# PICOact Antibody Conjugation (aAC) Kit

The **PICOact Antibody Conjugation Kit** (#PICO-000030) contents must be stored at RT or 4°C for up to 6 months (see product labels for exact storage conditions). **IMPORTANT:** Actomidin has a shelf life of 4 weeks, it can be reordered seperately. Find the date of expiry stated on the contents of the kit.

**Further information:**

- The PICO Handbook and the PICO aAC 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.
- Make sure that the antibody is stored in a buffer free of BSA, azide, TRIS and is generally free of primary amines. Otherwise use the **PICO Antibody Purification Kit** (#PICO-000050).
- Use a total of 100 µg antibody, not exceeding 400 µl. Otherwise use Ultrafiltration Columns (4°C, 14,000 rcf, 3 min) to concentrate the antibodies until requierements are fulfilled **(Figure 1)**.
- Always use **PBS without calcium or magnesium** when working with the **PICO aAC Kit.**

DAY 1

**The PICO aAC protocol (antibody conjugation):**

1. Vortex and quick spin **100 µg antibody** and load it onto a 100K Ultrafiltration Column placed into a collection tube. Save **4 µl antibody** as 'unconjugated control' for conjugation efficiency measurement.
2. Add up to **400 µl Conjugation Buffer** and wash by centrifugation (3 min). Discard flow-through.
3. Repeat step 2 with 10 min centrifugation time (leftover volume is ~20 µl).
4. Flip over the Ultrafiltration Column, place it into a new collection tube, recover the antibody with a quick spin and transfer it to a low protein binding tube.
5. Resuspend the **Activation Reagent** with **72.5 µl Conjugation Buffer**. Add **80 µl Conjugation Buffer** and **4.8 µl Activation Reagent** to the antibody, mix by pipetting and incubate for 20 min at RT.
6. Add **300 µl Conjugation Buffer** to the activated antibody, transfer it to a 10K Ultrafiltration Column and centrifuge it for 15 min. Discard flow-through.
7. Resuspend the **Actomidin** with **120 µl RNase-free water** and add **110 µl** to the 10K Ultrafiltration Column. Add **300 µl Conjugation Buffer** to the column, mix by pipetting and centrifuge for 20 min. Keep the flow-through. Close the column and incubate overnight at 4°C.

DAY 2

**Rebuffering of the antibody:**

8. Resuspend the **Maleimide** with **38.6 µl anhydrous DMSO** and mix by pipetting.
9. Recover the antibody with a quick spin (according to step 4) and transfer it to a low protein binding tube.
10. Add **1 µl Maleimide** to the antibody, vortex for 3 s and incubate for 60 min at RT.
11. Add **5 µl Quenching Buffer** to the antibody, vortex for 5 s and incubate for 10 min at RT.
12. Transfer the antibody into a 100K Ultrafiltration Column, add **350 µL PBS** and centrifuge for 3 min. Discard flow-through.

13. Repeat washing with **400 µl PBS** and centrifugation for 15 min (leftover volume is ~20 µl).

14. Recover the antibody with a quick spin (according to step 4) and transfer it to a low protein binding tube. Save 4 µl conjugated antibody for conjugation efficiency measurement.

15. Add **80 µl PBS**, **10 µl 10x Storage Buffer**, vortex for 5 s and store the antibody at 4°C (stable for 2 months).

16. See the user manual for the optional Antibody Conjugation Efficiency measurement using Agilent's Bioanalyzer.

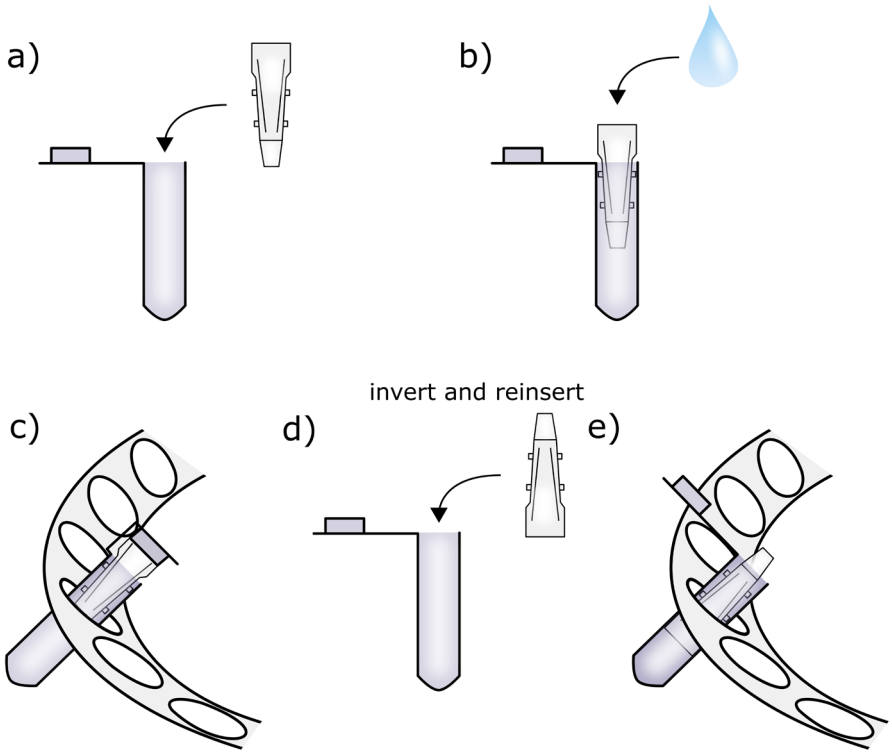


**Figure 1:** Handling of Ultrafiltration Columns. **a)** Place the Ultrafiltration Column into the collection tube. **b)** Load the liquid and cap the Ultrafiltration Column with the tube cap. **c)** Place the tube with the hinge of the cap and the side of the Ultrafiltration Column membrane outward.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

Scan the QR code for the user manual: