September 2025

Release Note: QIAcuity® Software Suite v3.2

The QIAcuity Software Suite version 3.2 is now available for download and installation.

Before updating your QIAcuity Software, please read the section “Known issues in the QIAcuity Software Suite version 3.2”.

Upgrading to QIAcuity Software version 3.2 requires upgrading both the QIAcuity Software Suite and the QIAcuity CSW to version 3.2.

New features of version 3.2

- The QIAcuity Software Suite now supports multiple targets of interest for Copy Number Variation as well as for Mutant Detection data analysis at once.
- An enhanced dilution and conversation function supports different dilution factors for samples having the identical sample name.
- Multiple pair (target/target or channel/channel) selections for 2D scatterplots analysis is now available, enabling multiple data analysis at once.
- The CSV output file of current results of analysis for absolute quantification now include the following QC parameters:
  - **Rain** — Percentage of positive partitions with RFU lower than the main positive population, determined by Lievens' algorithm.
  - **Resolution and bandwidth calculation** — Quantitative measure of how well two populations (positive and negative) are separated. Defined as the difference in fluorescence between two peaks, divided by the combined widths of the peaks.
  - **Real cycled volume per partition (nL)** — The actual volume per partition that is defined by the type of Nanoplate and the Volume Precision Factor (VPF)
  - **Mean RFU values for positive and negative bands** — Arithmetic average of RFU of all positive/negative partitions by a specific threshold.
  - **Lambda value** — Represents the average number of copies per target in a partition.
- The QIAcuity Lab Automation Service has been improved to support the following automated absolute quantification data analysis:
  - Fixed RFU threshold value for target- and channel-based data analysis.
  - **Controlled-based thresholding**: The auto-threshold of (i) a positive control, (ii) a negative control, or (iii) a non-template control will be applied to all unknown samples within the identical reaction mix.
  - Automatic RFU data request download via the Lab Automation Service.
  - A quality control rule related to minimal valid partition number that can be specified per reaction mix. The wells that fail the QC rule will be flagged in the result response, accordingly.

    Note: For more guidance, please refer to the *QIAcuity Lab Automation Service User Guide* (**www.qiagen.com/HB-3537-004**) available on the QIAcuity resource webpages.

Improvements to version 3.2

- In the case of threshold value adjustments during the 1D scatterplot data analysis, the recalculation is performed for all channels/all targets at once after pressing the **Recalculate** button.
- The 1D and 2D scatterplot displays have been improved by adding more space to the axes for improved visualization.
- For 2D scatterplot downloads and reporting purposes, a new **Setting** option is now available to unify the axis values for 2D scatterplot download and report addition.
- The polygon tool in 2D scatterplots now features a movable toolbar, allowing access to all partitions.

- Ongoing background actions (e.g., processing analysis results, reports, or importing/exporting a plate) are now highlighted in the Analysis view.
- For QIAcuity Eight instruments, the information about the cycler used is now included in the report PDF file.
- In case of significant differences among sample replicates, the CI value becomes very high for Copy Number Variation (CNV) and Gene Expression (GEX) analysis. If the CI value exceeds 100% during CNV and GEX analysis, a value of 100% is displayed in the results table together with a warning to indicate that these results are not reliable.
- **Custom cross talk matrix (CXTM) creation support**: The CXTM wizard in QIAcuity Software Suite version 3.2 now presents a saturation warning with a proposal for imaging parameters to prevent saturation if it is detected.
- The audit trail was improved to cover the occurrence of critical instrument errors, in addition to errors that were logged in the audit trail by the clearing event.

Bug fixes

- A saturation warning message that was wrongly displayed in the Analysis view of version 3.1 for all plates exhibiting one imaging step has been fixed.
- Increased software performance when a high number of plates are available in the Plates overview, which was impacted in software version 3.1 due to a constant CXTM suitability check.
- The analysis algorithm for the new reference channel detection has been improved.
- An incorrect sorting mechanism for controls, when using plate templates from previous software versions and observed in software version 3.1, has been corrected.
- The lab automation service now provides information on whether all imaging steps are completed by introducing a Get Experiment Status response.
- For the lab automation service, a typographical naming error in software version 3.1 for the drawer has been corrected to ensure that heartbeats sent by the device meet the naming convention.
- The analysis algorithm has been improved to avoid issues in partition detection in the case of dark images, which rarely occurred with software version 3.1.
- An incorrect RFU scale was presented in the report of software version 3.1 after increasing the maximum values for the axis of the 1D Scatterplot, which has now been corrected.
- Plates larger than 5 GB can now be imported and restored from the archive.
- In cases where a 2D Scatterplot threshold was defined using the lasso function and the threshold of only one of the channels was later modified in the 1D Scatterplot analysis, the unchanged channel contained the automatic threshold information in the audit trail instead of the threshold previously defined by the lasso. This issue is now solved and the audit trail information includes the correct threshold information.
- The export of the multiple occupancy CSV file is now also available for wells exhibiting a mixed scenario in the plate layout (e.g., some wells exhibiting reaction mixes while other samples and wells do not include these definitions).
- All second-level analyses such as Mutation Detection, Gene Expression, Genome Editing, and Copy Number Variation are not available for wells in amplitude multiplexing mode. However, when selecting reaction mixes that included a mixture of targets in both normal and amplitude multiplexing modes, data for all second-level analyses were displayed. This has been resolved.
- When using the temperature gradient function, the corresponding CSV result file from the Absolute Quantification result analysis now includes the temperature indication for the wells.
- When using the reaction mix import function, filtering for reaction mix templates now allows the distinction between uppercase and lowercase letters.
- If a plate includes multiple cycling steps with a mixed scenario — such as one cycling step using gradient functionality and another using a fixed temperature — the results and result reports now present all information on the applied temperatures in a single combined view.

Updating the QIAcuity Software Suite

The upgrade to this Software Suite version can be performed directly from QIAcuity Software Suite versions 3.1.1, 3.1, and 3.0.

Caution: All versions earlier than Software Suite version 3.0 are not supported for direct upgrade to version 3.2. Refer to the corresponding sections in the user manual for upgrade instructions.

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

Visit **www.qiagen.com** and go to the **Latest Software Version** 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 10 and 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 132.0*
- Microsoft Edge®: version 130.0.6723*
- Google Chrome®: version 130.0.2849*

Known issues in the QIAcuity Software Suite version 3.2

- When using the lasso function to change thresholds within the 2D scatterplot, a subsequent threshold change involving one of the previously used channel or target is not possible and will result in an error message. **If application require this option, use software version 3.1 and do not upgrade to software version 3.2.**
  Note: This issue does not affect multiple 2D scatterplot threshold changes via the lasso tool when different combinations of channels or targets are selected, which do not involve a channel or a target from the previous threshold change.
- If more than 24 reaction mixes are assigned to a plate, certain wells may appear black and only channel-based analysis can be performed. This issue is related to the color palette when creating the reaction mix and can occur after restoring plates from the archive and performing a new analysis. In such cases, download the CSV file of the plate layout and re-import the same file using the import function. After re-uploading, the issue is resolved and all wells will display the correctly assigned reaction mixes.
- In cases of high sample concentrations, cross talk between neighboring channels, and a very low background signal, the dust detection may falsely invalidate negative partitions. However, even in the worst case, the precision of the concentration is affected by less than 5%. For lower sample concentrations or non-template controls (NTC), this issue does not occur at all and dust correction is performed as intended. Therefore, please note that the precision of the analysis is not compromised at lower concentrations.
- 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 wells changed. This can take up to several minutes.
- If selected wells exhibit different modes (standard and amplitude multiplexing), no common threshold is displayed on the histogram of the Absolute Quantification analysis.
- In the report PDF file, the 1D scatterplot display is slightly compressed compared to its appearance in the web browser, resulting in misalignment of the “Ref” column header in every second well. Additionally, if an identical target is assigned and selected for different reaction mixes in a target-based analysis, the target appears twice in the drop-down selection list but the origin of the reaction mix is not listed.
- In rare cases involving plates originating from software version 1.2.18 or earlier, these plates cannot be exported, archived, imported, or restored in QIAcuity Software Suite version 3.1.1. Use QIAcuity Software version 2.5 to review these data.

* **Note**: The patch versions of the browsers are released very frequently. They contain mainly bug fixes and should not break browsers’ backward compatibility; thus, any of patch versions should be always compatible with QIAcuity Software Suite.

- If several wells are used to define a hyperwell in combination with the dilution function, the Software Suite does not round decimal values for the template volume and displays the whole number.
- If a conversion factor is defined for a sample but the dilution information is left empty, the list view in the Absolute Quantification results shows the “Undiluted sample” column but no data appear in that column. However, the correct data are available in the conversion factor column.
- For multiple occupancy target based data analysis, all result data are displayed, even if only some targets were selected for the target-based analysis.
- If the plate layout was uploaded via CSV import, plate edits and plate definition modifications are not tracked in the audit trail information.
- Hyperwells are not correctly displayed in the Plate Overview thumbnail for the heatmap and concentration diagram in the report PDF.
- If another well is added to an existing hyperwell or during the definition of additional hyperwells to an already existing one, the color and hyperwell ID of the selected hyperwell may change. However, this has no impact on the data.
- If multiple plates cannot be archived using the bulk archive function, not all affected plates are listed in the corresponding message.
- Detaching the archive location while archiving is still in progress correctly displays a message indicating that detachment is not possible. However, an additional message stating that the archive has been detached is incorrect. This message may be ignored.
- Each time a plate is exported, a plate audit trail — part of the exported plate — is created, resulting in multiple audit trails on the plate instead of a single cohesive audit trail.
- If a read-only plate gets upgraded and afterward a report is created for this read-only plate, the detailed run information table list in the report file appears empty. However, the corresponding upgraded plate includes all run detail information in its report. Already existing reports of read-only plates are not affected. It is recommended to generate any required reports before proceeding with the plate upgrade.
- If a plate is about to be archived to an archive location that no longer exists, a misleading error message appears.
- If read-only plate results cannot be displayed from a plate initially run using Software Suite version 1.2.18, archive the plate and restore it to retrieve the read-only plate results. If no archive is defined, export the affected plate and re-import it.
- If identical target names were defined for different channels across multiple reaction mixes within a single run, the channels indicated in the heatmap and concentration diagrams may be incorrect and result data are merged into one diagram.
- In case of a very low number of valid partitions, the histogram of the Absolute Quantification does not exhibit the data for the affected channel. However, in the PDF report, the histogram is displayed correctly.
- In case of Amplitude Multiplexing mode, the quality metrics like rain, resolution, and bandwidth are not calculated. However, a “0” is listed in the CSV export instead of a “-”.
- In case of using different reaction mixes for reference and target of interest and no replicates, the values for copies/genome and ratio are not calculated and “n.a.” is shown on the list view for Copy Number Variation calculation.
- In case of a run stop during the imaging process, the status “imaging completed” is shown instead of “imaging cancelled”.

Lab Automation Service

- In case of manual threshold changes of the reference channel resulting in a change in the number of valid partition number, the quality control rule for minimal valid partitions is not re-applied. If the QC rule is failed, the results are still displayed as “passed”.
- The new lambda value is missing in the result response for the lab automation and always contains a “0” instead of the correct value.