

README

QuantStudio™ 12K Flex Real-Time PCR Instrument Software v1.4

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Overview

QuantStudio™ 12K Flex Software v1.4 supports the QuantStudio 12K Flex Real-Time PCR System using qPCR (96-fast/standard well and 384-well), TaqMan® array cards and OpenArray™ blocks. This software allows users to perform the following tasks in a streamlined fashion:

- Setup experiments
- Send experiments to the instrument
- Control the thermal cycling process in the instrument
- Collect data and analyze results

Features

- QuantStudio™ 12K Flex Software allows users to easily design, run and analyze various experiments types for 96-fast/standard well, 384 well, TaqMan array cards and OpenArray block
- Experiment types supported: Standard curve experiments, Relative standard curve experiments, Comparative CT experiments, Genotyping experiments, Presence/Absence experiments, Melt-curve and HRM experiments
- QuantStudio 12K System home screen enables launch of analysis applications: Expression Suite, Genotyper and Digital Suite
- Customizable optical filter selection for more complex applications, such as multiplexing
- Quick-Start setup so you can begin a run immediately and enter plate information later
- Real-time monitoring of amplification curves enables you to view run progress (can be viewed from a remote PC as well)
- Auto-baseline and auto-threshold for simplified data analysis
- Alternative method for data analysis using a non-threshold Ct calling method
- Multiple Plots view for simultaneously assessing data from four perspectives
- Automated SNP genotype calling with intuitive graphical output and quality-value assignment
- Troubleshooting flags to help you diagnose and solve problematic experiments
- Tool tips for easy identification of sample wells when viewing amplification curves or SNP genotyping plots
- Email notifications to alert you when a run has started or ended
- Easy cut-and-paste functionality

- Export easily to Microsoft® PowerPoint® software, Excel® software, or directly as a .JPEG file
- Sample Tracker software for OpenArray plates
- Easy to set up experiments for both novice and advanced users for 384-well and OpenArray blocks
- Ability to run up to four OpenArray plate experiments at once

Updates and Fixed Defects

v1.4 Updates

- Changed system preference to allow changes made to be applicable to all users
 - Upon upgrade from v1.3 to v1.4
 - System preference will be reverted to default values
 - Administrator should update the necessary changes to the preference setting and it will be applicable to all users
 - To prevent other user from updating preference setting, Administrator should also disallow other user role the ability to update preferences
- Added Experiment Summary as cover page and pagination in footer in Audit Summary Report
- Added Experiment Summary as cover page in Audit Report
- Added option to enable/disable auto analysis in Amplification Plot when user adjusts threshold

v1.4 Defects Fixed

- Fixed the issue where extraneous audit records during every "SAVE"
- Fixed the issue where target specific audit is not clear
- Fixed the issue where user is unable to unselect NOAMP flag
- Fixed the issue where error message "Error occurred during analysis due to incorrect data." is displayed when user open experiment file
- Fixed the issue where no RQ result is available when multiple endogenous are selected
- Fixed the issue where user is not allowed to start ROI calibration run for Array Card or 384-wells block when SAE is enabled
- Fixed the issue where error message "An unexpected error occurred" is displayed when user performs analysis after importing .spf file
- Fixed the issue where user is not prompted to save experiment file during export or print report when there are unsaved changes
- Fixed the issue where plots printed in report are not in correct order
- Fixed the issue where identical flag is printed on multiple rows in printed report (QC Summary)

v1.3 Updates

- Develop a new Audit Summary Report that includes only result-impacting audit records.
- Enable audit records of new file that saved from other files started from scratch.
- Enable sorting in Export table
- Added Lower Specification Limit of - 0.2 for QC check of Pure Dye Calibration (Filter X1M5 and X1M6 were excluded)
- Included Rnase P Status in the exported file of non OA blocks' experiment.

V1.3 Defects Fixed

- New and robust instrument barcode reading algorithm for OpenArray block
- Fixed issue where the well information is displayed incorrectly in the plate layout of PDF report for Genotyping files.
- Fixed issue with analysis setting will revert back to default when user imports a plate setup file
- Fixed issue with the software produces an error when analyze Genotyping file with real-time data
- Fixed issue with the software prompts error message when user omitting wells in Open Array Genotyping file
- Fixed the issue where HRM file can't be analyzed when the passive reference dye Rox is used
- Fixed the memory leak issue where the software is freezing when user e-sign the result
- Fixed the issue when the first row of the sample is omitted in the analysis well table, the Quantity (Mean), Quantity (Std Dev), Ct (Mean) and Ct (Std Dev) columns for that sample is empty in the Result Summary table of the pdf report
- Fixed the issue where FAC role was not working properly for "run only" users
 - Added "Printing/Create Slide/View Report" and "Export Experiment/Audit Records/Security Settings" categories to the Setup's list
 - Added "Edit" category to the Dye's list
 - Added "Libraries" category to the main list
 - Remove "Transfer System Preferences In" and "Transfer System Preferences Out" categories from the "Preferences"
- Fixed the issue with incorrect run mode "Fast" was shown in the pdf report of the file that has "Standard" run mode

v1.2.4 Updates

- New and robust instrument spot finding algorithm for OpenArray block
- Enhanced Cq determination algorithm to align with Applied Biosystems® online qPCR analysis modules and offer improved target detection calling
- Include RQ analysis features similar to online application module. This includes use of equivalent Cq, which is the Cq value projected at 100% assay efficiency, when this information is available about a given assay
- Allow user export the QC image of OpenArray file via command line
- Allow user changes the colors for the sample in OpenArray file
- Display calibration history that has block and heated cover information to user
- Combine the assay ID and the gene symbol to create the target name when importing an AIF file in 96-well standard, 96-well fast and 384-well block
- Enable dual channel approach for OpenArray Gene Expression
- Change the default analysis setting for Genotyping experiments to “Analyze Real-Time Rn – Median (Rna to Rnb)” in line with TaqMan Genotyper software
- Backup calibration image file for OpenArray block
- Allow software connect to instrument that has firmware version "QS12KFlex-1.2.7.update" or later. This software has the latest firmware v1.2.9 (QS12KFlex-1.2.9.update) bundled together. User can upgrade the firmware via instrument's touch screen
- A back-up of OA calibration eds file will be stored in a folder (C:\Applied Biosystems\AppData\QuantStudio 12K Flex Software\UserData\calibrationTemp) for troubleshooting purpose
- dRn adjustment option for all experimental types included
- ‘Amp Status’ column to the Analysis Well Table, Export Table and Pdf report for all the experiments types now available
- Option to improve analysis time for OpenArray Genotyping files: Perform CRT analysis only when the CT setting/dRn value is changed and file is not analyzed before, or when AIG file is imported without re-analysis
- In SAE mode, the user name in experimental properties is automatically populated with SAE login user and cannot be edited. Open an older file required the file to be saved prior the printing or run
- With SAE, files cannot be overwritten directly from the software by using "Save as" button
- In SAE mode, the software will not allow the user to rename the file within QuantStudio 12K Flex v1.2.4 software's window

V1.2.4 Defects Fixed

- Restore the "clean Instrument" feature in touch screen for OpenArray block
- Support software upgrade from older version
- Fixed empty well smoothing error
- Fixed the import function for Standard Curve
- Fixed the import function for SDS/SDT file
- Fixed the reveal trace issue for OpenArray file
- Fixed the Open file permission issue
- Persist the sample sorting when user update the call cycle in reveal trace of Allelic Discrimination plot
- Fixed Positive control failed (PCFAIL) flag issue
- Display correct calibration status in exported file
- Display Tm value in exported file
- Fixed e-signature and audit trail issue
- Allow NOAMP flag to work in the absence of ROX passive reference
- Display correct AmpStatus when the well has target but no sample assigned
- Software can analyze the eds file that has many target assigned and will not display 'low memory'
- Only selected targets will be seen in pdf report's amplification plot

v1.2.3 Updates

- Removal of BASE license requirement for QuantStudio v 1.2.3 (license required for SAE and HRM)
- Improved reliability of saving run files to network drives
- Improved error logs for network issues
- Improved handling of temporary files and memory that may cause software to crash
- Improved file management within Windows® such that only the login user's home directory will require read/write access
- Added Target option as filter in Amplification Curve view
- When Touchscreen is in secure mode, remove access to change time, date, network information or instrument name, firmware upgrade/downgrade as well as restore settings
- Print report layout is improved
- Allow file to be opened when it is run with Automation Controller II software in headless mode
- Allow Genotyping run when the number of cycles is more than 40
- Improved opening time of OA files
- Allow user to export data with custom column order
- Allow opening of the same file by multiple users
- Retain Passive Reference setting from imported file
- Clear the analysis result after user changes the baseline and threshold in Amplification Plot
- On the Melt Curve plot, allow Tm vertical bar to be moved by user
- Add "time stamp" to the Experiment Name for .EDS file created from Batch Experiment Setup

V1.2.3 Defects Fixed

- Compatibility with Automation Controller software
- User selected password can be used
- Prevent user from closing the software when Automation Controller software is running

Setup

- For Standard Curves, under Define and Set Up Standards, allow setup for multiplex targets
- Fixed business logic for loaded SPF/TPF file
- Included new template file from Assay team

Import

- Experiments that import 7900 setup files can be run on instrument without error
- Fixed Assay Name during import

Analysis

- Plot settings will persist even after selecting different wells
- Fixed abnormal Amplification plot issue

Export

- Fixed decimal rounding in export result and well table
- Fixed Calibration Status captured in exported file
- Unselected export tabs will not auto-export
- Fixed incorrect default file name of export
- Fixed Quick Start function when SAE is enabled
- Ct values are exported as number instead of text
- Undetermined CT results will not be empty
- Melt Curve Raw tab is consistently available
- Fixed reports for results using CRT
- Allow export of Comparative CT (ddCT) in 7900 Format
- Slope, Y-Intercept and R2 values are available in 7900 Format for Standard Curves
- Multicomponent files are exported in 7900 Format

Report

- Print preview is available immediately after run
- Fixed incorrect timestamps
- Fixed e-signature report with operator full name included

Calibration

- Verification run can be opened when downloaded from Instrument Console

v1.2.2 Updates

- Algorithm update to improve OpenArray chip-finding
- Update operating temperature range setting for LED

v1.2.2 Defects Fixed

- Fix incorrect parameter and display issue in Define, Assign (including Plate Layout) and Run Method page
- Fix issue on order of Target Name in Export Result tab and files

v1.2.1 Updates

- Algorithm update to enhance OpenArray chip-finding
- Improve RNase P pass/fail criteria for OpenArray experiment

v1.2.1 Defects Fixed

- Fix issue on OpenArray real-time amplification plot freezing during run
- Fix files downloading issue upon completion of run

v1.2 Updates

- Allow assay specific cycle calling for genotyping experiment
- Enhance RNase P pass/fail criteria for OpenArray experiment
- Show VIC® amplification plots for OpenArray genotyping experiment
- Show Amplification Score (Amp Score) for VIC® plots
- Additional CqCONF flag in Analysis Settings, well table, export table and printed report
- Allow export of ROX™ values for OpenArray® experiment
- Allow analyzes experiment file (.EDS) from raw data in the event the software shows missing cycle data in the Amplification Plot
- Allow download of RNase P calibration file before saving
- Include sample name in the Print Report plate layout view
- Extend calibrations expiration dates to one year
- Two targets are displayed when only one target is selected in OpenArray dual reporter amplification plot (for genotyping)
- Ability to save OpenArray .EDS file after performing a calibration override
- e-Signature features enabled for Open Array® experiment run
- Enable command line export to ensure data for all wells of an OpenArray experiment are exported correctly via command line

v1.2 Defects Fixed

- Fix incorrect rounding for Amp Score values in Export Result and CRT/CT values in well table
- Fix the software to prevent user from closing the calibration run window when user clicks “Close”
- Fix incorrect color patterns swapping from 96-well to 384-well for Genotyping – 16 Adjustable Pipette format in OpenArray Sample Tracker Software.
- Fix incorrect mapping from 96-well to OpenArray for Genotyping – 16 Adjustable Pipette format in OpenArray® Sample Tracker Software.
- Firmware update to include a thermal protocol to clean the OpenArray block

v1.1.2 Updates

- Algorithm update to reduce false CRT calls of Undetermined
- Firmware update with algorithm enhancement to improve sensitivity of chip detection

v1.1.1 Updates

- Algorithm updates for system stability
- Defect repair during automation run
- Defect repair in Sample Tracker Software
- Defect repair for import of .TPF file when using 196-format and 168-format for OpenArray experiments

License

See accompanied End User License Agreement for details. You must agree to the terms of the license before installing or using the software.

Compatibility

- The software can open experiment files created with QuantStudio 12K Flex Software by v1.3 or before.
- The software cannot open files from ViiA™ 7, StepOne Plus®, 7900 HT Fast, 7500 Fast, or 7500 Instruments.
- The software can import setup files created by 7900 HT Fast SDS Software v2.3 and onwards
- The software cannot communicate/network with other non-QuantStudio 12K Flex instruments.

Known Issues for OpenArray Blocks

Import / Export / Report

- The system is unable to export automatically upon completion of an OpenArray block run. Therefore, open experiment file after the run has completed and export manually.
- Legend not shown fully in report and in an exported image file.
- Export with Filter Bookmark Data selected as 'Yes' incorrectly exports the wrong data from both the Raw Data and Multicomponent tabs. Therefore, export with Filter Bookmark Data selected as 'No' to export correct data.
- Instrument Details tab is not included in the Export.
- Calibration status is not included in the export.

Plot

- EMF format is not supported in this release of the software.
- Disabling Auto-adjust Range from Plot Properties of Allelic Discrimination Plot does not work. Therefore, do not disable Auto-adjust Range setting.

Settings

- Preferences settings of Sample Tracker software only take effect after software is re-launched.

Instrument Run

- Instrument may not boot up correctly if power is interrupted during firmware upgrade. Re-install firmware to ensure proper operation of instrument.
- Instrument display of real-time amp plot during a run may differ slightly from the software display due to additional processing applied in the software.

Chip Identification

- Chip detection at the beginning of a run can take up to 3 minutes, although typical processing period is approximately 40 seconds.

RNaseP Calibration

- User is unable to omit wells from the Plate Layout view. Select wells and navigate to Results table to omit wells.
- Omitting wells and then clicking 'Finish' without reanalyzing will display empty values for Amp Score and CRT. To view the actual values, transfer the experiment manually from the Instrument Console, then open and reanalyze the experiment.
- The system intermittently will not refresh the amplification plot upon completion of a run. To view the plot, close and re-open the RNase P calibration screen from the Instrument Console.

Connecting to Multiple Instruments

- It is advised to monitor only one OpenArray block run from a single computer due to the large volume of data being processed.

Opening Experiment Files

- The system may run out of memory after repeated opening and closing of experiment files. Close and re-open the software application to continue using the software.
- The system will not open an OpenArray genotyping experiment with a pre-read stage when transferred manually after the run from the Instrument Console. The work around is to transfer the experiment manually via USB stick.

Known Issues for non-OpenArray Blocks

Instrument Run

- If a run is terminated during an infinite hold, system incorrectly labels experiment with status of "Run Stopped," rather than "Run Completed." System is able to open and analyze file correctly.

Calibration

- Calibration runs may return a QC warning message in the QC tab that describes a failure condition. Disregard the warning message.

Connecting to the Instrument

- The status of the instrument remains as "Not Connected" in the Instrument Console after the IP address of the instrument has changed. Restart the software after changing the IP address of an instrument.

Notifications

- Calibration reminder settings cannot be tested from the calibration reminder screen. Therefore, to test email notification and SMTP settings, create a new experiment and test the notification settings from the experiment run screen.
- Email notification operation incorrectly does not function with SMTP encryption enabled. Uncheck the 'encryption required' setting in preferences for SMTP Settings to allow notifications.

Presence/Absence

- False presence calls. Due to the enhanced uniformity of the instrument, the threshold for the Presence/Absence call may be too close to the NTC wells. Increase the confidence level from 99% to move the threshold farther away from the NTC wells.

High Resolution Melt Experiments

- Manual calls cannot be made in the Variant Call column in the Well Table. To make a manual call, double-click a well in the Plate Layout tab and select the control from the Control drop-down list.
- Recommended hard drive space available when performing an instrument run for a High Resolution Melt experiment is 50MB.

System Requirements

The computer hardware and operating system requirements for the QuantStudio 12K Flex Software are:

- Windows® 7 (32-bit or 64-bit) with Service Pack or Windows® 10 (64-bit)
- Pentium 4 or compatible, with minimum 16GB of RAM and 20 GB of hard drive capacity
- Minimum monitor resolution of 1280x1024
- One open Ethernet port for connecting to the instrument directly
- Internet Explorer® 6.0 or higher (for online assay browsing and ordering)
- Microsoft® PowerPoint® software (for direct export into PowerPoint slides)
- Microsoft® Excel® software

Install the software

IMPORTANT - To help prevent data loss, it is strongly advised that all user data is backed up before upgrading the software.

- Log on to the Windows system with Administrator privileges.
- Obtain the software installation package.
- Double click the setup.exe file on the installation package to start the installation.
- Agree to the End User License Agreement (EULA) when prompted and complete the installation.
- The software will be installed, by default, to “C:\Applied Biosystems\QuantStudio 12K Flex” (referred to as the home directory of the QuantStudio 12K Flex Software).
- A program group, “Applied Biosystems/ QuantStudio 12K Flex Software” will be created during installation. In addition, a short cut to the application launcher, “QuantStudio 12K Flex Software”, will be installed on the computer desktop.
- When a previous version of the software is detected on the system, the installer will perform an upgrade. The user will be prompted to proceed with the upgrade or to cancel the operation.

Install the New Modules

- If you have purchased additional modules such as High Resolution Melt Module or Security and Auditing for QuantStudio 12K Flex Software, a license key is required to activate the modules.
- Select Tools > License Central from QuantStudio 12K Flex Software.
- Follow the instructions in the wizard to enter your license key. The wizard directs you to a QuantStudio 12K Flex Software license activation URL (<https://licensing.appliedbiosystems.com/activation/QuantStudio 12KFlex>) to receive your license file.
- After you have your license file, during the installation process, navigate to location of the license file to activate your module.
- Restart the QuantStudio 12K Flex Software after the license has been successfully applied.

Uninstall the Software

IMPORTANT: To help prevent data loss, it is strongly advised that all user data is backed up before uninstalling the software.

- From Windows “Start” button, find the “Applied Biosystems/QuantStudio 12K Flex Software” program group.
- Click on “Uninstall QuantStudio 12K Flex Software”
- In the wizard, follow the instructions and complete the uninstall operation.
- Optionally, rename or delete the home directory of the QuantStudio 12K Flex application. This ensures a clean environment for the next installation.

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