PICO Amplification Core Kit
USER MANUAL

Content

actome
a trademark of
PICO BioScience GmbH

PICO BioScience GmbH
Georges-Köhler-Allee 103
79110 Freiburg
Germany

01 Kit Contents and Storage

PICO Amplification Core Kit		
Catalog Number:		PICO-000010
Pouch - store at 4°C	**pcs**	**Item code**
Additive T	1	A
Additive C	5	B
Bovine serum albumin	5	C
Pouch - store at -20°C		
Additive L	5	D
Coupling dPCR Mix	1	E

The PICO Amplification Core Kit (AMC) ships at room temperature. Upon arrival, immediately store components at their optimal temperatures (noted on pouches). Note the different required storage temperatures. Expiration dates are on the back of the pouches.

02 Intended Use

The PICO AMC Kit is for research use only (RUO) and not for diagnosis, prevention, or treatment of disease. Handle the product with care, adhering to national safety guidelines.

03 Safety Information

Always wear a lab coat, disposable gloves, and protective goggles when handling chemicals. Consult the appropriate material safety data sheets (MSDS) for more information. These are available at: www.actome.de/resources/safety-data-sheets

For reagent spillage, absorb, dispose of, and clean with laboratory detergent and water. If the spilled liquid is potentially infectious, clean first with detergent and water, then with 1% (w/v) sodium hypochlorite.

04 Quality Control

Each PICO AMC Kit is tested against predetermined specifications to ensure consistent product quality.

05 Introduction

Protein Interaction Coupling (PICO) is an immunoassay for the characterization and quantification of EVs and detecting and quantifying proteins, protein interactions, and post-translational modifications using the QIAcuity Digital PCR System. The AMC Kit supports both 24- and 96-well QIAcuity Nanoplates (26k and 8.5k, respectively), allowing for 120 (five 24-well plates) or 480 (five 96-well plates) PICO reactions. Appropriate PICO BL, P8, N6, or O7 Probes are required based on the PICO Labels used. The PICO AMC protocol is a two-day procedure with minimal hands-on time (< 1 h 30 min) (**Figure 1**).

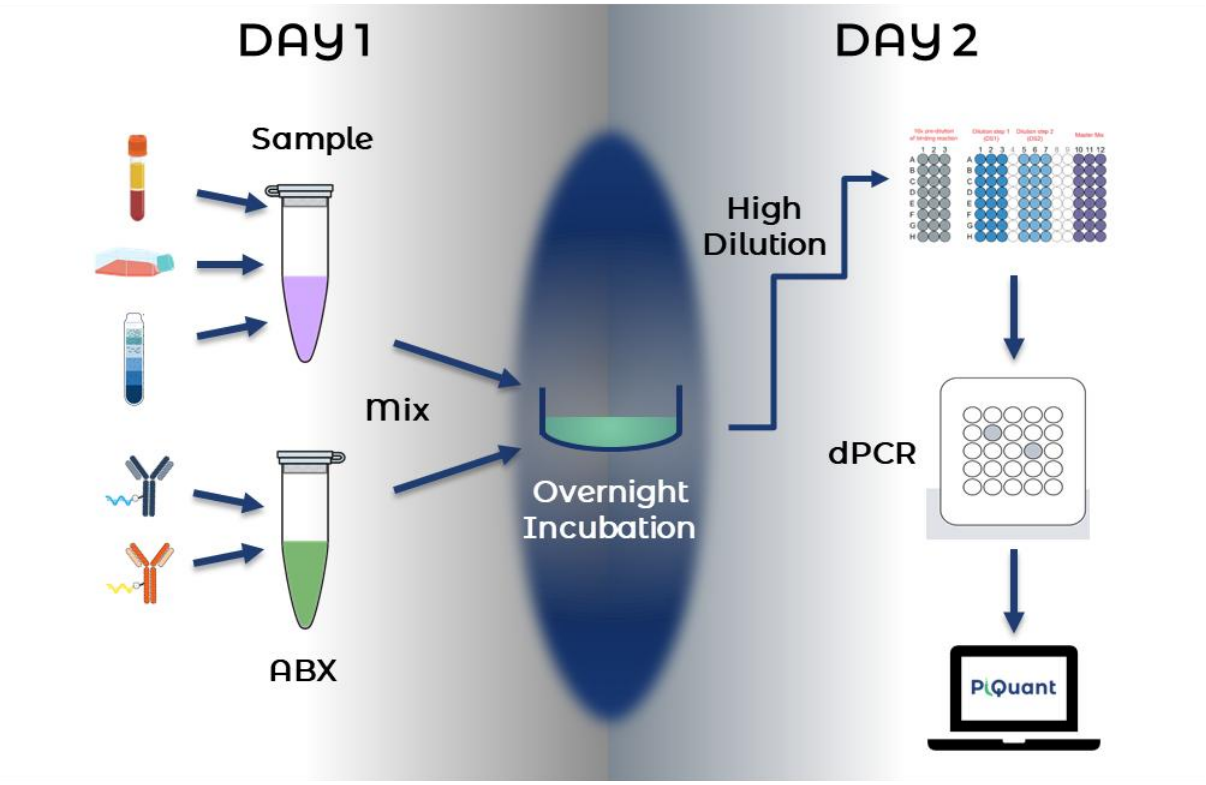


Figure 1 The PICO AMC Kit workflow

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.

06 Preparation for a PICO Assay

06.1 General Remarks

- The PICO assay requires precise pipetting. Always use calibrated pipettes for accurate results, especially at high dilution factors. Mix gently by pipetting up and down to avoid spills and material loss.
- Change gloves frequently to prevent contamination, which can be detected if the NTC (no-template control) shows positive signals.
- For best results, use four technical replicates per sample. Include both an NTC and an antibody control (ABC). The ABC contains only antibodies (no sample) and should ideally show zero couplex counts. Any deviation is automatically corrected by the PIQuant software.
- The Lambda value should be below 0.55, with 0.15 being optimal. A lower Lambda improves couplex-detection accuracy by reducing biases, but setting it too low decreases assay sensitivity and limits the detection of statistically significant couplexes.
- Note: Sensitivity depends on partition count. Using the 8.5k 96-well plate reduces sensitivity by ~3x compared to the 26k 24-well plate.

06.2 Concentration of Antibodies in the Binding Reaction

PICO assays require at least two antibodies per target, which should bind to two different, concurrently accessible epitopes.

- **For relative quantification:** Use **40 pM** antibody in the binding reaction.
- **For absolute quantification:** Use **500 pM** (saturated conditions).

06.3 Considerations Regarding Biological Material

Biological Material		
Feature	Main criteria	Additional criteria
Sample type	Any solubilized biological sample	Different sample preparation steps for homogenization is necessary
Sample preparation	Sample dependent	• Lyse cultured/primary cells using our lysis buffer (LB) • Mechanical/enzymatic homogenization of tissue material
Buffer	Lysis buffer (LB)	Lysis buffer (LB) is suitable for most biological materials; however, in some cases, adaptation might be necessary
Homogenization	Sonification and QIAShredder Spin Column treatment (Cat.#: 79656)	
Stabilization of proteins/ interactions	optional	• Use 1x PIC-PBS for all washing steps during cell preparation (cOmplete Protease™ Inhibitor Cocktail (Roche, Cat.#: 04693132001))
Stability of prepared sample	Sample dependent	Cell lysate can be stored at 4°C for up to 4 days, however we recommend using it immediately. Freezing of cell lysate is also an option (see *Section 08.2*)

- As an input for cell lysis, we recommend using a total of 1 million cells in a concentration of 1×10^4 cells/µl. However, total input and cell concentration can be adapted to the user's needs.
- For high expressing targets, we recommend using 6-fold dilution series. Thus, performing a total of five dilution steps results in a dilution series of undiluted, 6-, 36-, 216-, 1296- and 7776-fold diluted samples.
- For low expressing targets, we recommend using 2-fold dilution series. Thus, performing a total of five dilution steps results in a dilution series of undiluted, 2-, 4-, 8- and 16-fold diluted samples.

06.4 Exemplary Setup of a Binding Reaction for a 24-well dPCR Nanoplate

Plate layout of an example binding reaction for a 24-well PICO experiment. In this example, five samples, with four technical replicates each, were used. For the ABC, three technical replicates are recommended.

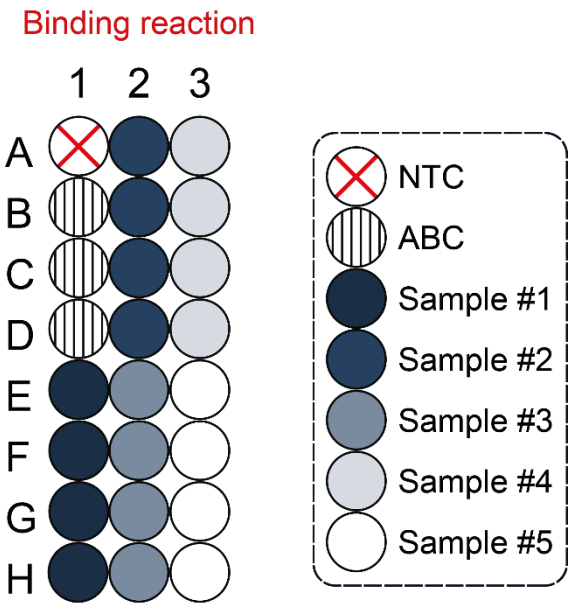


Figure 2 Example Binding Reaction

07 Equipment and Reagents to be Supplied by User

Notes

Note: Equipment and reagents required for the preparation of biological material are not listed below and depend on the input material.

Devices/Equipment

- QIAcuity Digital PCR System (QIAGEN, Cat.#: 911001)
- Table-top mini centrifuge for quick spins (~1,000 rcf)
- Plate centrifuge (e.g., Megafuge 8 (Thermo Fisher, Cat.#: 75007210)
- Vortex mixer
- Ultrasonic bath
- Multichannel pipette, 8-channel (1-10 µl)
- Multichannel pipette, 8-channel (10 – 100 µl)
- Multichannel pipette, 8-channel (30 – 300 µl) (optional)
- Regular 1-channel pipettes (1 – 1,000 µl)
- Electronic Multichannel pipette (INTEGRA, Cat.#: 4722), 12-channel, 5-12.5 µl (optional)

Consumables

- QIAcuity Nanoplate 26k 24-well (QIAGEN, Cat.#: 250001) or QIAcuity Nanoplate 8.5k 96-well (QIAGEN, Cat.#: 250021)
- PCR microplates, 96-well, Polypropylene, V-bottom or similar
- Sealing foil Adhesive Film (e.g., Thermo Fisher, Cat.#: 10696771)
- 12.5 µl GRIPTIP, Sterile, Filter 5 Racks of 384 Tips, long; for electronic multichannel pipette (INTEGRA, Cat.#: 3405) (optional)
- 1.5 ml reaction tubes
- 0.5 ml reaction tubes
- 0.5 ml low protein binding tubes (e.g., Eppendorf, Cat.#: 0030108094)
- 10 µl, 200 µl, 1,000 µl standard pipette tips
- 15 ml Falcon tubes

Chemicals and Kits

- QIAcuity Probe PCR Kit (QIAGEN, Cat.#: 250101) (~ 1.5 ml)
- PICO Probes (PICO P8, BL, N6, O7 Probe, Cat.#: PICO-000070-73)
- Phosphate-Buffered Solution (PBS), without calcium or magnesium ions (e.g. Thermo Fisher Scientific, Cat.#: 12037539)
- cOmplete Protease™ Inhibitor Cocktail (Roche, Cat.#: 04693132001)
- Ultrapure water (e.g. Thermo Fisher Scientific, Cat.#: 15667708)

08 Protocol of PICO Amplification Core Kit

Note: This protocol applies to PICO assays using up to four antibodies labeled with PICOglue BL, P8, N6, or O7. It covers the setup for both QIAcuity Nanoplate 26k 24-well and QIAcuity Nanoplate 8.5k 96-well plates. Note that the 8.5k 96-well plate, with fewer partitions per sample, reduces assay sensitivity.

08.1 Buffer Preparation

1. Prepare the chemicals and buffers as listed below. The volumes can be adapted to the needs (e.g. if required more LB can be prepared for the dilution series).

   The buffers can be kept for the specified periods of time and used for further experiments. Prepare the buffers directly when they are used.

Additive C (5x stock)
Add 500 µl PBS
Stable for 1 week at 4°C

BSA (5x stock)
Add 400 µl PBS
Stable for 3 days at 4°C

EDTA-free Protease Inhibitor Cocktail (PIC), (25x stock)
1 tablet of cOmplete Protease™ Inhibitor Cocktail
2 ml PBS
Stable for 12 weeks at -20°C

Lysis Buffer Stock (LB-Stock), (2x stock)
200 µl Additive T
400 µl Additive C
80 µl PIC
200 µl Additive L
120 µl PBS
Stable for 3 days at 4°C

Lysis Buffer (LB)
300 µl LB-Stock
300 µl PBS
Stable for 3 days at 4°C

Control Buffer (CB)
250 µl LB-Stock
100 µl BSA
150 µl PBS
Prepare the Control Buffer fresh

PICOglue Storage Buffer
20 µl 10x PICOglue Antibody Storage Buffer
180 µl PBS
Stable for 6 months at 4°C

Buffer is optional for the Preparation of Biological Material

PIC-PBS (1x)
1 PIC tablet
50 ml PBS
Stable for 2 weeks at 4 to 8°C
Stable for 12 weeks at -15 to -25°C

08.2 Preparation of Biological Material - Exemplary for Cultivated Cells

Note: Adaptations and changes are necessary depending on the biological material used. For recommendations, see *06.3 Requirements for Biological Samples*.

2. Prepare PIC-PBS solution and LB according to *step 1*. For weak or transient protein interactions, cross-linking may be considered.
   The advantages or disadvantages of cross-linking must be discussed individually for each setup. Please select a crosslinker suitable for the type of target proteins.

3. Remove the medium from the flask, add PIC-PBS to wash the cells carefully, and remove the PIC-PBS.
   The amount of PIC-PBS depends on the flask size. We recommend using between 3 ml and 15 ml.

4. Harvest the cells using a cell scraper (e.g. Sigma Aldrich, Cat.#: C5981-100EA).

5. Add PIC-PBS and transfer the cells to a 15 ml Falcon. Centrifuge (400 rcf, 5 min) and discard the supernatant.
   The amount of PIC-PBS depends on the flask size. We recommend using between 3 ml and 15 ml.

   Resuspend the cells in 1 ml of PIC-PBS, transfer to a 1.5 ml reaction tube, centrifuge (400 rcf, 5 min), and discard the supernatant. Repeat the washing with 1 ml of PIC-PBS, centrifuge (400 rcf, 5 min), and discard the supernatant.

6. Resuspend the cells in 1 ml of PIC-PBS. Count the cells and transfer 1×10^6 cells into a new 1.5 ml reaction tube.
   Use the total cell count, not the viable cell count. In case you have less than 1×10^6 cells, reduce the LB volume in step 12 to have a cell concentration of 1×10^6 cells per 100 µl (=1×10^4 cells/µl). Depending on the experimental setup and the desired input, the concentration of the cells can be adapted.

7. Centrifuge (400 rcf, 5 min) and carefully discard the supernatant.
   Discard the supernatant carefully, not to lose cells, as the number of cells in the assay is defined at this step.

8. Resuspend the cells in 100 µl of LB and vortex for 10 s. Lyse the cells for 3 h at 4°C.
   Prepare the antibody mix (ABX) during the incubation time (see *08.3 Binding Reaction 16-19*). Keep leftovers of the LB and LB-Stock for the following steps.

9. Sonicate the lysate for 5 min at full power in an ultrasonic bath at room temperature. Use ice if the temperature reaches a critical level.

10. Transfer lysate into a QIAshredder spin column (QIAshredder, QIAGEN, Cat.#: 79656) and centrifuge at full speed (~20,000 rcf) for 2 min. Transfer the flow-through to a new 1.5 ml reaction tube. The lysate is ready to use.
    Lysate is stable for 4 days at 4°C, though direct use is recommended. Aliquoted lysate can be frozen at -20°C for at least a month. Treat and store all comparative samples identically is recommended.

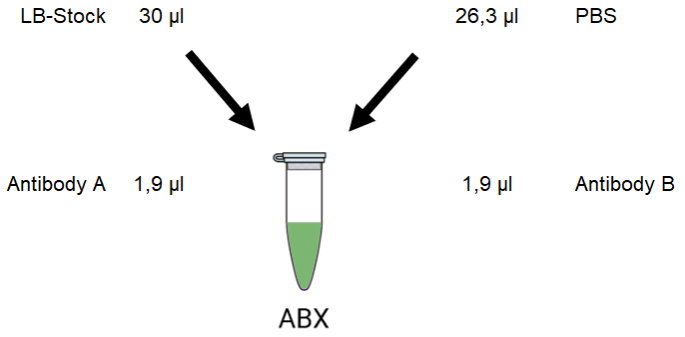
08.3 Binding Reaction

11. Calculate the volume of antibody stocks, LB-Stock, and PBS required for the antibody mix (ABX) using the PICO Calculator (Step 2 in the 'PICO Calculator' tab).
    Antibody dilutions for binding reactions, if prepared in 1x Storage Buffer, can be stored up to 12 months at 4°C. Highly diluted stocks 200-500 times, may be unstable; an intermediate dilution of 20-200 times in 1x Storage Buffer is recommended for storage.

12. Prepare the ABX using LB-Stock. Then add the calculated volume of the antibody stock solutions or antibody pre-dilutions and fill up the remaining volume up with PBS.

    Prepare a minimum of 60 µl of ABX for a QIAcuity Nanoplate 26k 24-well and 210 µl for a QIAcuity Nanoplate 8.5k 96-well. Recommended antibody working concentrations are 40 pM for relative quantification and 500 pM for absolute quantification (selectable in the PICO Calculator).

    Example for 2 Antibodies with a concentration of 1,93E+10 cp/µl:



13. After preparation of the ABX, the biological material prepared in *section 08.2* can be diluted or used directly for the following binding reaction.

14. Set up the binding reaction in a 96-well PCR microplate (v-bottom) (see example in **Section 06.4**). Add 4 µl of LB to a dedicated well for the NTC

15. Combine 2 µl of CB with 2 µl of ABX and make at least three technical replicates for the ABC control.
    Due to the small volume of the binding reaction, do not mix by pipetting. The volume of lysate and ABX can be adapted. This must be considered in the calculations of the ABX and the pre-dilution. Please keep in mind that multiple different ABC control samples might be necessary, if different antibody mixes are used in one experimental setup.

16. Combine 2 µl of the biological sample with 2 µl of ABX. Good laboratory practice recommends using at least four replicates.

17. Seal the plate (without the lid) with an adhesive foil. Vortex the plate for thorough mixing. Centrifuge the plate to collect the liquid at the bottom (~1,000 rcf, 30 s) and incubate at 4°C overnight.
    The incubation time can be varied between 12-24 hours.

08.4 Pre-dilution and Digital PCR

18. Prepare the Master Mix for 24 or 96 samples, ensuring it contains the required PICO Probe(s). If fewer than four labeled antibodies are used, substitute the PICO Probe volume with ultrapure water (see amounts below). Vortex for 10 s and spin down (~1,000 rcf, 5 s). The Master Mix can be prepared up to three days in advance.

Master Mix		
Reagents	**24-well plate**	**96-well plate**
Ultrapure water	606 µl	834 µl
QIAcuity Probe Master Mix	284 µl	390 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
PICO Probe (P8, BL, N6, or O7)	45 µl	62 µl
Coupling dPCR Mix	36 µl	50 µl

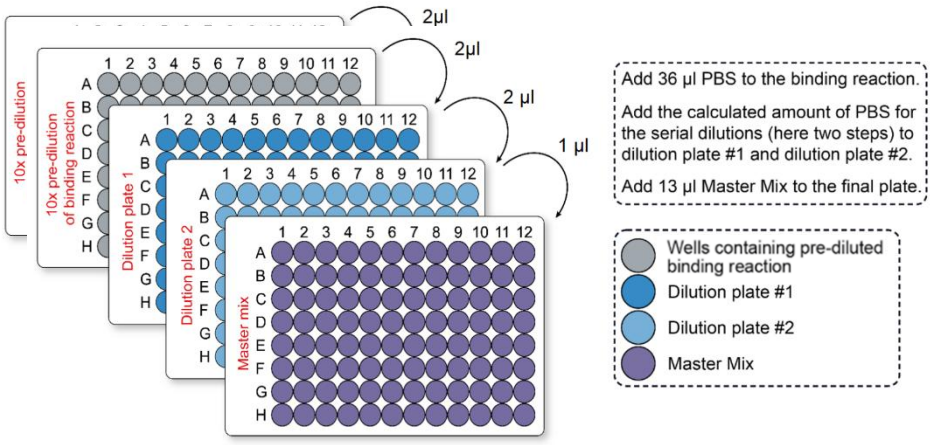
Note: If you use less than 4 Probes add the respective amount of ultrapure water to the Master Mix.

19. PICO Assay Preparation Instructions

For a 24-well PICO Assay:

a. Prepare a new 96-well plate for the dilution steps.
b. Add 120 µl of PBS for a 500 pM ABX concentration or 33 µl of PBS for a 40 pM ABX concentration to columns 1-3 (for Dilution Series 1, DS1) and columns 5-7 (for DS2).
c. Add 41 µl of Master Mix to columns 10-12.

For a 96-well PICO Assay:



a. Prepare three 96-well plates: Dilution Plate #1, Dilution Plate #2 filled with 143 µl PBS when using a 500 pM ABX concentration or 39 µl PBS for an ABX concentration of 40 pM.
b. Master Mix Plate #3 is filled with 13 µl Master Mix.

20. Remove the adhesive foil from the incubated sample plate. Add 36 µl of PBS to the 96-well plate containing the binding reactions (represents the recommended first 10x pre-dilution). Mix thoroughly by pipetting up and down.

    Perform the following steps without unnecessary breaks, as the dilution disrupts the equilibrium binding conditions and induces antibody dissociation.

21. Transfer 2µl volume from each pre-diluted sample into the corresponding wells of the dilution plate (DS1/Dilution plate #1). Mix by pipetting up and down 10 times.

22. When performing the second dilution step, transfer 2 µl volume from the first dilution step into the corresponding wells of the dilution plate (DS2/Dilution plate #2). Mix thoroughly by pipetting up and down.

23. Finally, transfer **1 µl** from the last dilution step into the wells containing the Master Mix. Mix thoroughly by pipetting up and down.

24. Transfer 40 µl (24-well) or 12 µl (96-well) of the diluted sample Master Mix to the QIAcuity Nanoplate. Seal and insert the Nanoplate into the QIAcuity dPCR System. Run the dPCR program using the following dPCR settings:

Priming			
QIAGEN Standard Priming Profile			
PCR conditions			
Step	**Temperature**	**Time**	
Hot-start	95°C	2 min	
Denaturing	95°C	15 s	Cycle 40 times
Annealing	58°C	30 s	

Imaging conditions			
PICO Probe	**QIAcuity channel**	**Integration time**	**Gain**
P8 Probe	FAM, green channel	500 ms	6
BL Probe	HEX, yellow channel	400 ms	6
N6 Probe	TAMRA, orange channel	400 ms	6
O7 Probe	ROX, red channel	300 ms	4

09 Evaluation

For the Evaluation with the PIQuant Software please use this link. To get more information consult the PIQuant User Manual or contact our support team here.

10 Troubleshooting Guide

If you encounter any issues, please refer to the troubleshooting guide provided below. Additional support is available here. Alternatively, you can contact our customer support team using the online contact form.

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 (which can be found at the bottom of 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 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 QIAcuity software suite and adapt the thresholds.
Sample concentration outside the dynamic range of the PICO assay	Perform a longer dilution curve to find the optimal concentration of target detection. Too high target concentration can result in lack of couplex detection.

11 Ordering Information

PICO kits can be purchased directly from shop.actome.de or a quote can be requested from sales@actome.de. Supporting materials are available on www.actome.de/resources/downloads or can be requested from our Customer Support (support@actome.de).

Ordering		
Product	Description	Cat.#
PICO Amplification Core Kit	dPCR detection for PICO assays (5 x 24 reactions; 5 x 96 reactions)	PICO-000010
PICO Probes	P8 (FAM), BL (HEX), N6 (TAMRA), O7 (ROX) for detection in dPCR (5 x 24 reactions)	PICO-000070 - 73