PICOglue Antibody Labeling Kit

The **PICOglue Antibody Labeling (gAL) Kit** (#PICO-000110) must be stored at RT, 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 gAL User Manual and the Safety Data Sheets are available at: **www.actome.de/resources/downloads**

Notes before starting:

- Digital PCR is very sensitive to DNA contamination, take all precautions to avoid it.
- For the '**Quality Control of Labeled Antibodies**' step contents of the **PICO AMC Kit** as well as **PICO Probes** are required additionally.
- All steps are valid for the labeling of one antibody. The PICO gAL Kit contains sufficient reagents for the labeling of 4 antibodies.
- Make sure the antibody fulfills the requirements stated in *Section 06* in the PICO gAL Kit User Manual.
- **100 µg of antibody** is required as input.
- Quick spins are performed at ~1,000 rcf, 20 s, RT.
- Perform all other centrifugation steps at 14,000 rcf, 3 min, 4°C.
- Load ~1 ml air into the syringe (additional to the liquid) to remove excess liquid from the column during the washing steps.
- Mixing by pipetting should be done slowly (30 times) to avoid air bubbles.

DAY 1

Antibody Rebuffering:

1. Prepare **2 ml 1x Ultrafiltration Buffer (UB)** by diluting the **10x Ultrafiltration Buffer** ● with **ultrapure water**.
2. Vortex the antibody for 5 s, quick spin and load **100 µg antibody** onto a **100K Ultrafiltration Column** (**Figure 1**).
3. Fill up to **400 µl** with **1x UB**, centrifuge, discard flow-through.
4. Repeat washing with **400 µl 1x UB**, discard flow-through.
5. Recover the antibody in a new collection tube by inverting the column, quick spin and transfer the antibody (15 - 50 µl, record exact volume) to a 0.5 ml reaction tube (**Figure 1d-e**). Save 1 µl as 'unconjugated control'. Store at 4°C until further use.

Antibody Deglycosylation

6. Pre-warm the heating block to **37°C**.
7. Add **3 µl 10x PICOzyme Buffer** ● and **3 µl PICOzyme** ● to the antibody solution and fill up to **30 µl** with **1x UB** (if the volume is less than 30 µl). Incubate for 1 h at 37°C.
8. Vortex for 5 s and quick spin. Save 1 µl as 'deglycosylated control'. Store at 4°C until further use.

Azide Attachment

9. Pre-warm the heating block to **30°C**.
10. Reconstitute one vial of **PICOtransferase Substrate** ⊘ with **10 µl ultrapure water**, incubate for 20 min at RT, vortex for 5 s and quick spin.
11. Transfer the antibody solution to this tube, add **5 µl 10x PICOtransferase Buffer A** ⊘ and **5 µl 10x PICOtransferase Buffer B** ⊘, fill up to **48.7 µl** with **ultrapure water** and mix by pipetting.
12. Add **1.3 µl PICOtransferase** ⊘, mix by pipetting and incubate overnight at 30°C protected from light (incubation time of 12 - 24 h).

DAY 2

Antibody Clean Up

13. Vortex for 5 s, quick spin, transfer the antibody solution to a **100K Ultrafiltration Column** and fill up to **400 µl** with **1x UB**, centrifuge and discard flow-through.
14. Repeat washing with **400 µl 1x UB** and 10 min of centrifugation.
15. Recover the antibody in a new collection tube by inverting the column and quick spin (**Figure 1d-e**).

Label Attachment

16. Transfer the antibody to a new 0.5 ml reaction tube, add **4 µl PICOglue Label**, mix by pipetting and incubate overnight (up to 48 h) at 4°C protected from light.
17. After incubation save **1 µl** as 'labeled antibody' for labeling efficiency analysis.

DAY 3

Free Label Removal

18. Prepare **6 ml 1x Wash Buffer I** (**WBI**) by diluting **2x Wash Buffer I** with **ultrapure water** and set up a waste vessel.
19. Vortex the antibody solution for 5 s, quick spin, transfer the antibody solution to a **100K Ultrafiltration Column** and fill up to **400 µl with 1x WBI**, centrifuge and discard flow-through.
20. Repeat washing with **400 µl 1x WBI** and 10 min centrifugation.
21. Recover the antibody in a new collection tube by quick spin (**Figure 1d-e**) and fill up to **40 µl** with **1x WBI**.
22. Vortex the **PICOglue Antibody Binding Resin** ○ and quick spin. Transfer **100 µl resin** to a **Loading Column**.
23. Wash **twice** (with closed cap) with **2 ml 1x WBI** using a syringe.
24. Place the Loading Column in a new reaction tube (1.5 ml), quick spin, pipette the antibody onto the resin (**Figure 2b**) and incubate for 1 h at RT.
25. Prepare **25 ml 1x Wash Buffer II** (**WBII**) by diluting **2x Wash Buffer II** with **ultrapure water** and set up a waste vessel.
26. Wash the Loading Column **3 times** with **7 ml 1x WBII** using a syringe. Place the column in a new reaction tube and quick spin. Keep the **flow-through** of the last washing step as 'last-wash' sample for quality control.
27. Transfer the Loading Column to a 2 ml reaction tube, add **300 µl PICOglue Elution Buffer** ○ to the resin, carefully mix by pipetting and incubate for 30 min at RT.
28. Recover the antibody by quick spin (**Figure 1d-e**).

Antibody Rebuffering

29. Transfer the labeled antibody to a **100K Ultrafiltration Column**, fill up to **400 µl** with **PBS**, centrifuge and discard flow-through.
30. Repeat washing with **400 µl PBS** and 10 min of centrifugation.
31. Recover the antibody in a new collection tube by quick spin (**Figure 1d-e**).
32. Transfer the antibody (15 to 40 µl) to a low protein binding tube. Add one tenth of the total volume of **10x PICOglue Antibody Storage Buffer** ●, vortex for 5 s and store at 4°C.

Optional: The steps for Antibody Deglycosylation and Labeling Efficiency Analysis can be found in the PICO gAL User Manual

The 'unconjugated control', 'deglycosylated control', and the 'labeled antibody' samples are used in this step.

Quality Control of Labeled Antibodies

33. Prepare the chemicals and buffers listed below. Contents of the **PICO AMC Kit** and corresponding **PICO Probes** are required additionally.

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 (PIC) in 2 ml PBS
Lysis Buffer Stock (LB-Stock), (2x) 200 µl Additive T 400 µl Additive C 80 µl PIC 200 µl Additive L 120 µl PBS	**Control Buffer (CB)** 250 µl LB-Stock 100 µl BSA 150 µl PBS	**PICOglue Antibody Storage Buffer** 20 µl 10x PICOglue Antibody Storage Buffer 180 µl PBS (For long-term storage we recommend adding an additional 8.3 µl PIC)

34. Prepare the **Master Mix**, vortex for 10 s and quick spin. If two antibodies are analyzed in parallel, replace 45 µl ultrapure water with the second **PICO Probe**.

742 µl Ultrapure water
284 µl QIAcuity Probe Mix
45 µl PICO Probe (P8, BL, N6, or O7)
36 µl Coupling dPCR Mix

35. Dilute the labeled antibodies and the 'last-wash' sample 1:40 in **1x Storage Buffer** in low protein binding tubes (Dilution Step (**DS**) **1**), vortex for 10 s and quick spin (**Figure 3**). Sonicate for 1 min in an ultrasonic bath.

36. Add **39 µl CB** in column 1 of a 96 well plate and add **1 µl sample** into column 1 (**DS2**).

37. Make at least **3 technical replicates**, mix by pipetting. Seal the plate and sonicate for 5 min.

38. Add **39 µl PBS** into columns 2 to 5 of a 96-well plate (**Figure 3**).

39. Serially transfer **1 µl sample** four more times in columns 2, 3, 4, and 5 (**DS3 - 6**). Mix by pipetting.

40. Add **41 µl Master Mix** into columns 10 - 12. Transfer **1 µl** from each serial dilution (columns 3 - 5) of the samples to columns 10 - 12. Don't add any sample to the A10 well (non template control (NTC)) (**Figure 3**).

41. 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
PICO P8 Probe - FAM green channel, 500 ms integration time, gain 6
PICO BL Probe - HEX yellow channel, 400 ms integration time, gain 6
PICO N6 Probe - TAMRA orange channel, 400 ms integration time, gain 6
PICO O7 Probe - ROX red channel, 300 ms integration time, gain 4

42. Instructions for evaluation can be found in the user manual.

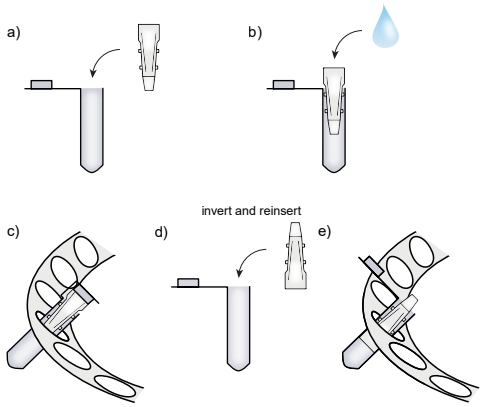

Figure 1: Handling of Ultrafiltration Columns. **a)** Place the Ultrafiltration Column into the collection tube. **b)** Load the liquid and cap the Ultrafiltration Column with the tube cap. **c)** Place the tube with the hinge of the cap and the side of the Ultrafiltration Column membrane outward. **d)** To recover the sample, place the Ultrafiltration Column into a new collection tube in an inverted position. **e)** Centrifuge.

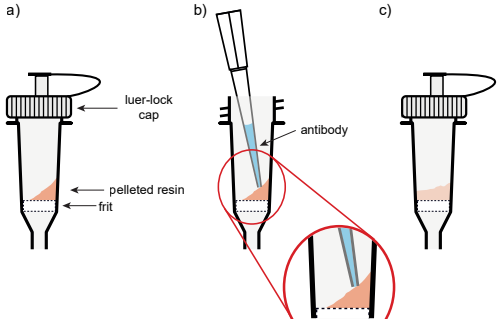

Figure 2: Handling of Loading Columns. **a)** After centrifugation, the resin forms a tilted surface. **b)** Pipette the liquid solution onto the middle of the tilted resin surface. Do not touch the plastic walls or the frit. **c)** Close the lid after pipetting.

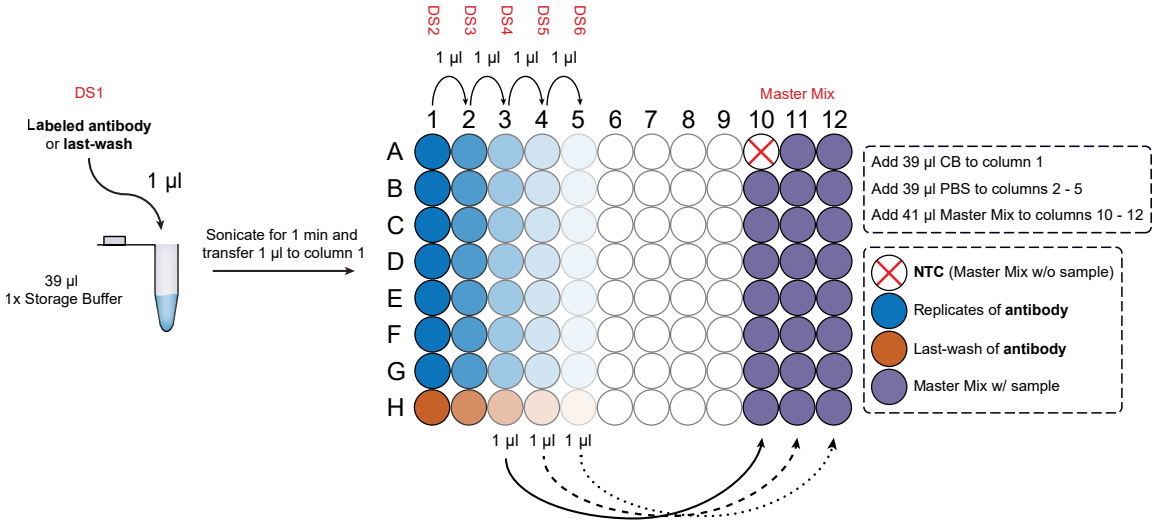

Figure 3: Exemplary setup to perform the Quality Control dPCR of one labeled antibody. Here, seven replicates of the labeled antibody and one replicate of the last-wash sample are prepared. If two antibodies are analyzed at the same time, prepare three replicates for each labeled antibody and one replicate for the corresponding last-wash sample. DS - dilution step; NTC - non template control

Scan the QR code for the user manual: