PICO Protein Detection Trial Kit

The **PICO Protein Detection Trial Kit** (#PICO-000090) must be stored at 4°C and -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 Protein Detection Trial Kit User Manual, the PICO Calculator, and the Safety Data Sheets are available at: **www.actome.de/resources/downloads**

Notes before starting:

- Read the **PICO Protein Detection Trial Kit User Manual** for detailed description of the steps and to learn more about the scientific background of a **calibration curve (CLC) experiment** and **relative quantification (RQ)**.
- The cOmplete Protease™ Inhibitor Cocktail tablet and the QIAGEN consumables (QIAcuity Probe PCR Mix, QIAcuity Nanoplate 26k 24-Well) are **not included** in the kit.
- 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 unless stated otherwise.
- The concentration of the pre-labeled antibodies is: **4.82×10^9 cp/µl**.

DAY 1

Preparation of the dilution series and the binding reaction:

1. Prepare the following chemicals and buffers:

Additive C (5x stock) add 500 µl PBS	**BSA (5x stock)** add 400 µl PBS	**EDTA-free Protease Inhibitor Cocktail (PIC), (25x stock)** dissolve 1 tablet of cOmplete Protease™ Inhibitor Cocktail in 2 ml PBS
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	**Lysis Buffer (LB)** 300 µl LB-Stock 300 µl PBS	**Control Buffer (CB)** 250 µl LB-Stock 100 µl BSA 150 µl PBS

2. Thaw the vial of the supplied **recombinant human ErbB2/HER2 protein** and spin it down.
3. Prepare a dilution series of the protein in **LB** in a volume of at least **30 µl** each. Use the **PICO Calculator** (step 1 in the 'PICO Calculator tab') to plan the dilution series:

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. Prepare the **antibody mix (ABX)** using the **PICO Calculator** (step 2 in the 'PICO Calculator tab'). We recommend using **100 µl** for the total volume of ABX.
5. Mix 2 µl sample with 2 µl ABX (**binding reaction**) in a 96-well PCR microplate following the pipetting scheme (**Figure 1**). For the **NTC** sample use **4 µl LB**. For the **ABC** sample mix **2 µl ABX** with **2 µl CB**.
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

Pre-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 µl PICO BL Probe
    45 µl PICO P8 Probe
    36 µl Coupling dPCR Mix

9. Calculate the required dilutions to target a **lambda of 0.15** using the **PICO Calculator** (step 3 in the 'PICO Calculator tab').

10. 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**).

11. Remove the adhesive foil from 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. Transfer 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**). Mix by pipetting.

13. Transfer **40 µl Master Mix**, containing the diluted sample, 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 BL Probe - HEX yellow channel, 400 ms integration time, gain 6
    PICO P8 Probe - FAM green channel, 500 ms integration time, gain 6

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

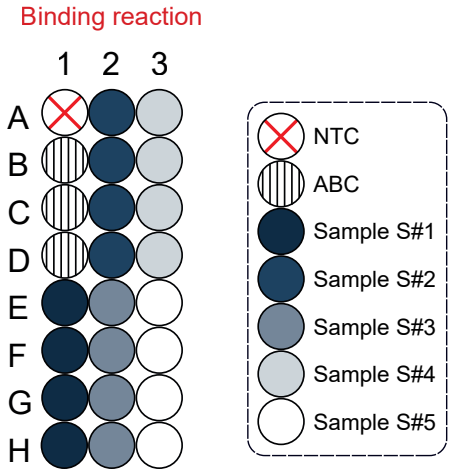

Figure 1: Plate layout of the binding reaction. Five dilution steps, with four technical replicates each, were made. For the ABC three technical replicates are sufficient. ABC - antibody control; NTC - non template control.

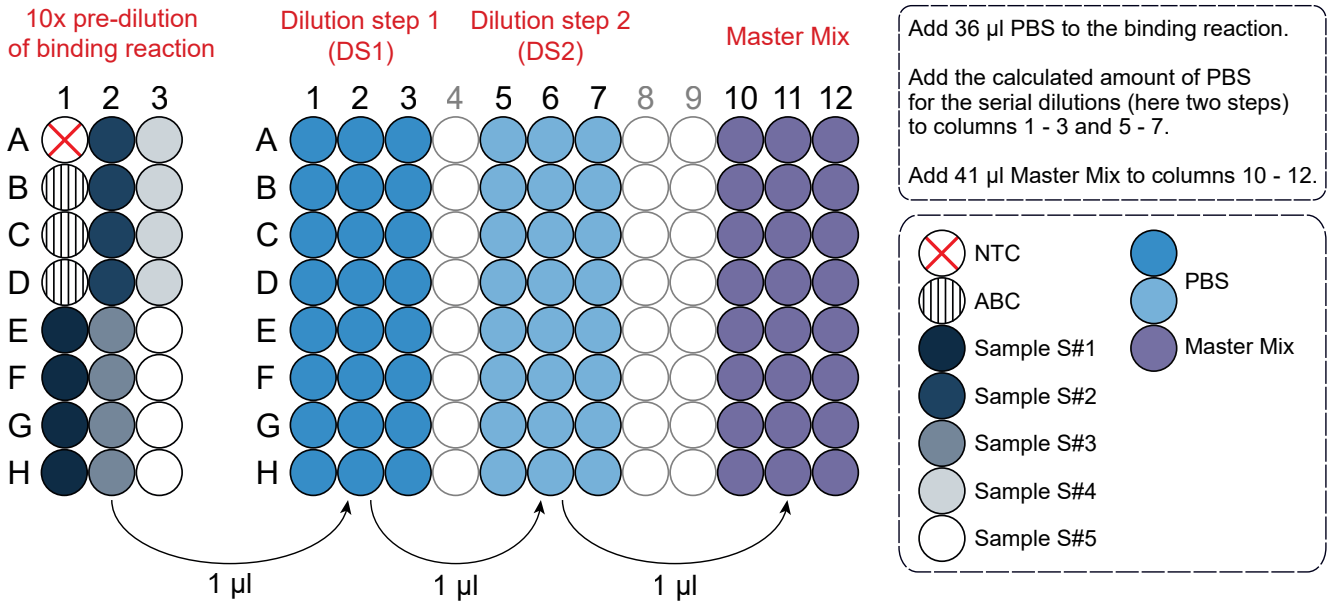

Figure 2: Plate setup for dPCR pre-dilution. The '10x pre-dilution of binding reaction' is prepared in the plate containing the binding reaction (see **Figure 1**). The two step dilution is prepared in an additional 96-well plate. ABC - antibody control; NTC - non template control.

Scan the QR code for the user manual: