Quick-Start Protocol

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:

- **First-time customers:** we highly recommend reading the **user manual** before starting with your first antibody labeling experiment.
- For the 'Quality Control of Labeled Antibodies' section contents of the **PICO Amplification Core (AMC) Kit** as well as **PICO Probes** are required additionally.
- All steps are valid for the labeling of one antibody. The kit contains sufficient reagents for four labeling reactions.
- Make sure the antibody fulfills the requirements stated in *Section 06* in the PICO gAL Kit User Manual.
- **50 - 100 µg of antibody** is required as input.
- Quick spins are performed at ~1,000 rcf, 20 s, RT with a table-top mini centrifuge.
- 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 generation of 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 for 20 s and load **50 - 100 µg antibody** onto a 100K Ultrafiltration Column.
3. Fill up to **400 µl** with **1x UB**, centrifuge at 14,000 rcf, for 3 min at 4°C. Discard flow-through.
4. Repeat washing with **400 µl 1x UB**, centrifuge at 14,000 rcf, for 3 min at 4°C. Discard flow-through.
5. Recover the antibody by placing a new collection tube over the column and inverting it. Quick spin and transfer the antibody (15 - 50 µl, **record exact volume**) to a new 1.5 ml reaction tube (**Figure 1d-e**). If intended, 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**. Incubate for 1 h at 37°C.
8. Vortex for 5 s and quick spin. If intended, 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 can vary between 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 at 14,000 rcf, 3 min at 4°C. Discard flow-through.
14. Repeat washing with **400 µl 1x UB** and centrifuge at 14,000 rcf, for 10 min at 4°C. Discard flow-through.
15. Recover the antibody by placing a new collection tube over the column and inverting it and quick spin (**Figure 1d-e**).

Label Attachment:

16. Transfer the antibody to a new 1.5 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, if intended, 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 and quick spin. Transfer the antibody solution to a **100K Ultrafiltration Column** and fill up to **400 µl with 1x WBI**, centrifuge at 14,000 rcf, 3 min at 4°C. Discard flow-through.
20. Repeat washing with **400 µl 1x WBI** and centrifuge at 14,000 rcf, for 10 min at 4°C. Discard flow-through.
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 (2s). Transfer **100 µl resin** to a **Loading Column**.
23. Wash carefully **twice** with **2 ml 1x WBI**. Use a syringe with gentle but constant pressure, directing the flow into the waste vessel (**Figure 2b**).
24. Place the Loading Column in a new reaction tube (1.5 ml), quick spin, pipette the antibody onto the resin (**Figure 2c**) 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**.
26. Wash the Loading Column carefully **3 times** with **7 ml 1x WBII**. Use a syringe with gentle but constant pressure, directing the flow into the waste vessel.
27. Place the column in a new reaction tube and quick spin (**Figure 2b**). **Keep the flow-through** as 'last-wash' sample for quality control.
28. 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.
29. Recover the antibody by quick spin (**Figure 1d-e**).

Antibody Rebuffering:

30. Transfer the labeled antibody to a **100K Ultrafiltration Column**, fill up to **400 µl** with **PBS**, centrifuge at 14,000 rcf, for 3 min at 4°C. Discard flow-through.
31. Repeat washing with **400 µl PBS** and centrifuge at 14,000 rcf, for 10 min at 4°C. Discard flow-through.
32. Recover the antibody by placing a new collection tube over the column, inverting it and quick spin (**Figure 1d-e**).
33. 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 **user manual**.

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

Quality Control of Labeled Antibodies:

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

Additive C (5x stock)	BSA (5x stock)	EDTA-free Protease Inhibitor Cocktail (PIC), (25x stock) - not provided
add 500 µl PBS	add 400 µl PBS	dissolve 1 tablet of cOmplete Protease™ Inhibitor Cocktail (PIC) in 2 ml PBS
Lysis Buffer Stock (LB-Stock), (2x)	**Control Buffer (CB)**	**PICOglue Antibody Storage Buffer**
200 µl Additive T 400 µl Additive C 80 µl PIC 200 µl Additive L 120 µl PBS	250 µl LB-Stock 100 µl BSA 150 µl PBS	20 µl 10x PICOglue Antibody Storage Buffer 180 µl PBS (For long-term storage we recommend adding an additional 8.3 µl PIC)

35. 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

36. 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.

37. Add **39 µl CB** to column 1 of a 96 well plate and transfer **1 µl sample** to column 1 (**DS2**).

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

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

40. Serially transfer **1 µl sample** four more times from columns 2 to 5 (**DS3 - 6**). Mix by pipetting.

41. Add **41 µl Master Mix** to columns 10 - 12.

42. Transfer **1 µl** from each serially diluted sample (columns 3 - 5) to columns 10 - 12 (**Figure 3**). Don't add any sample to the A10 well (non template control (NTC)).

43. 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			
Step	**Temperature**	**Time**	
Hot-start	95°C	2 min	
Denaturing	95°C	15 s	Cycle 40 times
Annealing	58°C	30 s	

Imaging conditions			
PICO Probe	**QIAcuity channel**	**Integration time**	**Gain**
P8 Probe	FAM, green channel	500 ms	6
BL Probe	HEX, yellow channel	400 ms	6
N6 Probe	TAMRA, orange channel	400 ms	6
O7 Probe	ROX, red channel	300 ms	4

An example screen recording for setting up a Nanoplate in the QIAcuity Software Suite can be watched here:

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

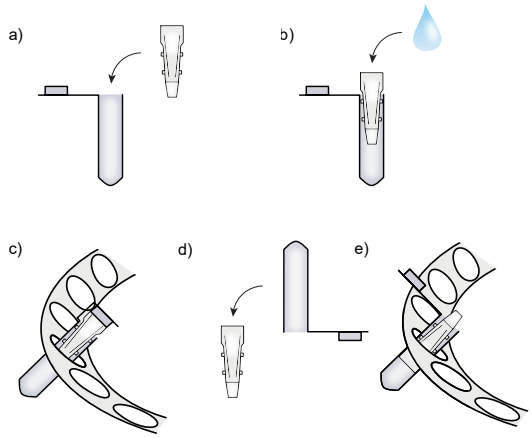

Figure 1. Handling of Ultrafiltration Columns. **a)** Place the Ultrafiltration Column into the collection tube. **b)** Load the liquid and cap the Ultrafiltration Column with the tube cap. **c)** Place the tube with the hinge of the cap and the side of the Ultrafiltration Column membrane outward. **d)**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

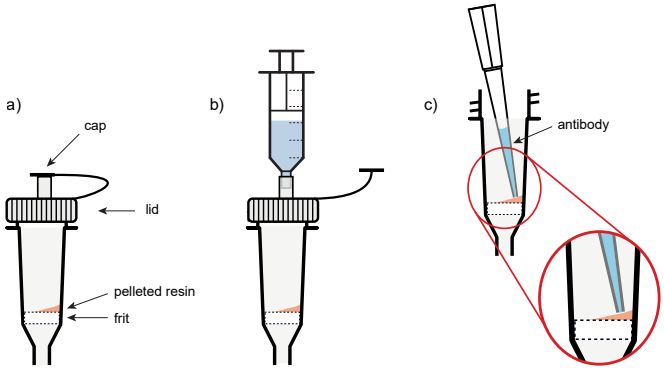

Figure 2. Handling of Loading Columns. **a)** After centrifugation, the resin forms a tilted surface. **b)** For washing, close the lid and open the cap. Attach the syringe to the opening and wash carefully with gentle but constant pressure. **c)** For pipetting, unscrew the lid of the Loading Column and open it. The antibody solution should be dispensed onto the middle of the tilted resin surface directly.

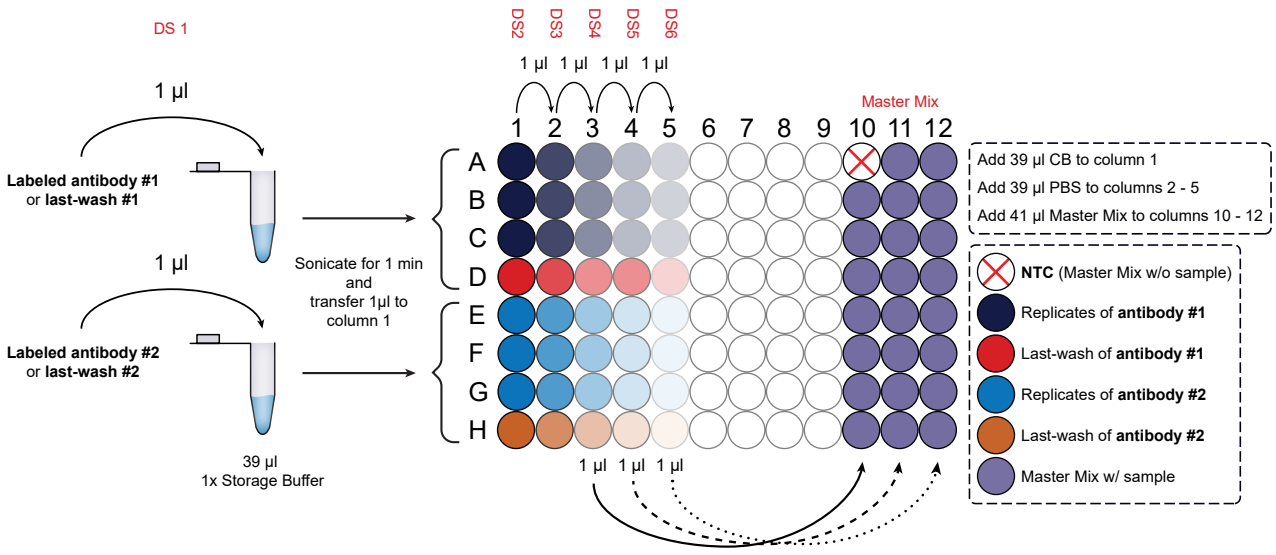

Figure 3. Exemplary setup to perform the Quality Control dPCR of two labeled antibodies. Here, three replicates of each labeled antibody and one replicate of each last-wash sample are prepared. DS - dilution step; NTC - non template control

Scan the QR code for the user manual: