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Release Note: QIAcuity® Software Suite (v3.1)

Dear valued customer,

The QIAcuity Software Suite version 3.1 is now available for download and installation.

Upgrading to QIAcuity Software version 3.1 requires the upgrade of both the QIAcuity Software Suite and the QIAcuity CSW to version 3.1.

New features

- The QIAcuity Software Suite supports dPCR assays up to a 12-plex by using amplitude multiplexing. In addition to the six optical channels available, this option enables two amplicons to be detected in the same channel. During 1D Scatterplot analysis, three thresholds can be applied, resulting in four sub-population analysis: negative partitions, positives for the low target, positives for the high target, and the double positives for both targets.
  Note: This feature requires use of a new master mix (QIAcuity High Multiplex Kit). dPCR assays up to 5-plex are still supported using all other available QIAcuity probe master mixes. In addition, this feature is available for channel-based absolute quantification analysis only, and a custom cross-talk matrix is strongly recommended.

- A new functionality for the integration of a QIAcuity Lab Automation Service allows third-party lab automation software to control robotic devices to interact with the QIAcuity system, run dPCR experiments, and analyze results without any human interaction.
  Note: For more guidance, please refer to the *QIAcuity Lab Automation Service User Guide* (**www.qiagen.com/HB-3537**) available on the QIAcuity resource webpages.

Improvements

- The multiple occupancy result output file is enhanced up to 8-plex, supporting assays using amplitude multiplexing mode.
- The multiple occupancy result output file now allows for the selection of sub-populations of interest (e.g., for the analysis of double positives) to reduce the size of the output file.
- The hyperwell function for channel-based analysis is now available for the absolute quantification and for all second-level analyses.

Bug fixes

- When using the dilution and replicate function, the mean diluted concentration was not uniform in the multiple occupancy result CSV file. This has been corrected in the QIAcuity Software Suite version 3.1.
- In very rare cases, plates were duplicated after the bulk archiving, resulting in a plate being available in the archive and its duplicate being available in the plate overview. This has been corrected in the QIAcuity Software Suite 3.1.
- When the default report name was changed during report generation, this name was not updated in the separate report generation window even though the correct report name was assigned to the report PDF file. This has been corrected in the QIAcuity Software Suite version 3.1.

Updating the QIAcuity Software Suite

The upgrade to this Software Suite version may be performed directly from the QIAcuity Software Suite v3.0, v2.5.0.0, and v2.5.0.1.

Caution: All versions older than the Software Suite version 2.5 are not supported for a direct upgrade to version 3.1. 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 starts. 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 in the QIAcuity Software Suite:

- Mozilla® Firefox®: version 132.0*
- Microsoft Edge®: version 130.0.6723*
- Google Chrome®: version 130.0.2849*

Note: Asterisk is used on last position because 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.

Known issues in the QIAcuity Software Suite version 3.1

- For all plates exhibiting one imaging step, the warning message "*The signal for channel reached the saturation. Try re-image the plate with reduced exposure time and gain settings to fix this issue"* is wrongly displayed in the Analysis view. However, the result data that do not contain any saturation are displayed correctly in the List view and the corresponding samples do not exhibit a warning symbol. Also, this incorrect warning message is not included in the report PDF. As soon as a second imaging step is added to the plate, this erroneous display is no longer available. Results that contain a real saturation also contain a warning symbol for each affected sample in the List view and show a summary of all warnings in the report PDF. Saturation can occur for positive signals above RFU value of 180.
- If the plate layout has been changed with the assignment of reaction mixes exhibiting a custom cross talk matrix, the plate cannot be operated for a certain time depending on amount of well changed (this can take up to several minutes).
- All second-level analyses like Mutation Detection, Gene Expression, Genome Editing, and Copy Number Variation are not available for wells in amplitude multiplexing mode. However, when selecting reaction mixes exhibiting a mixture of targets in normal and amplitude multiplexing mode, the data are displayed for all second-level analysis.
- In case of selected wells exhibiting different modes (standard and amplitude multiplexing), no common threshold is visible on the Histogram of the absolute quantification analysis.
- In case a 2D Scatterplot threshold was defined by the lasso function and the threshold of only one of the channels was later modified in the 1D Scatterplot analysis, the unchanged channel will contain the automatic threshold information in the audit trail instead of the threshold previously defined by the lasso.
- In the report PDF file, the 1D scatterplot display is slightly compressed in comparison to how it displays in the web browser, resulting in misalignment of the "Ref" column header in every second well. If an identical target was assigned and selected for different reaction mixes in a target-based analysis, the target is listed twice in the drop-down selection list, but the origin of the reaction mixture is not listed.
- In rare cases, instances where plates originated from software version 1.2.18 or lower, these plates cannot be exported, archived, imported, nor restored in the QIAcuity Software Suite version 3.1. Use the QIAcuity Software version 2.5 for reviewing these data.
- In case several wells are used for defining a hyperwell in combination with using the dilution function, the Software Suite does not round the decimal numbers for the template volume and displays the whole number.
- In case a conversion factor is defined for a sample but the dilution information is left empty, the list view for the absolute quantification result shows the column "Undiluted sample" but no data is shown in that column. However, the correct data can be found in the column for the conversion factor.
- For multiple occupancy target based data analysis, all result data are presented, even if only some of the targets were selected for a target-based analysis.
- In case the plate layout was uploaded via CSV import, the plate edits and the plate definition modifications are not tracked in the audit trail information.
- For usage of temperature gradient function, the corresponding CSV result file of the absolute quantification result analysis does not exhibit the temperature indication for the wells. However, the data are present in the list view on the user inface as well as in the temperature map of the result report PDF.
- If a plate exhibits multiple cycling steps with a mixed scenario, such as one cycling step with use of gradient functionality and one without, the resulting display, including the results report, contains all information on the applied temperatures in one combined view.
- In case the reaction mix import function is used, the filtering for the reaction mix template of interest does not work when using at least one capital letter. However, the search functionality works using lowercase letters, and the scrolling option then works as expected.
- In case of hyperwell usage, the hyperwells are not correctly reflected in the thumbnail of the plate overview for the heatmap and the concentration diagram in the report PDF.

- In case another well is added to an existing hyperwell or during definition of additional hyperwells to an already existing one, the color and hyperwell ID number of the selected hyperwell change. However, this has no influence on the data.
- If multiple plates cannot be archived using the bulk archive function, not all of the affected plates are listed in the corresponding message.
- Detaching of the archive location while archiving is still ongoing displays the correct message that detaching the archive is not possible. However, an additional incorrect message that archive has been detached is displayed as well. This message can be ignored.
- Every time a plate is exported, a plate audit trail, which is part of the exported plate, is created, resulting in multiple audit trails on the plate instead of one cohesive audit trail.
- In case a read-only plate gets upgraded and afterwards a report is created for this read-only plate, the detailed run information table list in the report file is empty. The corresponding upgraded plate exhibits all information of the run details in the report. Already existing reports of read-only plates are not affected. It is recommended to create first required report/s before continuing with the plate upgrade.
- The export of the multiple occupancy CSV file is only possible if the wells selected for analysis have a uniform plate layout definition with regard to **Reaction Mix** and **Sample and Controls**. All selected wells to be analyzed must either not have a plate layout definition at all or have a defined plate definition. It is not possible to export the multiple occupancy CSV file if there is a mixed scenario in regard to the plate layout (e.g., some wells exhibiting reaction mix while some samples and wells are without any definition). In case no reaction mix is defined, an incorrect error message is displayed.
- In case 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 this plate and restore it again to obtain the read-only plate results. If no archive is defined, please export the affected plate and import it again.
- In case identical target names were defined for different channels of several reaction mixes used within one run, the indicated channels of the heatmap and concentration diagrams are wrong and result data are merged in one diagram.

Best regards,

Your QIAGEN Team

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