

Release Note: QIAcuity® Software Suite v3.5

The QIAcuity Software Suite version 3.5 is now available for download and installation. It is recommended to install or upgrade to this software version.

Upgrading to QIAcuity Software version 3.5 requires upgrading both the QIAcuity Software Suite and the QIAcuity CSW to version 3.5.

Both software versions are Long-Term Support (LTS) releases, each with an extended support period of 7 years following its release date.

New features of version 3.5

The QIAcuity Software Suite version 3.5 introduces new features for automated data analysis and reporting. Threshold selection, as well as quality control (QC) parameters, is available through the new analysis rules for automated data analysis.

Threshold selection

For automated thresholding, the following options can be selected for each target/channel within a reaction mix:

- Auto threshold
- Fixed RFU threshold value for target- and channel-based data analysis
- New threshold methods:
 - **Delta Bandwidth Average-based (DBA)** threshold for controls and unknown sample
 - **Delta Min Max-based (DMM)** threshold for controls and unknown samples
 - **Positive Control-based Threshold (PCT)** for positive controls
 - **Negative Control-based Threshold (NCT)** for negative controls and non template controls
- **Control-based thresholding:** The threshold option for (i) a positive control, (ii) a negative control, or (iii) a non-template control is propagated to all unknown samples within the same reaction mix.
- **Quality Control (QC) parameters** – The following control rules are available for selection:
 - Minimum valid partitions for controls and samples
 - Expected concentration rules for positive controls
 - Maximal positive partitions for negative controls and non template controls

Definition of severity level and scope of application

The severity level for QC rule failures can be set to either a warning or an invalidation of the affected results. The scope of application can also be defined to specify whether only the affected channel or the entire well is marked. QC rule failures are flagged accordingly in the results.

Report template

A new feature, Report Template, has been introduced for automated result reporting. The report content for all absolute quantification analysis options can be preselected. A PDF report is automatically generated after run completion. In addition, the desired CSV files can be downloaded automatically after run completion.

Improvements in version 3.5

- Password expiration is now configurable.
- Concentration units can now be converted into predefined or user-defined units for dPCR reaction results, and for the initial concentration of diluted controls (positive and negative) and non-template controls (NTCs).
- A copy-to-clipboard feature is now available for all graphs, plots, and diagrams, allowing them to be copied with a single click.
- Absolute Quantification CSV result files now include the new threshold strategies and updated QC information. The previously reported rain, resolution, and bandwidth metrics have been replaced by a new set of user-defined quality parameters. These parameters are not equivalent to the previous metrics and are intended for QC rather than descriptive quality assessment.
- The Plate Layout CSV file has been redesigned to improve usability and now includes all Analysis Rules and Report Template information.
- RFU CSV exports now also include RFU values for invalid partitions.
- The QIAcuity Software Suite can now be installed in any installation directory and is no longer limited to the C: drive.
- The QIAcuity Lab Automation Service has been enhanced to support the full Analysis Rules functionality.
- Multiple Occupancy support via the QIAcuity Lab Automation Service (API) has been enhanced with automatic CSV download and download when using plate templates.

Bug fixes

- Resolved an issue where enabling or disabling the Audit Trail did not correctly track all events. Audit Trail event tracking now functions as expected.
- Resolved an issue where, under rare conditions (high sample concentrations, cross-talk between neighboring channels, and very low background signal), dust detection could incorrectly invalidate negative partitions. Even in the worst-case scenarios, the impact on concentration precision was limited to less than 5%.
- Resolved an issue where changing a threshold after using the lasso tool to adjust thresholds in a 2D scatterplot could result in an error message if one of the previously selected channels or targets was modified.
- The well status for Amplitude Multiplexing is now retained after a plate upgrade.
- Resolved an issue where assigning more than 24 reaction mixes to a plate could cause some wells to appear black and limit the analysis to channel-based mode because of color palette limitations.
- Resolved an issue where the 1D Scatterplot in PDF reports could appear slightly compressed compared with the web browser view, causing the "Ref" column header to be misaligned in every second well.
- Resolved an issue in target-based analysis where assigning and selecting the same target across different reaction mixes could result in duplicate entries in the drop-down list without indicating the associated reaction mix.
- The checkbox "Use dilution factor" is now displayed in the Heatmap and Concentration diagram tabs even when none of the selected wells contains concentration results.
- Resolved an issue where plate modifications and edits were not correctly tracked in the Audit Trail information when the plate layout was imported from a CSV file.
- Resolved an issue in Copy Number Variation analysis where, if different reaction mixes were used for the reference and the target of interest and no replicates are present, the values for copies per genome and the ratio are now calculated and displayed correctly in the List view.
- Resolved an issue where the histogram for Absolute Quantification did not display the data correctly when the number of valid partitions was very low.

Lab Automation Service

- If a manual threshold change in the reference channel results in a different number of valid partitions, the quality control rule for the minimum number of valid partitions is now correctly applied. If the QC rule is not met, the results are displayed as “Failed”.
- Resolved an issue in which the lambda value in the lab automation result response was always returned as “0”. The correct value is now displayed.

Upgrading the QIAcuity Software Suite

The upgrade to this Software Suite version can be performed directly from QIAcuity Software Suite versions 3.1, 3.1.1, and 3.2.

Caution: Direct upgrade to Software Suite version 3.5 is not supported from versions earlier than 3.1. Refer to the corresponding sections in the user manual for upgrade instructions.

Note: The latest Software Suite version 3.5 is only compatible with CSW version 3.5. If only one software component is updated, the Software Suite cannot establish a connection to the CSW.

Important: It is strongly recommended to update the Software Suite first before proceeding with the Control Software update.

Not following the instructions may result in a loss of your previous plate data!

Visit www.qiagen.com and go to the **Operation Software** section under the **Resources** tab of the QIAcuity product page to check for the latest QIAcuity Software Suite version and the latest user manual. On a computer running Microsoft® Windows®, download the software, and unzip the file. Locate the **QIAcuitySuite.exe** file and run it with full administrator rights in Windows. The installation process begins. Follow the instructions given in the user manual.

The QIAcuity Software Suite is designed to work with Windows 11 Professional Edition. It is recommended to upgrade your Windows operating system to the latest available build version from Microsoft. The following browsers are supported by the QIAcuity Software Suite:

- Mozilla® Firefox®: version 149.0.2*
- Microsoft Edge®: version 146.0.3856.109*
- Google Chrome®: version 147.0.7727.56*

Known issues in the QIAcuity Software Suite version 3.5

- When changing a user's password, the form fields remain populated after closing and reopening the dialog box. The information is cleared only after the page is reloaded.
- When using Amplitude Multiplexing mode with defined analysis rules for negative controls, the report incorrectly shows “Auto threshold” instead of “-”.
- When “Analyze per target” is configured for the 1D Scatterplot in the report template and Amplitude Multiplexing mode is enabled for all wells in the reaction mix, the plot for the reference channel does not appear in the generated report.
- In the report file, the graph on the 2D Scatterplot tab is positioned too close to the header, making the Y-axis difficult to read. However, the underlying data are not affected. Control and non-template control names are not considered when sorting wells, resulting in a discrepancy between the data displayed in the user interface and in the Concentration diagram report.
- In Mozilla Firefox, exiting the audit trail view by using the navigation buttons (e.g., “Plates”) may not work. Repeated clicks may resolve the issue. Chrome and Microsoft Edge are not affected.
- When entering invalid values for quality control rules (e.g., values below the minimum), the error message is displayed for only one field instead of all affected fields.

* Patch versions of the browsers are released very frequently that mainly contain bug fixes; therefore, any of patch versions are compatible with the QIAcuity Software Suite.

- When a custom cross-talk matrix is added to or removed from a reaction mix in a plate layout, the **Refresh** button may be displayed incorrectly.
- When importing a CSV file for channels in Amplitude Multiplexing mode, duplicate channel names are not validated (e.g., “Green (Low)” may be entered twice). Ensure that channel names are specified correctly when using CSV import for plate layout definition.
- When a report template is used to select groups such as all positives or all negatives for multiple occupancy CSV export in wells with Amplitude Multiplexing mode, the output file may include more positives than selected in the report template. However, all displayed data are correct, and all groups are displayed correctly for selected wells in which Amplitude Multiplexing mode is not enabled.
- If the plate layout has been modified by assigning reaction mixes with a custom cross-talk matrix, the plate may become temporarily inoperable, depending on the number of changed wells. This may last several minutes.
- When selected wells use different modes (Standard and Amplitude Multiplexing), no common threshold is displayed in the histogram of the Absolute Quantification analysis.
- In the report PDF, the 1D Scatterplot appears slightly compressed compared with its display in the web browser, which causes the “Ref” column header to be misaligned in every second well. In addition, when the same target is assigned to different reaction mixes in a target-based analysis, it appears multiple times in the drop-down selection list without indicating the corresponding reaction mix.
- In rare cases, plates from Software Suite version 1.2.18 or earlier are not compatible with export, archive, import, or restore functions in version 3.5. Use version 2.5 to review these data.
- When multiple wells are combined to define a hyperwell together with the dilution function, Software Suite does not round decimal values for the template volume and instead displays the full-precision value.
- When a conversion factor is defined but no dilution information is provided, the “Undiluted sample” column appears without data in the Absolute Quantification results. However, the correct values are shown in the conversion factor column.
- For target-based multiple occupancy analysis, all result data are displayed, even if only some targets were selected.
- Hyperwells are not displayed correctly in the Plate Overview thumbnail for the heatmap and concentration diagram in the report PDF.
- When another well is added to an existing hyperwell or additional hyperwells are defined, the color and hyperwell ID of the selected hyperwell may change. This has no impact on the data.
- When multiple plates cannot be archived using the bulk archive function, the corresponding message may not list all affected plates.
- When the archive location is detached while archiving is still in progress, a message correctly indicates that detachment is not possible. However, an additional message incorrectly states that the archive has been detached. This message can be ignored.
- Each time a plate is exported, a new plate audit trail is created as part of the exported plate, resulting in multiple audit trails instead of a single consolidated audit trail.
- When a read-only plate is upgraded and a report is generated afterward, the Detailed Run Information table in the report file appears empty. However, the corresponding upgraded plate report contains all run details. Existing reports generated before the upgrade are not affected. Generate any required reports before performing the plate upgrade.
- If a plate is about to be archived to a location that no longer exists, a misleading error message may be displayed.
- If results from a read-only plate created with Software Suite version 1.2.18 cannot be displayed, archive and restore the plate to retrieve the results. If no archive location is defined, export the plate and then import it again.
- When identical target names were defined for different channels across multiple reaction mixes within a single run, the channels displayed in the heatmap and concentration diagrams may be incorrect, and result data may be merged into a single diagram.

- If a run is stopped during the imaging process, the status “Imaging completed” is displayed instead of “Imaging cancelled”.
- In case user’s password expired, the “New password” field on the Change password page displays a warning when a 8-character password is entered. The warning remains until the user clicks another field or enters an additional character. It disappears immediately when the user moves to the next field.
- When a conversion factor is used, the Y-axis description in the Concentration diagram of the Copy Number Varion results overlaps in the report PDF. However, the Y-axis data and labels remain readable.

Lab Automation Service

- The Lab Automation Service user can delete a locked plated while it is being processed by the instrument (loaded, during run, run completed). Do not delete a locked plate by using the Lab Automation Service user.