PIQuant User Manual

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01 Intended Use

The PICO technology is for research use only (RUO) and not for diagnosis, prevention, or treatment of disease. The following protocol intends to give guidance on how to obtain absolute quantitative data from a PICO experiment.

02 Quality Control

The Software has been tested extensively under a defined test protocol to verify that it performs as intended under expected operating conditions.

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03 Introduction

Protein Interaction Coupling (PICO) is an immunoassay for the characterization and quantification of EVs and for detecting and quantifying proteins, protein interactions, and post-translational modifications.

Two different evaluation options are available for PICO: 1) relative quantification (RQ), using an external standard or control sample for internal reference, or 2) absolute quantification (AQ), analysis without an external standard. To learn more about the different PICO quantification methods, check out our PICO Quantification application note.

The following manual intention is to give guidance on how to use the software PIQuant For the wet lab protocol, please refer to the AMC Kit Manual.

04 Evaluation

04.1. QIAcuity Software Suite

1. It is best practice to assign clear, consistent sample names in the QIAcuity Software Suite. Note that "Reaction Mixes" in the QIAcuity Software Suite correspond to "Antibody Mixes" in PIQuant. In the "Samples & Controls" section, label the control wells (e.g., ABC and NTC) and name the wells containing your samples using descriptive identifiers such as "Cell Lysate A," "Cell Lysate B," "10× dilution," and "100× dilution.". Those will be used in PIQuant to identify your samples.

2. After the dPCR run, select the QIAcuity Software Suite, choose your plate in the 'Plates Overview', click 'Analyze', and select all wells. In the 'Select targets' drop-down window, click 'Select All' and press 'Show results'.

3. Select '1D Scatterplot' in the menu above the results and adjust the thresholds in all channels. We recommend placing the threshold close to the negative population. Please note that directly after adjusting the threshold for a channel, the values have to be recalculated.

4. After adjusting the thresholds, control the raw data and images of the plate in the QIAcuity Software Suite to ensure that the data is valid and the experimental setup was performed correctly.

04.2 PIQuant Relative Quantification Analysis

5. PIQuant analysis software can be accessed here: https://pico-bioscience.shinyapps.io/piquant. Note that you need a token to access the software, which you receive when purchasing a kit or a service. For request you can contact us here: https://landing.actome.de/support

6. Select the device where the PICO experiment was run. PIQuant currently supports dPCR devices from Qiagen and Bio-Rad (beta).

7. Upload the requested file(s).

8. On the left side, the content to analyze (antibody mix, antibody pairs, samples) can be selected, as well as how the couplex data will be processed.

    - For maximal accuracy of Absolute Quantitative data, we recommend using lambda normalization, ABC, and labeling efficiency corrections (Couplexes LN-ABC-LE) to compensate for unexpected deviations. The labeling efficiency for each antibody is provided in the labeling report.

    - Do not apply the labeling efficiency correction to extracellular vesicle (EV) samples. Since the correction is designed for monovalent targets with a single epitope per antibody, it is unsuitable for multivalent targets like EVs, which present multiple epitopes on their surface.

9. The Quality Control tab details performance features (e.g., number of valid partitions, positive non-template control (NTC) partitions, average lambda, and antibody binding control (ABC) values). PIQuant suggests acceptable ranges for these parameters.

10. The Evaluation tab displays the experimental results for the samples and processing steps selected in the left column.

    - The couplex graphs give a first insight into the relative abundance of your target. If a dilution series was performed, the couplex plot may follow the trend of a bell-shaped curve, in which, after a certain target concentration, higher amounts result in a lower number of couplexes detected (high-side).

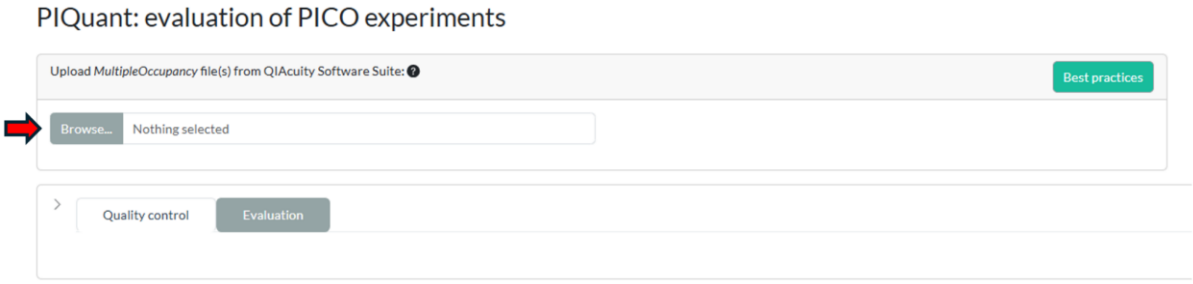
    - For Absolute quantification, the antibody concentration and target lambda employed need to be indicated. For regular AQ experiments in saturated conditions, this is 500 pM with a target lambda of 0.15.

    - After clicking on the “Run absolute quantification” button, the AQ results will be depicted. But for AQ only data from the high or low side can be processed alone. Select those samples which are either on the low- or high-side.

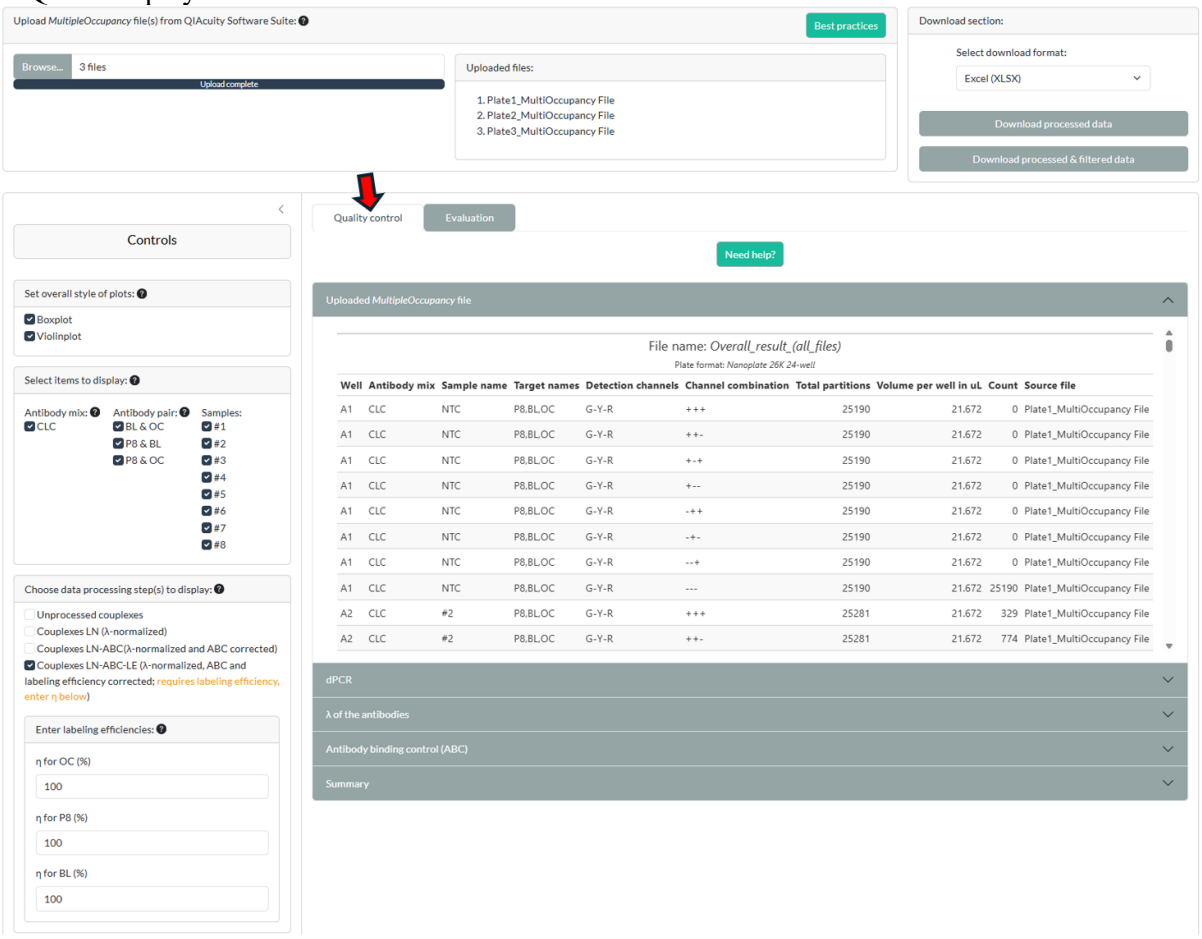
11. After the analysis is completed, the data and graphs can be downloaded by clicking on the appropriate buttons in the “Absolute quantification” tab, as well as the upper-right corner of the software.

04.3 PIQuant Absolute Quantification Analysis

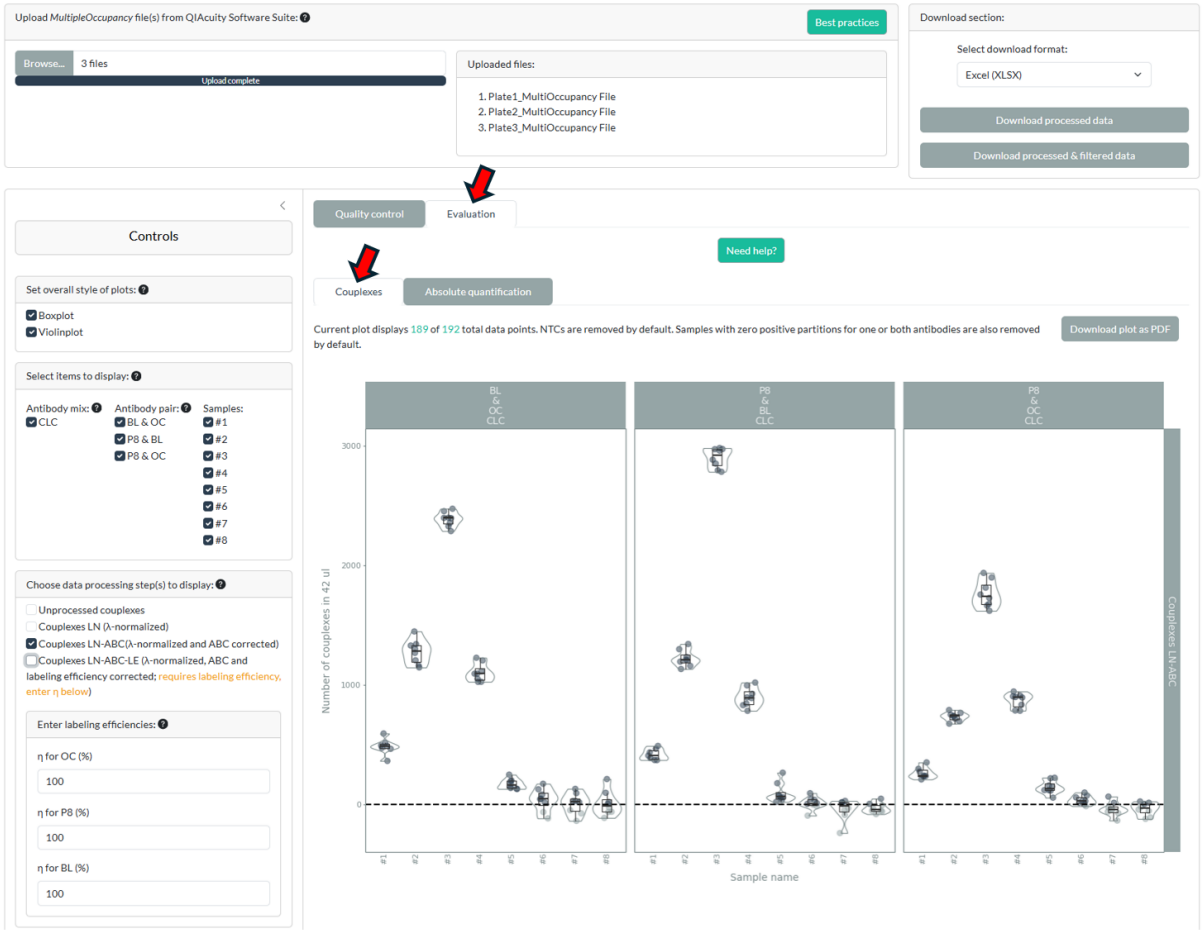
- Upload your multioccupancy file(s)



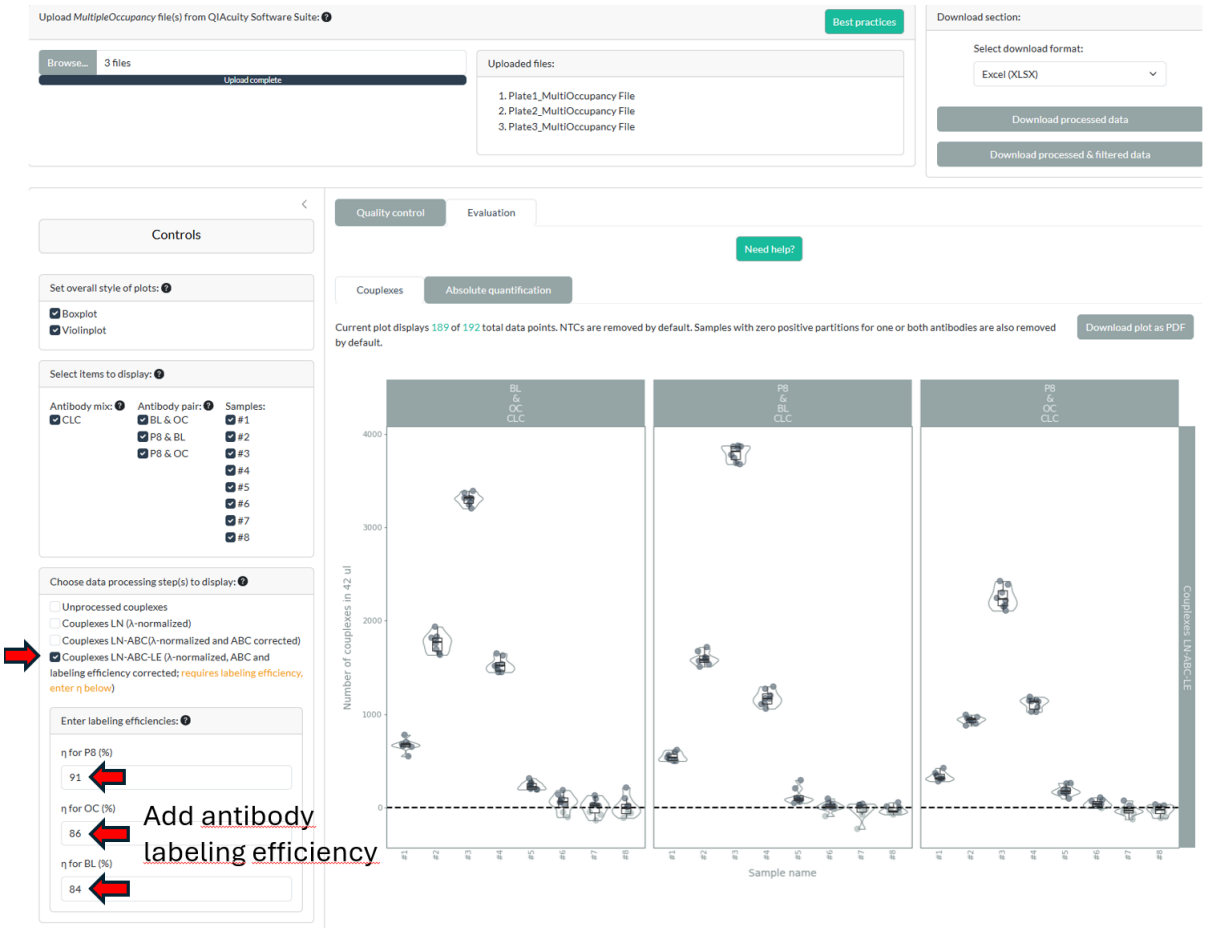
- On the "Quality Control" tab, validate your data following the recommendations of PIQuant displayed



- Change to the “Evaluation” tab and the sub-tab “Couplexes”



For the highest precision in absolute quantification, it is necessary to know the labeling efficiency of the antibodies. Add them to the corresponding box. Select the data processing step *Couplexes LN-ABC-LE*



- In this example, sample #1 has the highest target concentration and sample #8 the lowest. The data follows a bell-shaped curve as a consequence of the so-called hook effect, which can occur in immunoassays that use two antibodies for detection. When the target concentration exceeds the antibody concentration, the couplex count can INCREASE as the target concentration DECREASES and is considered as the "high-side". Samples in which a DECREASE in couplex count is observed with DECREASING target concentration are considered the "low-side" (See Figure 1).

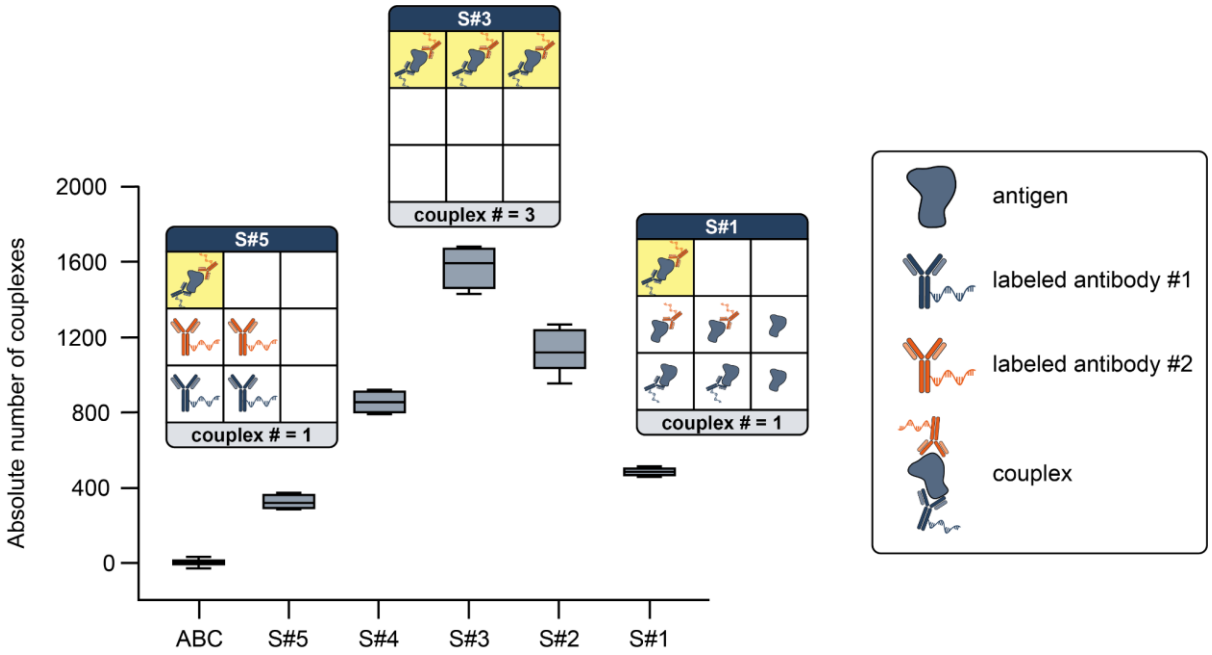
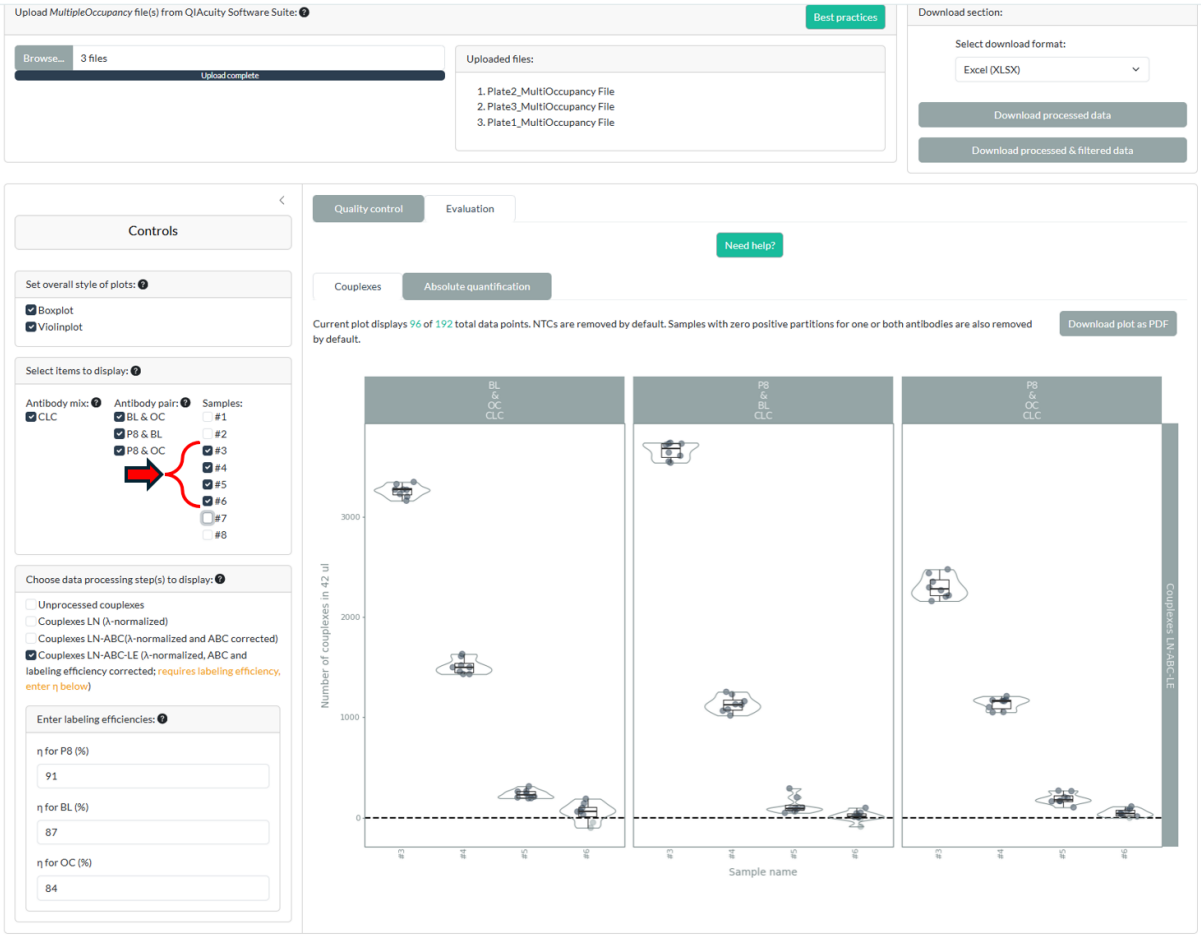
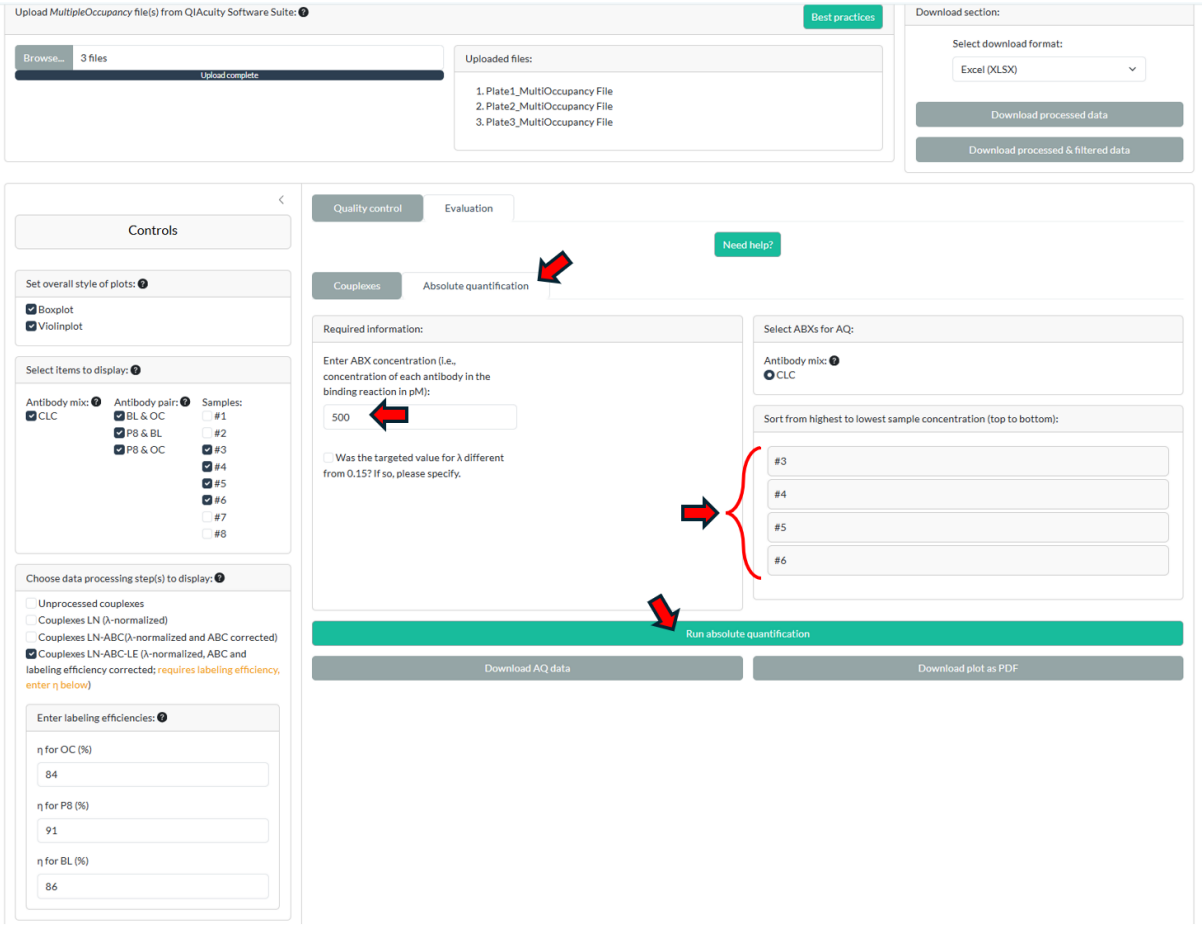


Figure 1. Typical result of measuring a dilution series with PICO: Boxplots show the absolute number of couplexes (yellow) detected across dilution steps (S#5 to S#1), forming a **bell-shaped curve**. Ratios of antigen to labeled antibodies are displayed for **S#5, S#3, and S#1**. The boxplots indicate the **mean (solid line)**, **25th/75th percentiles (box edges)**, and **10th/90th percentiles (whiskers)**. ABC = antibody control.

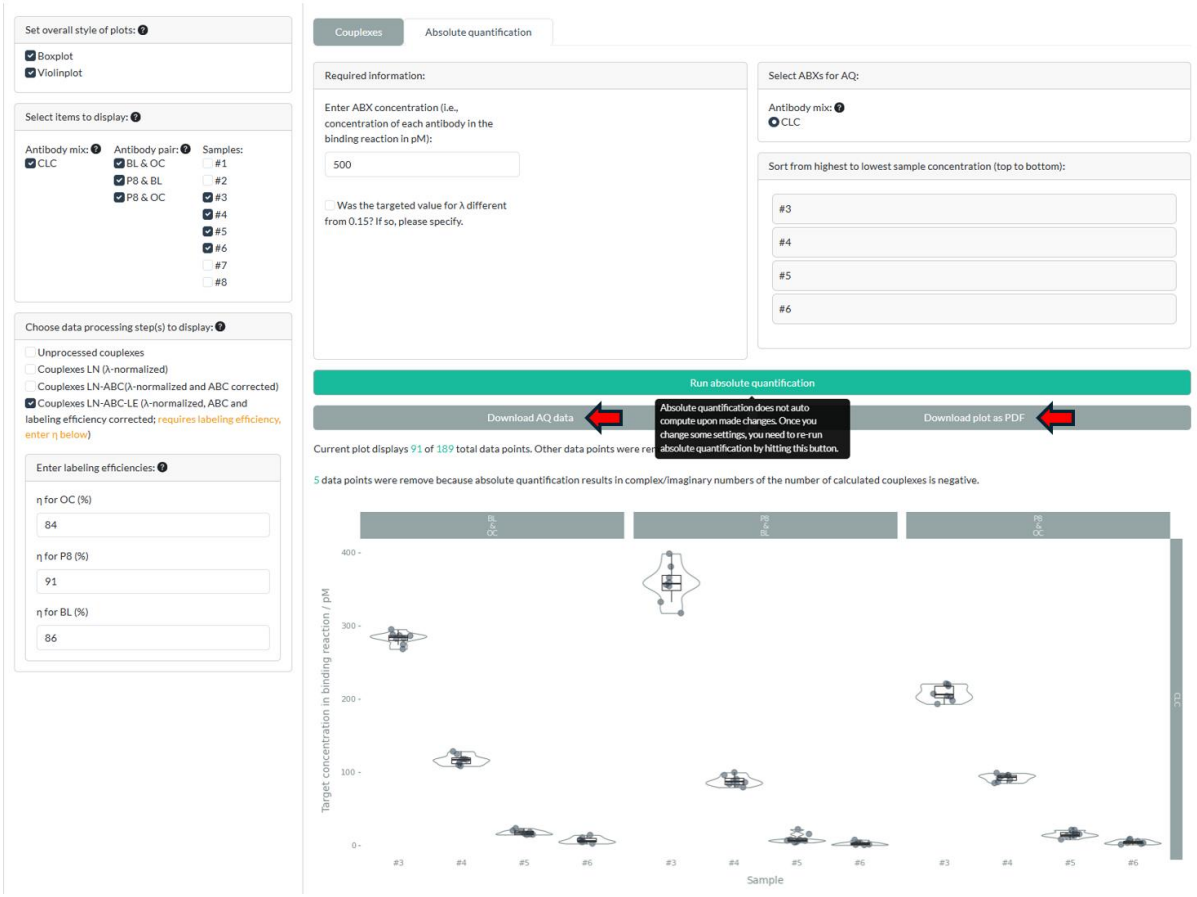
- PIQuant can use the high- or low-side for quantification. Choose either the low- or high side for absolute quantification evaluation. In this example, samples #3, # 4, #5, and #6 were chosen for absolute quantification on the low side:



- Change to the "Absolute quantification" tab and set the antibody concentration that has been used in the binding reaction (500 pM is recommended for saturating conditions). Sort the samples from highest to lowest concentration. Press the button "absolute quantification".



- Check the chart as a quick sanity check to confirm whether the data matches your expectations. You can download the plot and the absolute quantitative data. Note that the concentrations are usually low; if the chart appears to show zeros throughout, adjust the axis scaling (e.g., use a logarithmic or scientific/exponential format) to better visualize low concentrations.



05 Troubleshooting Guide

Consult the troubleshooting guide below to solve problems that may arise. For more information, visit our support page.

Troubleshooting	
Issue	Comments and Suggestions
Lambda value in PICO assay not in range (0.01-0.6)	
Antibody concentration determined during quality control of labeled antibodies was not correct	Recalculate antibody concentrations using the data of the PICO assay and repeat the assay with the new concentrations. For this, the antibody concentration of each antibody found in the 'Current Results' file of the PICO assay is multiplied with the dilution factor back to the antibody stock for the corresponding antibody (DF_{BS}). The dilution factor back to the antibody stock concentration can also be calculated using the PICO Calculator.
No couplexes or low numbers of couplexes detected	
Antibody concentration determined during quality control of label-loaded antibodies was not correct	Check if the lambda value is in range (0.01-0.6), if not, recalculate concentrations based on the data of the PICO assay and repeat the assay with the new concentrations.
Wrong default threshold of fluorescence intensity (RFU) was set in the QIAcuity software suite	Select '1D Scatter Plot' in analysis mode of the QIAcuity software suite and adapt the thresholds.

06 End User License Agreement (EULA)

License scope: Non-exclusive, non-transferable right to use PIQuant (incl. SaaS) per purchased license (single/multi-user).

Permitted use: Only for data generated with PICO Kits purchased from PICO BioScience.

Prohibited actions: No reverse engineering; no unlawful use; no sharing SaaS links/logins/tokens; no distribution/rental/lease/lending without consent.

IP ownership: PICO BioScience retains all intellectual property rights (patent-protected).

Warranty & liability: Software provided "as is"; warranties disclaimed; liability for damages broadly limited/excluded.

Indemnification: User must defend/indemnify PICO BioScience for claims arising from misuse or breach.

Termination: License ends if the user stops and destroys copies/credentials, or if PICO terminates for noncompliance.

Governing law & disputes: German law; negotiation first, then mediation in Freiburg, Germany before litigation.

Privacy/data: User owns the data and is responsible for storage; PICO states it doesn't collect/use user data.

Compliance/other: Export control compliance; third-party/open-source licenses apply; force majeure and standard contract clauses.

The detailed End User License Agreement can be found on our webpage.