PICO Amplification Core (AMC) Kit

The **PICO Amplification Core Kit** (#PICO-000010) contents must be stored at 4°C or -20°C for up to 6 months (see product labels for exact storage conditions). Find the date of expiry stated on the contents of the kit.

Further information:
- The PICO Handbook and the PICO AMC Kit User Manual: **www.actome.de/resources.html**
- Safety Data Sheets: **www.actome.de/resources.html**

Notes before starting:
- Perform **all centrifugation steps** with 400 rcf for 5 min at RT in a centrifuge equipped with a rotor for 15 ml falcon tubes if not stated otherwise.
- Mix the dilutions in the 96-well plates by **gently pipetting up and down 30 times** while avoiding air bubbles.
- We recommend using at least **four technical replicates** for each sample.
- We recommend using a total of **1 million cells** in a concentration of $1x10^4$ cells/µl. However, the total input can be adapted. We also 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. For preparation of other samples see the full manual. Steps involving cross-linking are optional.

DAY 1

Start of the PICO AMC workflow (sample preparation):

1. Prepare the following chemicals and buffers:

Additive C (5x stock) add 500 µl PBS	**BSA (5x stock)** 20 mg BSA + 400 µl PBS	**EDTA-free Protease Inhibitor Cocktail (PIC), (25x stock)** dissolve 1 tablet of cOmplete Protease™ Inhibitor Cocktail (PIC) in 2 ml PBS	**PIC-PBS (1x)** dissolve 1 PIC tablet in 500 µl PBS
Cell Lysis Buffer (LBT), (2x) 200 µl Additive T (10x) 400 µl Additive C (5x) 80 µl PIC (25x) 200 µl Additive L 120 µl PBS	**Cell Lysis Buffer (LBTW), (1x)** 300 µl LBT (2x) 300 µl PBS	**Antibody Binding Control (ABC) Buffer, (1x)** 250 µl LBT (2x) 100 µl BSA (5x) 150 µl PBS	**BS3 Cross-linker Solution (BS3S)** 2 mg BS3 in 37.8 µl DMSO **BS3 Working Solution (BS3W), (1x)** 6 µl BS3S 114 µl PBS

2. Harvest the cells with the preferred method and wash them in an appropriate volume **(3 - 15 ml) of PIC-PBS** by centrifugation.
3. Resuspend the cell pellet in **1 ml PBS**, transfer it to a 1.5 ml reaction tube and centrifuge again. Repeat this step once more. **IMPORTANT:** Do **not** use PIC-PBS, otherwise the PIC will quench the BS3 cross-linker.
4. Count cells in **1 ml PBS**, transfer **$2x10^6$ cells** into a new 1.5 ml reaction tube, centrifuge and discard supernatant.
5. Add **100 µl of BS3W**, mix by pipetting up and down and incubate for 30 min at RT.
6. Add **900 µl of PIC-PBS**, centrifuge and discard supernatant.
7. Count the cells again in **1 ml PBS**, transfer **$1x10^6$ cells** into a new 1.5 ml reaction tube, centrifuge and discard supernatant.
8. Resuspent the cells in **100 µl LBTW** and vortex for 10 s. Incubate the cells for 3 h at 4°C.
9. Sonicate the lysate for 5 min at full power in an ultrasonic bath at room temperature.

DAY 1

10. 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.

11. Calculate the volume of antibody stocks, LBT Buffer and PBS for the antibody mix using the **Actome PICO Calculator** provided online.

12. Prepare **four technical replicates** of each sample in a 96-well PCR microplate. Combine **2 µl of sample with 2 µl of ABX** per well. Include at **least three technical** replicates of ABC **(2 µl of ABC Buffer with 2 µl of ABX).** Add **4 µl of LBTW** to an empty well as **NTC**.

13. Seal the plate with adhesive foil, sonicate for 1 min at full power in an ultrasonic bath at room temperature, centrifuge the plate (~1,000 rcf, 30 s) and incubate at 4°C overnight.

DAY 2

Preparing and performing of the dPCR:

14. Prepare the **Master Mix**, vortex for 10 s and spin down (~1,000 rcf, 5 s).

    697 µl RNAse-free water
    284 µl QIAcuity Probe Mix
    45.4 µL PICO BL Probe
    45.4 µL PICO P8 Probe
    36.3 µL Coupling dPCR Mix

15. Calculate the required dilutions to target a **lambda of 0.15** using the **Actome PICO Calculator.**

16. Prepare a new 96-well plate for the dilution steps. Add the calculated amount of **PBS and 41 µl of Master Mix** to the wells shown in the dilution scheme **(Figure 1).**

17. Remove the adhesive foil of the plate containing samples and add **36 µl of PBS** to all wells. Mix by pipetting.

18. Dilute the samples by transferring **1 µl to the first set of wells** in the 96-well plate and mix by pipetting (DS1). Repeat this once more (DS 2) and finally transfer **1 µl to the wells containing the Master Mix.**

19. Mix by pipetting and transfer **40 µl into a QIAcuity Nanoplate 26k 24-well.** Seal the plate according to the QIAcuity user manual and run a dPCR using the following parameters

    Priming - QIAGEN Standard Priming Profile

    PCR conditions

Hot-start	95°C for 2 min
Cycling	40 times
Denaturing	95°C for 15 sec
Annealing	58°C for 30 sec

    Imaging conditions
    PICOact P8 Label - FAM green channel, 500 ms integration time, gain 6
    PICOact BL Label - HEX yellow channel, 400 ms integrationtime, gain 6

20. Instructions for evaluation and calculators can be found in the user manual or at www.actome.de

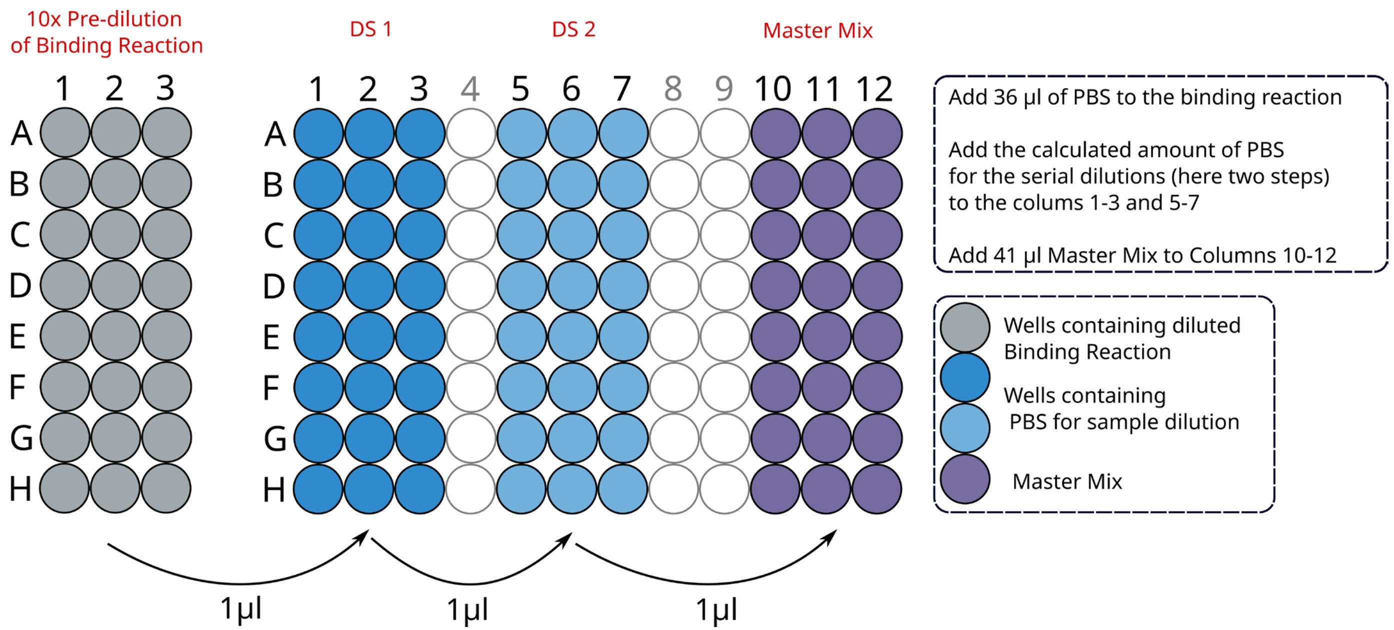

Figure 1: Setup for dPCR pre-dilution. The '10x Pre-dilution of Binding Reaction' is prepared in a separate plate. Subsequently a two step dilution (can be adjusted if needed) is prepared in an additional 96-well plate.

Scan the QR code for the user manual: