PICO Protein Detection Trial Kit

The **PICO Protein Detection Trial Kit** (#PICO-000090) can be stored at 4°C and -20°C (see product labels for storage conditions). Find the date of expiry on the contents of the kit.

Further information:
- The PICO Protein Detection Trial Kit User Manual and the PICO Calculators: **www.actome.de/resources.html**
- Safety Data Sheets: **www.actome.de/resources.html**

Notes before starting:
- Read the full PICO Protein Detection Trial Kit manual to learn more about the scientific background of a **CLC experiment** and **Relative Quantification**.
- Mix the dilutions in the 96-well plates by **gently pipetting up and down 30 times** while avoiding air bubbles.
- Perform **all centrifugation steps** with 1,000 rcf for 5 s at RT if not stated otherwise.

DAY 1

Preparation of biological material:

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
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

2. Thaw a vial of the supplied **recombinant human ErbB2/HER2 protein** and spin it down.
3. Prepare a dilution series of the protein in **LBTW** with a volume of **30 µl** each:

Stock $\xrightarrow{1 \text{ in } 100}$ **S#1** (100x) $\xrightarrow{1 \text{ in } 60}$ **S#2** (6,000x) $\xrightarrow{1 \text{ in } 2}$ **S#3** (12,000x) $\xrightarrow{1 \text{ in } 2}$ **S#4** (24,000x) $\xrightarrow{1 \text{ in } 10}$ **S#5** (240,000x)

4. Calculate the volume of antibody stocks, LBT buffer, and PBS for the antibody mix (ABX) using the **Actome PICO Calculator** available online.
5. Set up the **binding reaction** in a 96-well PCR microplate (v-bottom) following the pipetting scheme (**Figure 1**).
6. Seal the plate, sonicate at full power for **1 min** and centrifuge the plate (~1,000 rcf, 30 s).
7. Incubate the plate at **4°C for 12 - 24 h**.

DAY 2

Dilution and Digital PCR:

8. Prepare the Master Mix, vortex for **10 s** and spin down.

697 µl Ultrapure water
284 µl QIAcuity Probe Mix
45.4 µl PICO BL Probe
45.4 µl PICO P8 Probe
36.3 µl Coupling dPCR Mix

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

10. Prepare a new 96-well plate for the dilution steps. Add the calculated amount of **PBS and 41 µl Master Mix** to the wells shown in the dilution scheme **(Figure 2)**.

11. 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**). Mix by pipetting.

12. Dilute the samples by transferring the predetermined volume (usually 1 µl) from each sample to the corresponding wells of the dilution plate and mix by pipetting (DS 1). Repeat the dilution once more (DS 2) and finally transfer **1 µl** to the wells containing the Master Mix (**Figure 2**).

13. 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
PICO P8 Probe - FAM green channel, 500 ms integration time, gain 6
PICO BL Probe - HEX yellow channel, 400 ms integration time, gain 6

20. Instruction for evaluation of raw couplex calculation with **AMULATOR** and **Relative Quantification** can be found in the full user manual.

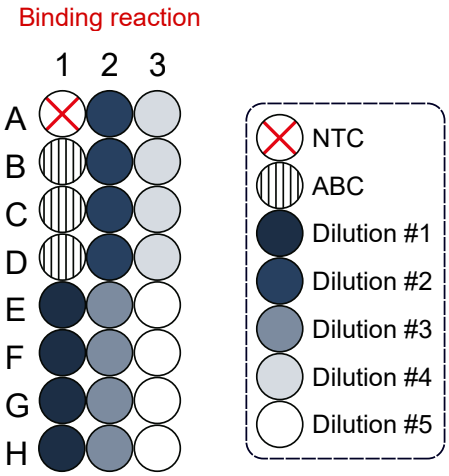

Figure 1: Plate layout of the binding reaction. Five dilution steps, with four technical replicates each, were made. For the ABC three technical replicates are sufficient.

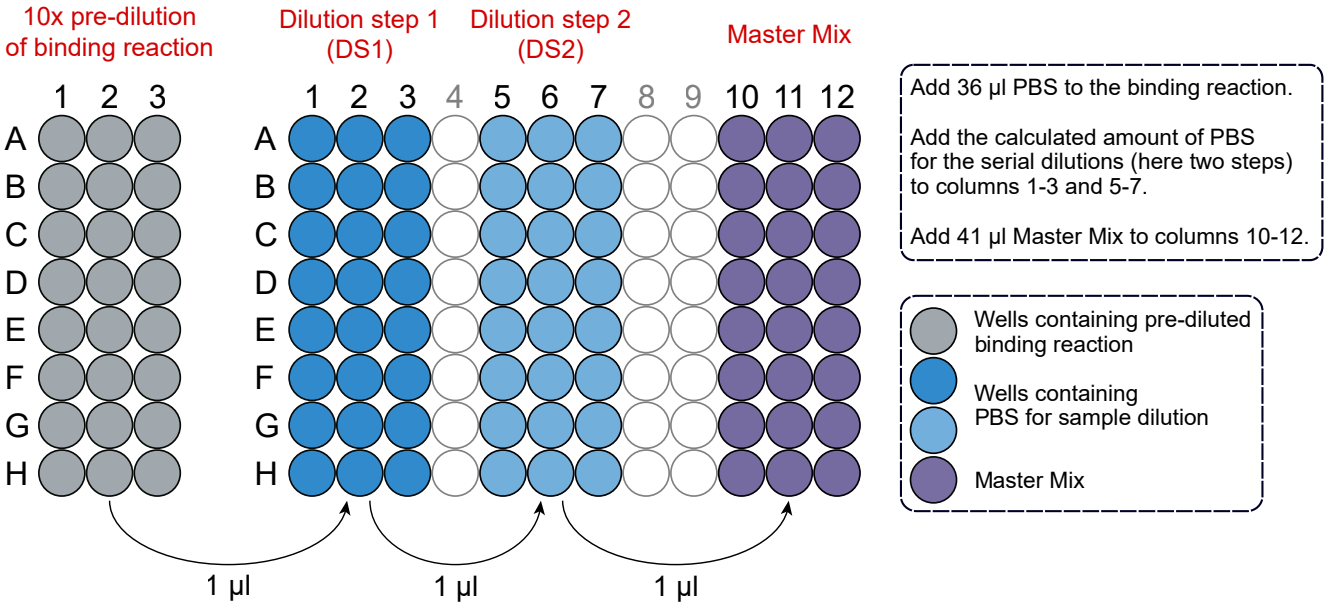

Figure 2: Plate setup for dPCR pre-dilution. The '10x pre-dilution of binding reaction' is prepared in the plate containing the binding reaction (see **Figure 1**). The two step dilution is prepared in an additional 96-well plate.

Scan the QR code for the full user manual: