

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.

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 complex data will be processed.
 - For maximal accuracy of Absolute Quantitative data, we recommend using lambda normalization, ABC, and labeling efficiency corrections (Complexes 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 complex graphs give a first insight into the relative abundance of your target. If a dilution series was performed, the complex 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 complexes 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)

PIQuant: evaluation of PICO experiments

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

Upload MultipleOccupancy file(s) from QIAcuity Software Suite: Best practices

Browse... 3 files Upload complete

Uploaded files:

1. Plate1_MultiOccupancy File
2. Plate2_MultiOccupancy File
3. Plate3_MultiOccupancy File

Select download format:
Excel (XLSX)

Download processed data

Download processed & filtered data

Quality control Need help? Evaluation

Uploaded MultipleOccupancy file

File name: Overall_result_(all_files)
Plate format: Nanoplate 25K 24-well

Well	Antibody mix	Sample name	Target names	Detection channels	Channel combination	Total partitions	Volume per well in uL	Count	Source file
A1	CLC	NTC	P8.BLOC	G-Y-R	+++	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	++-	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	++	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	+--	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	+-	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	--+	25190	21.672	0	Plate1_MultiOccupancy File
A1	CLC	NTC	P8.BLOC	G-Y-R	---	25190	21.672	25190	Plate1_MultiOccupancy File
A2	CLC	#2	P8.BLOC	G-Y-R	+++	25281	21.672	329	Plate1_MultiOccupancy File
A2	CLC	#2	P8.BLOC	G-Y-R	++-	25281	21.672	774	Plate1_MultiOccupancy File

dPCR

λ of the antibodies

Antibody binding control (ABC)

Summary

Set overall style of plots:

Boxplot

Violinplot

Select items to display:

Antibody mix: CLC

Antibody pair: BL & OC, P8 & BL, P8 & OC

Samples: #1, #2, #3, #4, #5, #6, #7, #8

Choose data processing step(s) to display:

Unprocessed complexes

Complexes LN (λ-normalized)

Complexes LN-ABC (λ-normalized and ABC corrected)

Complexes LN-ABC-LE (λ-normalized, ABC and labeling efficiency corrected: requires labeling efficiency, enter η below)

Enter labeling efficiencies:

η for OC (%)
100

η for P8 (%)
100

η for BL (%)
100

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

Upload MultipleOccupancy file(s) from QIAcuity Software Suite: Best practices

Browse... 3 files Upload complete

Uploaded files:

1. Plate1_MultiOccupancy File
2. Plate2_MultiOccupancy File
3. Plate3_MultiOccupancy File

Download section:

Select download format:

Excel (XLSX)

Download processed data

Download processed & filtered data

Controls

Set overall style of plots:

- Boxplot
- Violinplot

Select items to display:

Antibody mix: CLC

Antibody pair: BL & OC, PB & BL, PB & OC

Samples: #1, #2, #3, #4, #5, #6, #7, #8

Choose data processing step(s) to display:

- Unprocessed complexes
- Complexes LN (λ-normalized)
- Complexes LN-ABC (λ-normalized and ABC corrected)
- Complexes LN-ABC-LE (λ-normalized, ABC and labeling efficiency corrected; requires labeling efficiency, enter η below)

Enter labeling efficiencies:

η for OC (%)

η for PB (%)

η for BL (%)

Quality control **Evaluation**

Complexes Absolute quantification Need help?

Current plot displays 189 of 192 total data points. NTCs are removed by default. Samples with zero positive partitions for one or both antibodies are also removed by default. Download plot as PDF

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*

The screenshot shows the software interface for data analysis. At the top, there is a file upload section with 3 files uploaded. Below this is the 'Controls' panel on the left, which includes options for plot style (Boxplot, Violinplot) and data processing steps. The 'Choose data processing step(s) to display' section has 'Couplexes LN-ABC-LE' selected. Below this, the 'Enter labeling efficiencies' section has input fields for PB (91%), OC (86%), and BL (84%) with red arrows pointing to them. The main area shows a box plot titled 'Couplexes' with 'Absolute quantification' selected. The plot displays the number of complexes in 42 µl for 24 samples, grouped by antibody mix (BL & OC, PB & BL, PB & OC). A red arrow points to the 'Couplexes LN-ABC-LE' option in the controls panel. Another red arrow points to the 'Add antibody labeling efficiency' text next to the input fields.

- 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 complex count can INCREASE as the target concentration DECREASES and is considered as the “high-side”. Samples in which a DECREASE in complex count is observed with DECREASING target concentration are considered the “low-side” (See Figure 1).

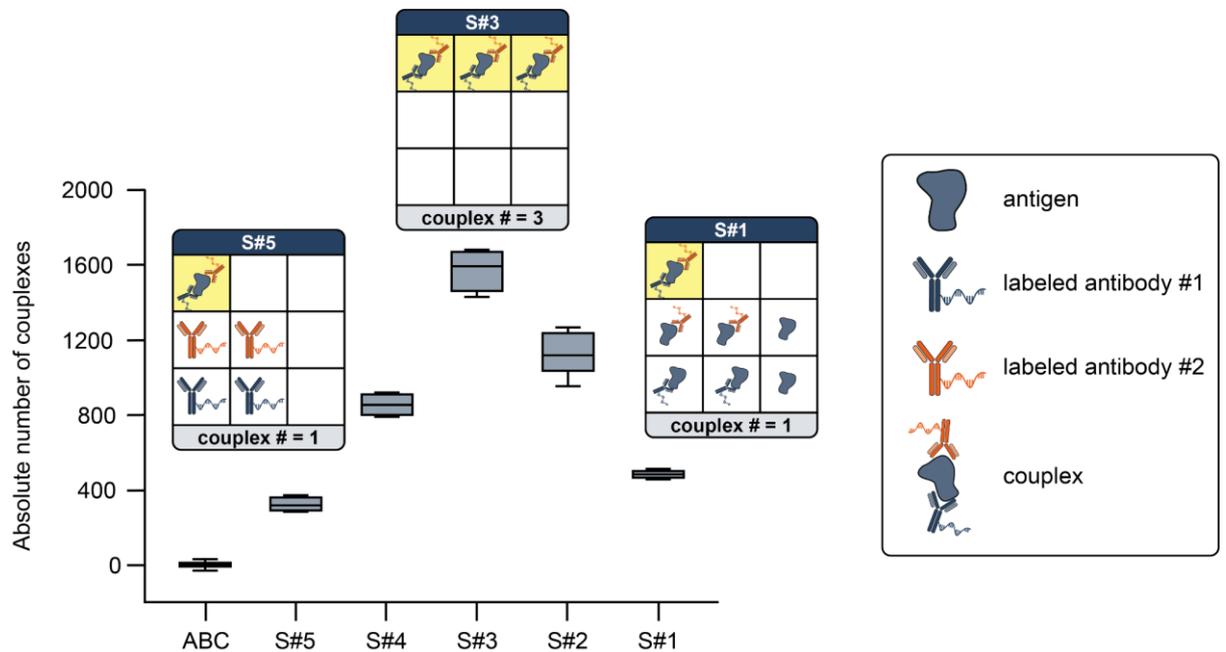


Figure 1. Typical result of measuring a dilution series with PICO: Boxplots show the absolute number of complexes (yellow) detected across dilution steps (S#5 to S#1), forming a **bell-shaped curve**. Ratios of antigen to labeled antibodies are displayed for **S#5, S#3, and S#1**. The boxplots indicate 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:

Upload MultipleOccupancy file(s) from QIAcuity Software Suite: Best practices

Browse... 3 files Upload complete

Uploaded files:

1. Plate2_MultiOccupancy File
2. Plate3_MultiOccupancy File
3. Plate1_MultiOccupancy File

Select download format:
Excel (XLSX) ▼

Download processed data

Download processed & filtered data

Controls

Set overall style of plots:

Boxplot
 Violinplot

Select items to display:

Antibody mix: CLC

Antibody pair: BL & OC
 P8 & BL
 P8 & OC

Samples: #1
 #2
 #3
 #4
 #5
 #6
 #7
 #8

Choose data processing step(s) to display:

Unprocessed complexes
 Complexes LN (λ -normalized)
 Complexes LN-ABC (λ -normalized and ABC corrected)
 Complexes LN-ABC-LE (λ -normalized, ABC and labeling efficiency corrected; requires labeling efficiency, enter η below)

Enter labeling efficiencies:

η for P8 (%)
91

η for BL (%)
87

η for OC (%)
84

Quality control Evaluation Need help?

Complexes Absolute quantification

Current plot displays 96 of 192 total data points. NTCs are removed by default. Samples with zero positive partitions for one or both antibodies are also removed by default. Download plot as PDF

Number of complexes in 42 ul

Sample name

- 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".

The screenshot displays the QIAcuity software interface for absolute quantification. At the top, there is a file upload section where three files (Plate1, Plate2, Plate3 MultiOccupancy Files) have been uploaded. The 'Download section' on the right allows for downloading processed data in Excel (XLSX) format.

The main interface is divided into several sections:

- Controls:** Includes options for plot styles (Boxplot, Violinplot) and items to display (Antibody mix: CLC, Antibody pair: BL & OC, P8 & BL, P8 & OC, Samples: #1-#8).
- Quality control / Evaluation:** The 'Absolute quantification' tab is selected. It contains:
 - Required information:** A field for 'Enter ABX concentration (i.e., concentration of each antibody in the binding reaction in pM):' with the value '500' entered.
 - Select ABXs for AQ:** 'Antibody mix: CLC' is selected.
 - Sort from highest to lowest sample concentration (top to bottom):** A list of samples #3, #4, #5, and #6.
 - Buttons:** 'Run absolute quantification', 'Download AQ data', and 'Download plot as PDF'.
- Labeling efficiencies:** A section for entering efficiencies: η for OC (%) is 84, η for P8 (%) is 91, and η for BL (%) is 86.

- 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.

Set overall style of plots:

Boxplot
 Violinplot

Select items to display:

Antibody mix: CLC

Antibody pair: BL & OC
 PB & BL
 PB & OC

Samples: #1
 #2
 #3
 #4
 #5
 #6
 #7
 #8

Choose data processing step(s) to display:

Unprocessed couplexes
 Couplexes LN (λ -normalized)
 Couplexes LN-ABC (λ -normalized and ABC corrected)
 Couplexes LN-ABC-LE (λ -normalized, ABC and labeling efficiency corrected; requires labeling efficiency, enter η below)

Enter labeling efficiencies:

η for OC (%)
84

η for PB (%)
91

η for BL (%)
86

Couplexes | Absolute quantification

Required information:

Enter ABX concentration (i.e., concentration of each antibody in the binding reaction in pM):
500

Was the targeted value for λ different from 0.15? If so, please specify.

Select ABXs for AQ:
Antibody mix: CLC

Sort from highest to lowest sample concentration (top to bottom):

#3
#4
#5
#6

Run absolute quantification

Download AQ data

Download plot as PDF

Current plot displays 91 of 189 total data points. Other data points were removed because absolute quantification results in complex/imaginary numbers of the number of calculated complexes is negative.

5 data points were removed because absolute quantification results in complex/imaginary numbers of the number of calculated complexes is negative.

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 complexes or low numbers of complexes 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](#).