Bench Protocol – PICO AMC Kit

Additional Resources

- Manuals and Calculator: www.actome.de/resources/downloads
- PI-Quant Software: pico-bioscience.shinyapps.io/piquant/

Notes Before Starting

- Centrifuge: 400 rcf, 5 min, RT (unless otherwise specified)
- Antibody concentration: – 500 pM for absolute quantification
  – 40 pM for qualitative measurement
- Pipette slowly → avoid air bubbles
- Recommended: 3 technical replicates per sample
- For cell analysis: 1 million cells (1×10^4 cells/µl)
- High protein abundance: 6-fold dilution series
- Low protein abundance: 2-fold dilution series
- Example protocol describes cultured cells

A. Sample Preparation

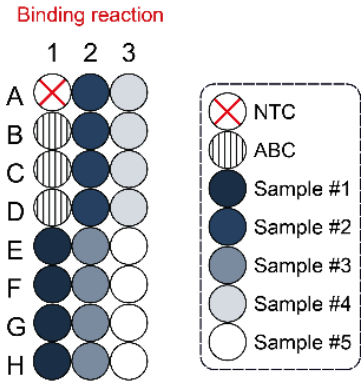
1. Prepare Buffers & Solutions

Reagent	Preparation Instructions
Additive C (5×)	500 µl PBS
BSA (5×)	400 µl PBS
Protease Inhibitor Cocktail (25×)	Dissolve 1 tablet in 2 ml PBS
PIC-PBS	Dissolve 1 tablet in 50 ml PBS
Lysis Buffer (2×)	200 µl Additive T; 400 µl Additive C; 200 µl Additive L; 120 µl PBS; 80 µl PIC
Control Buffer (CB)	250 µl LB-Stock; 100 µl BSA; 150 µl PBS
Storage Buffer	20 µl 10×; 180 µl PBS (for long-term storage + 8.3 µl PIC)

2. Cell Lysis

- Harvest cells, wash with ~5 ml PIC-PBS, centrifuge, and discard supernatant.
- Resuspend in 1 ml PIC-PBS, transfer to a 1.5 ml tube, centrifuge.
- Count cells, and transfer 1×10^6 cells to a new tube, centrifuge.
- Resuspend in 100 µl LB, vortex for 10 s. Lyse for 3 h at 4°C.
- Sonicate for 5 min (ultrasonic bath, RT).
- Apply lysate to QIAshredder, centrifuge at 20,000 rcf for 2 min. Transfer flow-through to a new tube.

3. Binding Reaction



- Calculate ABX-Mix (Antibodies + LB-Stock + PBS) using the PICO Calculator.

Set up Binding Reaction in a 96-well PCR plate:

NTC	4 µl LB
ABC	2 µl CB + 2 µl ABX (3 replicates)
Sample	2 µl Lysate + 2 µl ABX (4 replicates)

- Seal plate → vortex for convenient mixture, spin at 1,000 rcf for 30 s → incubate overnight at 4°C.

B. dPCR-Mix & Pre-Dilution

- Prepare Master Mix (24-well or 96-well)

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/O7)	45 µl	62 µl
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Coupling dPCR Mix	36 µl	50 µl

Note: If fewer than four PICO Probes are used, fill the missing volume with water.

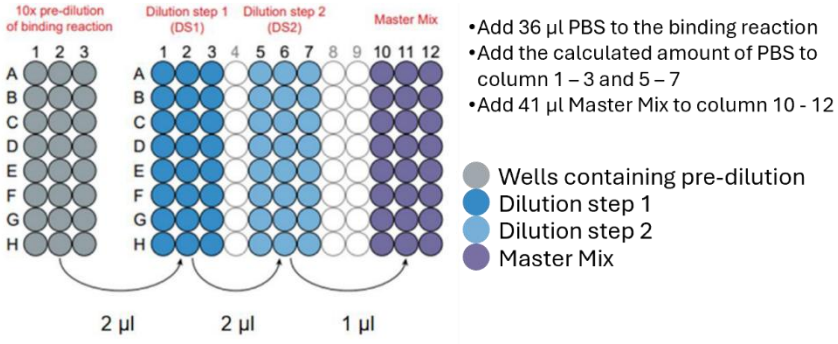
Pre-Dilution

- Remove adhesive foil from Binding Reaction. Add 36 µl PBS to each well. Mix well.

For a 24-well PICO Assay:

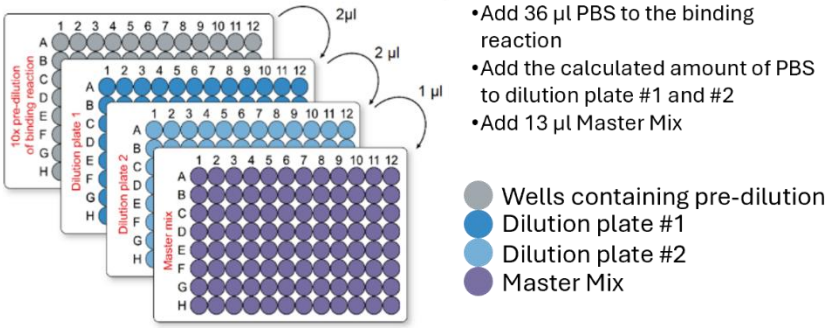
a. Prepare a new 96-well plate for the dilution steps.
b. Add PBS to columns 1-3 (for Dilution Series 1, DS1) and columns 5-7 (for DS2). For a 40 pM ABX concentration, add 33 µl of PBS; for an ABX concentration of 500 pM, add 120 µl PBS.
c. The carry-over volume is always 2 µl.
d. Add 41 µl of Master Mix to columns 10-12.

e. From DS2, carry over 1 µl to columns 10-12.



For a 96-well PICO Assay:

a. Prepare three 96-well plates: Dilution Plate #1, Dilution Plate #2 filled with 39µl PBS (for ABX concentration of 40pM) or 143µl PBS (for a 500 pM ABX concentration), and Master Mix Plate #3 with 13µl Master Mix.
b. Dilution Plates (#1 and #2): The carry-over volume is 2 µl.
c. From Plate #2, carry over 1µl to the Master Mix.



- Seal plate according to QIAcuity Manual and start PCR:

Priming: Standard Qiagen Priming Profile

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c. PCR:

- 95°C for 2 min
- 40× (95°C for 15 s + 58°C for 30 s)

Imaging:

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

- For data analysis, use the PIQuant Software and User Manual www.actome.de/resources/downloads