that you follow the instructions in the “Calculations and Test Interpretation” section of the QuantiFERON-TB Gold Plus (QFT-Plus) ELISA Package Insert.

The QuantiFERON-TB Gold Plus Analysis Software has already been validated to ensure that the Quality Control checks — and the results obtained — are accurate and reproducible. The QuantiFERON-TB Gold Plus Analysis Software also has the added flexibility of simple one-click formatting of standards and samples, allowing for the format to be easily updated as changes to your ELISA test layout arise.

Q. When a newer version of the software is available, should I uninstall the old version of the QuantiFERON-TB Gold Plus Analysis Software? How do I do this?

A. Yes, you should always uninstall obsolete versions of the software before installing the new software. The new version of the QFT-Plus software may contain changes to the test criteria, therefore it is essential that only the current version of the software be available for use.

To uninstall the old software, simply locate the default QuantiFERON folder in the Start Menu (Start > QuantiFERON) and select “Uninstall”.

Alternatively, locate and remove the software using Start > Control Panel > Add/Remove Programs.

Q. I would like to contact QIAGEN to discuss my data/results/technique. What information should I provide in order to obtain a prompt reply?

A. It is best to provide the QuantiFERON-TB Gold Plus Analysis Software results file (*.qdf) which by default is located in the folder “My Documents\QuantiFERON\Save”. It is best to provide a detailed outline of your enquiry, kit lot number and any other information you feel is relevant.

Q. Why can’t data cells for standards be blank or contain text?

A. Because the standard curve is used to derive QuantiFERON-TB Gold Plus ELISA results, blank values or text may reduce the quality of the standard curve.

Q. When I open the QuantiFERON-TB Gold Plus Analysis Software, some of the text appears to be missing, as though it is covered by other text. What is the problem?

A. The computer’s Display Settings may be incorrectly setup for the software. Make sure that the Display settings are set to “default”.

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