Quick-Start Protocol

PICO Amplification Core Kit

The **PICO Amplification Core (AMC) Kit** (#PICO-000010) contents must be stored at 4°C or -20°C (see product labels for exact storage conditions). Find the date of expiry on the back side of the pouches.

Further information:

- The PICO AMC User Manual, the PICO Calculator, and the Safety Data Sheets are available at: **www.actome.de/resources/downloads**
- The AMULATOR can be accessed here: **https://amulator.actome.de/**

Notes before starting:

- The PICO AMC workflow is compatible with both 24- and 96-well QIAcuity Nanoplates.
- Perform all centrifugation steps with 400 rcf for 5 min at RT unless stated otherwise.
- **Important:** For absolute quantification (AQ), we recommend an antibody concentration of 5×10^{-10} M in the binding reaction. If AQ is *not* required, we recommend an antibody concentration of 4×10^{-11} M.
- Mixing by pipetting should be done slowly (30 times) to avoid generation of air bubbles.
- We recommend using at least four technical replicates for each sample.
- For the analysis of cultivated cells, we recommend using a total of 1 million cells in a concentration of 1×10^4 cells/µl as an input.
- We recommend using 3-fold dilution steps for high abundance proteins and 2-fold dilution steps for low abundance proteins.
- Sample preparation is dependent on the sample type used. Here, cultivated cells are described.
- During the analysis of protein interactions using a cross-linker is optional.

DAY 1

Preparation of biological material:

1. Prepare the following chemicals and buffers:

Additive C (5x stock)	BSA (5x stock)	EDTA-free Protease Inhibitor Cocktail (PIC), (25x stock) - not provided	PIC-PBS
add 500 µl PBS	add 400 µl PBS	dissolve 1 tablet of cOmplete Protease™ Inhibitor Cocktail (PIC) in 2 ml PBS	dissolve 1 PIC tablet in 500 µl PBS
Lysis Buffer Stock (LB-Stock), (2x stock)	**Lysis Buffer (LB)**	**Control Buffer (CB)**	**BS3 Stock Solution (BS3S), (20x stock) - optional**
200 µl Additive T 400 µl Additive C 80 µl PIC 200 µl Additive L 120 µl PBS	300 µl LB-Stock 300 µl PBS	250 µl LB-Stock 100 µl BSA 150 µl PBS	dissolve 2 mg BS3 in 37.8 µl DMSO
			BS3 Working Solution (BS3W) - optional
			6 µl BS3S 114 µl PBS

2. Harvest the cells with the preferred method and wash them in **~5 ml PIC-PBS**. Centrifuge and discard supernatant.
3. Resuspend the cells in **1 ml PIC-PBS** and transfer them to a 1.5 ml reaction tube. Centrifuge and discard supernatant.
4. Repeat the washing step with **1 ml PIC-PBS**, centrifuge, and discard supernatant. If cross-linking is required use **PBS** instead and continue with step 5, otherwise skip ahead to step 8.

DAY 1

5. Resuspend the cells in **1 ml PBS**, count and transfer 2 × 10^6 cells into a new 1.5 ml reaction tube. Centrifuge and discard supernatant.
6. Add **100 µl BS3W**, mix by pipetting, and incubate for 30 min at RT.
7. Add **900 µl PIC-PBS**, centrifuge, and discard supernatant.
8. Resuspend the cells in **1 ml PIC-PBS**, count, and transfer 1 × 10^6 cells into a new 1.5 ml reaction tube. Centrifuge and discard supernatant carefully.
9. Resuspend the cells in **100 µl LB** and vortex for 10 s. Lyse the cells for 3 h at 4°C.
10. Sonicate the lysate for 5 min at full power in an ultrasonic bath at RT.
11. Transfer the lysate into a QIAshredder spin column. Centrifuge (~20,000 rcf, 2 min) and transfer the flow-through to a new 1.5 ml reaction tube.

Binding reaction:

12. 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). Make sure the correct Nanoplate is selected in cell E7.
13. Set up the binding reaction in a 96-well PCR microplate (see example layout for a 24-well PICO assay in **Figure 1**). For the **NTC** add **4 µl LB** to the A1 well. For the **ABC** combine **2 µl CB** with **2 µl ABX** (prepare three technical replicates). For the **samples** combine **2 µl biological sample** with **2 µl ABX** (prepare four technical replicates).
14. Seal the plate with adhesive foil and sonicate for 1 min at full power in an ultrasonic bath at RT. Centrifuge the plate (~1,000 rcf, 30 s) and incubate at 4°C overnight.

DAY 2

Pre-dilution and digital PCR:

15. Prepare the **Master Mix** for 24 or 96 samples, vortex for 10 s, and quick spin (~1,000 rcf, 5 s).

Important: *If less than four PICO Probes are used, replace the volume of not used PICO Probe(s) with ultrapure water.*

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	62 µl

16. Calculate the required dilutions to target a **lambda of 0.15** in step 3 of the **PICO Calculator**.
17. For a 24-well PICO assay prepare a new 96-well plate for the dilution steps. Add the calculated amount of **PBS** to columns 1 - 3 and 5 - 7, and **41 µl Master Mix** to columns 10 - 12 (**Figure 2**). For a 96-well PICO assay prepare three additional 96-well plates. Add the calculated amount of **PBS** to Dilution plate #1 and #2 and add 13 µl Master Mix to the third 96-well plate (**Figure 3**).
18. Remove the adhesive foil of the plate containing the binding reaction and add **36 µl PBS** to all wells (reflects the '10x pre-dilution of binding reaction' in **Figure 2 and 3**). Mix thoroughly by pipetting.
19. Transfer the predetermined volume (usually 1 µl) from each pre-diluted sample to the corresponding wells of the dilution plate and mix by pipetting (DS1/Dilution plate #1). Repeat the dilution once more (DS2/Dilution plate #2) and finally transfer **1 µl** to the wells/plate containing the **Master Mix** (**Figure 2 and 3**). Mix by pipetting.

DAY 2

20. Transfer **40 µl or 12 µl Master Mix**, containing the diluted samples, to a 24- or 96-well QIAcuity Nanoplate respectively. Seal the plate according to the QIAcuity user manual and run a dPCR using the following parameters:

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

21. Instructions for the data analysis with AMULATOR can be found in the user manual.

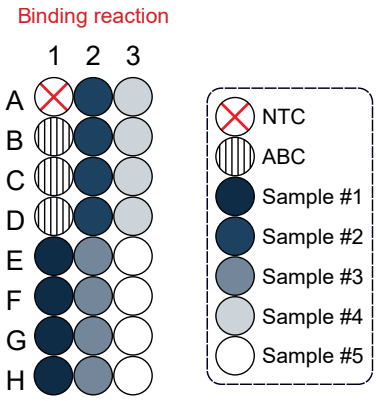

Figure 1: Plate layout of an example binding reaction for a 24-well PICO assay. In this example five samples, with four technical replicates each, were used. For the ABC three technical replicates are sufficient. ABC - antibody control; NTC - non template control

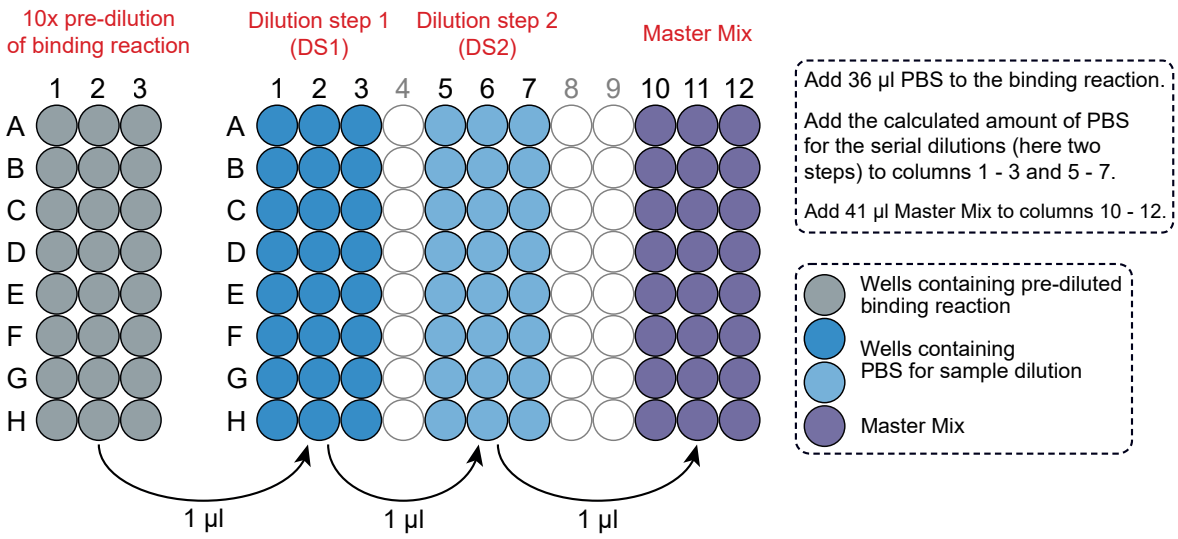

Figure 2: Exemplary setup to perform the dPCR pre-dilution for a 24-well PICO assay. The '10x pre-dilution of binding reaction' is prepared in the plate containing the binding reaction.

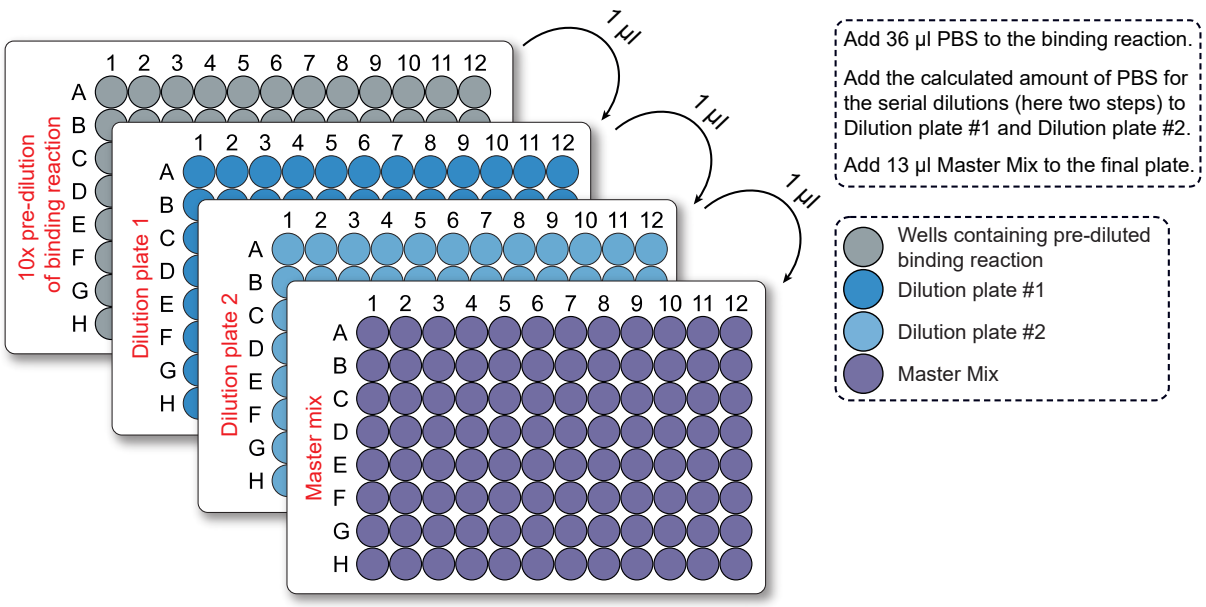

Figure 3: Exemplary setup to perform the dPCR pre-dilution for a 96-well PICO assay. The '10x pre-dilution of binding reaction' is prepared in the plate containing the binding reaction.

Scan the QR code for the user manual: