PICOact Conjugated Antibody Label Loading (aCALL) Kit

The **PICOact Conjugated Antibody Label Loading Kit** (#PICO-000040) contents must be stored at RT or 4°C (see product labels for storage conditions). Find the date of expiry stated on the contents of the kit.

Further information:

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

Notes before starting

- Perform **all centrifugation steps** at 4°C, ~14,000 rcf and stated duration.
- **Quick spins** are performed at RT, ~1,000 rcf, 20 s.
- **10 µl Actomidin-conjugated antibody** (prepared with the PICOact Antibody Conjugation Kit) is needed. We recommend a conjugation efficiency of > 50% and a concentration of > 50 ng/µl.
- Always load **~1 ml air** into syringes (additional to liquid) when washing to remove excess liquid from the column.
- Since digital PCR is very sensitive to **contamination**, take all precautions to avoid it.
- This protocol is eligible for the labeling of **one antibody**. Multiple antibodies can be labeled in parallel. Adjust the amount of buffers accordingly.
- For the **Quality Control** step the **PICO Probes** as well as contents of the **PICO AMC Kit** are required additionally.

DAY 1

The PICO aCALL protocol (antibody labeling):

1. Prepare 3x 50 ml falcon tubes (filled with PBS, water and one waste vessel) and **25 ml 1x Wash Buffer** (dilute 10x Wash Buffer with PBS).
2. Homogenize the resin by pipetting and load **20 µl of resin** onto a Loading Column placed in a 1.5 ml reaction tube (break off the nozzle of the Loading Column).
3. Wash the resin with **2 ml PBS** (into the waste vessel) and quick spin.
4. Vortex the **conjugated antibody** for 5 s, quick spin and pipette **10 µl** onto the resin **(Figure 1)**.
5. Wash the resin with **5 ml PBS** and quick spin.
6. Vortex the **PICO Label** for 5 s, quick spin and add **10 µL** to the resin. Incubate for 30 min at RT.
7. Wash the resin with **20 ml 1x Wash Buffer** and then with **5 ml RNAse-free water**. Quick spin to remove the last traces of the buffer. **Keep the flow-through as 'last-wash' sample**.
8. Put the Loading Column in a 1.5 ml reaction tube, already containing **4 µl Buffer A**. Add **36 µl Elution Buffer** to the column, mix by pipetting and incubate for 3 min at RT **(Critical! Don't exceed the 3 min!)**. Recover the antibody by quick spin.
9. Transfer the antibody into a 100K Ultrafiltration Column, add **350 µl PBS** and centrifuge for 3 min **(Figure 2)**. Discard flow-through. Repeat washing with **400 µl and 15 min** centrifugation time.
10. Flip over the column, place it into a new reaction tube and recover the antibody by quick spin. Transfer the antibody to a low protein binding tube, add **2 µl 10x Storage Buffer** and vortex for 5 s.
11. The labeled antibody can be stored for two months at 4°C.

Quality Control of the labeled antibodies:

12. Prepare chemicals and buffers listed below for **Quality Control**.

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 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	**Storage Buffer (1x)** 20 µl Storage Buffer (10x) 180 µl PBS	**Antibody Binding Control (ABC) Buffer (1x)** 250 µl LBT (2x) 100 µl BSA (5x) 150 µl PBS

13. Prepare the **Master Mix** (dependent on how many antibodies have to be analyzed), vortex for 10 s and quick spin.

1 Antibody
742.4 µl RNAse-free water
284 µl QIAcuity Probe Mix
45.4 µl PICO Probe
36.3 µl Coupling dPCR Mix

2 Antibodies
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

14. Dilute the labeled antibodies and the 'last-wash' sample 1:40 in **1x Storage Buffer** in low protein binding tubes, vortex for 10 s and quick spin. Sonicate for 1 min in an ultrasonic bath (Dilution Step (DS) 1).

15. Dilute all samples 1:40 in **ABC Buffer** in a 96-well plate (DS 2; **Figure 3**). Make at least **3 replicates**, mix by pipetting and avoid air bubbles. Seal the plate and sonicate for 5 min.

16. Serially repeat the 1:40 dilution **three more times** in the 96-well plate (DS 3 - 5).

17. Add **41 µl Master Mix** into columns 10-12 of the 96-well plate and add **1 µl of** each serial dilution of the samples. Don't add any sample to the A10 well (NTC control sample).

18. Transfer **40 µl sample** (from columns 10-12) to 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 integration time, gain 6

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

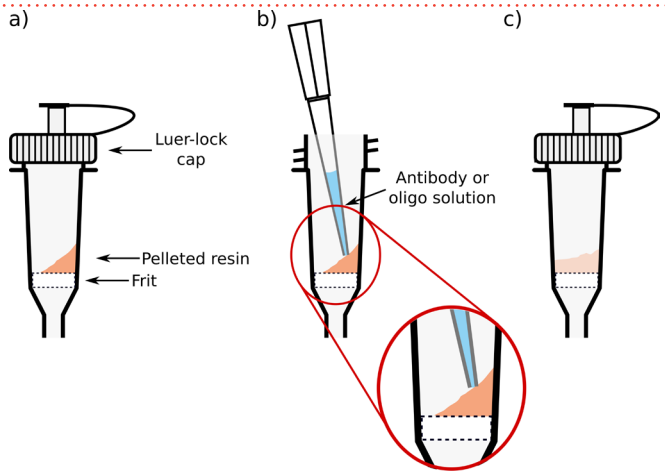

Figure 1: Handling of Loading Columns. **a)** After centrifugation, the resin forms a tilted surface. **b)** Pipette the liquid solution onto the middle of the tilted resin surface. Do not touch the plastic walls or the frit. **c)** Close the lid after pipetting.

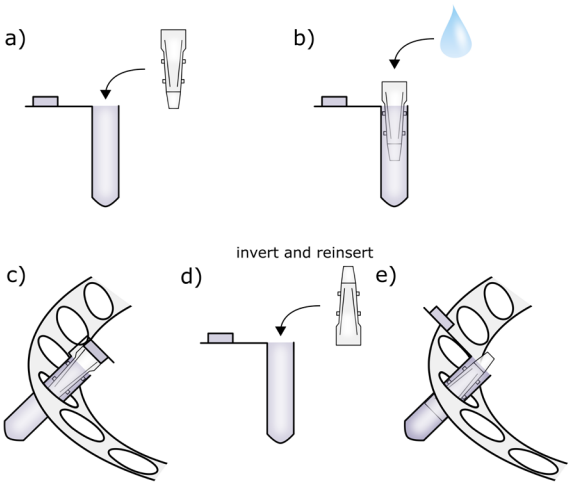

Figure 2: Handling of Ultrafiltration Columns. **a)** Place the Ultrafiltration Column into the collection tube. **b)** Load the liquid and cap the Ultrafiltration Column with the tube cap. **c)** Place the tube with the hinge of the cap and the side of the Ultrafiltration Column membrane outward. **d)** To recover the sample, place the Ultrafiltration Column into a new collection tube in an inverted position. **e)** Centrifuge.

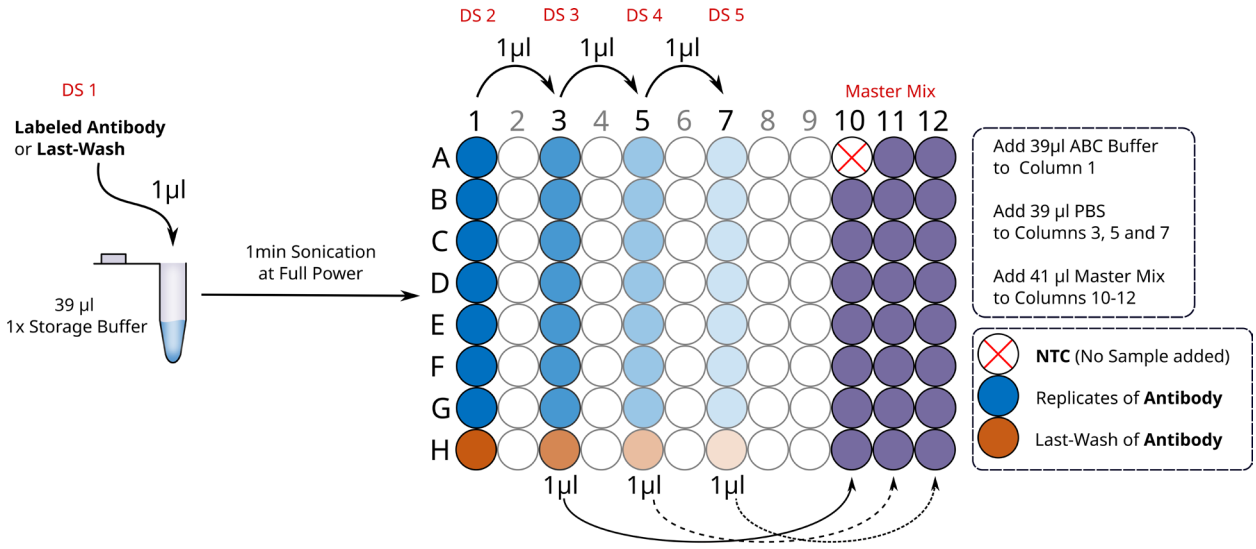

Figure 3: Setup to perform Quality Control dPCR of one labeled antibody. Here, seven replicates of each antibody dilution and one replicate of the last-wash sample are prepared. If two antibodies are analyzed at the same time, use three replicates each for the antibody dilution together with the corresponding last-wash sample. (DS: Dilution Step)

Scan the QR code for the user manual: