

# Quick Reference Guide: EZGlyco O-Glycan Prep Kit

## Releasing

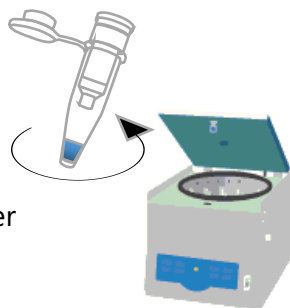
※Samples should be less than 10  $\mu\text{L}$  of aqueous solution.

1. Combine 5 vol of reagent **2** and 2 vol of reagent **3**, and then mix well.
2. In a fresh 1.5-mL microcentrifuge tube, combine 15  $\mu\text{L}$  of the reagent mixture prepared as above and sample solution.
3. Mix well by a Vortex mixer followed by a brief spin down
4. Incubate the tube for **75 min** at **37°C** leaving the cap open.



## Elution

12. Add 50  $\mu\text{L}$  of the 2AB labeling solution to the Column **5**.
13. Centrifuge the column at 3,000 x g for 1 min to recover O-glycans in the 2AB labeling solution as a filtrate.
14. Discard the Filter Column **5**, and mix the recovered solution by a Vortex mixer followed by a brief spin down.

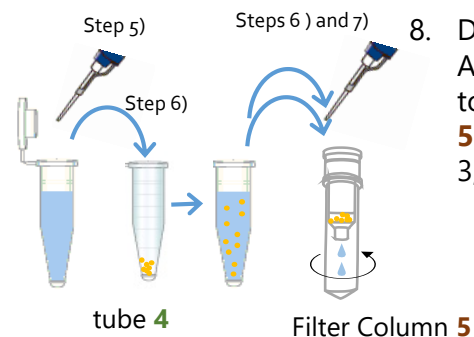


## Labeling

15. Incubate the tube on a heat block at **50°C** for **2.5 hr** to perform a labeling reaction, leaving the cap open..



5. Dilute the reaction mixture by adding 1 mL of acetonitrile (ACN). Mix well by vortexing followed by a brief spin down.
6. Transfer the solution to the tube **4** containing Glycan-Capturing beads. Transfer half of the suspension to a Filter Column **5**. Centrifuge the Filter Column at 3,000 x g for 1 min.

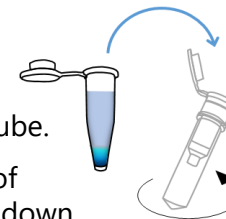


7. Discard the filtrate, and set back the Filter Column. Then transfer the rest of the bead suspension in the tube **4** to a Filter Column **5**. Centrifuge the Filter Column at 3,000 x g for 1 min.

8. Discard the filtrate. Add 200  $\mu\text{L}$  of ACN to the Filter Column **5** and centrifuge at 3,000 x g for 30 sec.

## Cleanup

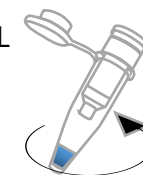
16. During the labeling reaction, condition a Cleanup Column **8** :
  - Take off and discard the lid from a Cleanup Column **8**, and then add 200  $\mu\text{L}$  of water to the Column followed by a centrifugation at 3,000 x g for 1 min.
  - Add 200  $\mu\text{L}$  of ACN and centrifuge at 3,000 x g for 1 min. Repeat again. Discard the filtrate in tube.
17. After the labeling reaction, add immediately 1 mL of ACN to the tube(s) and mix well by pipetting up & down.
18. Transfer the ACN solution to the preconditioned Cleanup Column **8**. Leave it for 10 min letting the solution partially pass through the Column.
19. Centrifuge the Column **8** at 500 x g for 1 min. Discard the filtrate.
20. Add 600  $\mu\text{L}$  of ACN to the Column **8**, and then centrifuge at 3,000 x g for 1 min. Discard the filtrate.
21. Repeat the wash: add 600  $\mu\text{L}$  of ACN to the Column **8**, and then centrifuge at 3,000 x g for 1 min. Discard the filtrate.



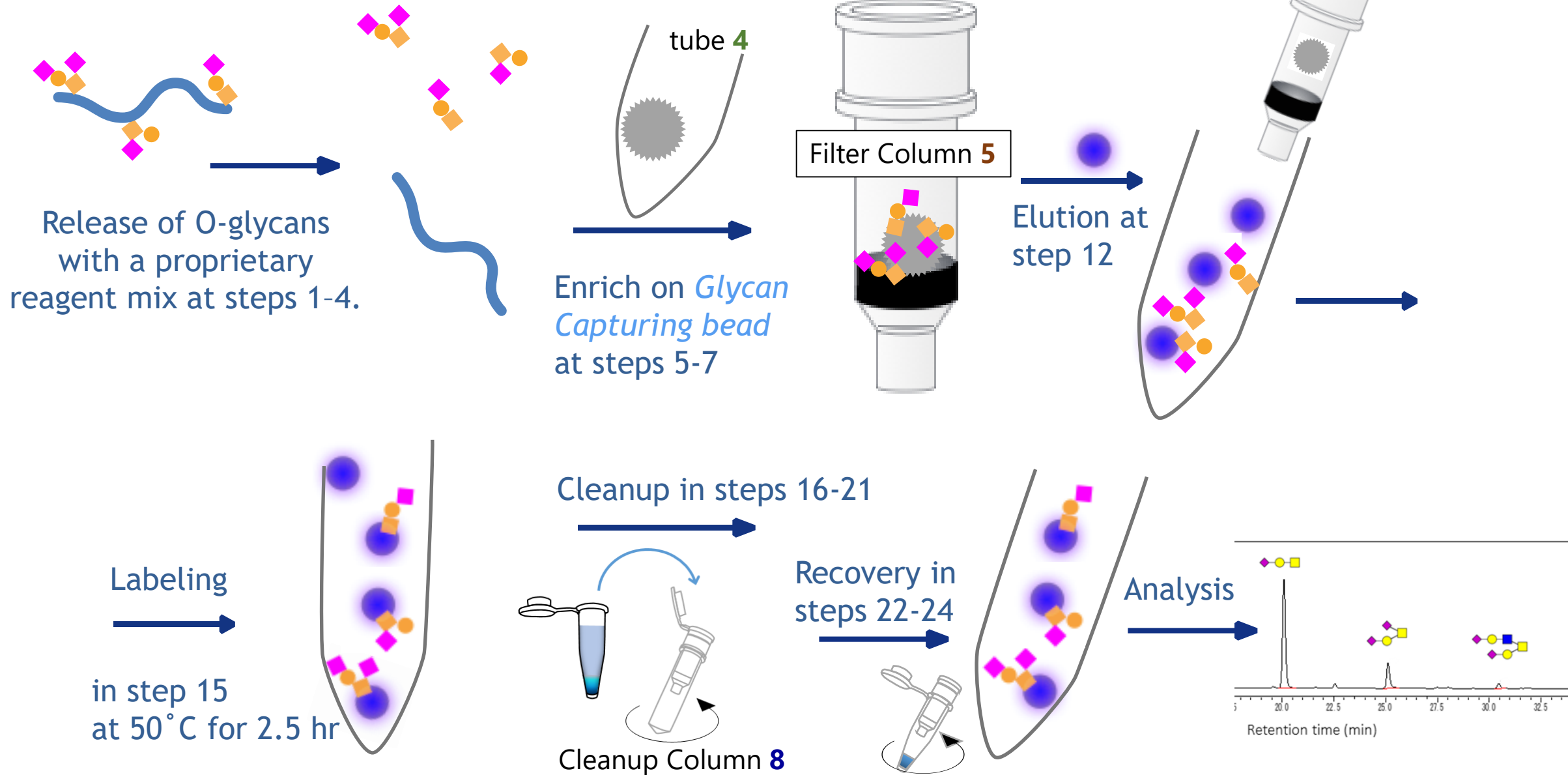
9. Repeat the wash: Add 200  $\mu\text{L}$  of ACN to the Filter Column **5**, and centrifuge at 3,000 x g for 30 sec.
10. Transfer the Filter Column **5** to a fresh 1.5-mL tube.
11. Prepare 2-AB labeling solution as follows;
  - Add 450  $\mu\text{L}$  of MeOH and 100  $\mu\text{L}$  of acetic acid (AcOH) to a reagent **6**. Dissolve the content by vortexing the tube with the cap closed. Spin down briefly. Add 450  $\mu\text{L}$  of water. Mix well by vortexing. Spin down briefly.
  - Add 450  $\mu\text{L}$  of MeOH and 100  $\mu\text{L}$  of AcOH to a reducing reagent **7**. Dissolve the content by vortexing the tube with the cap closed. Spin down briefly. Add 450  $\mu\text{L}$  of water. Mix well by vortexing. Spin down briefly.
  - Transfer 20  $\mu\text{L}$  of the reducing reagent **7** solution to the reagent **6** solution, making 2-AB labeling solution. Mix well by vortexing

## Recovery

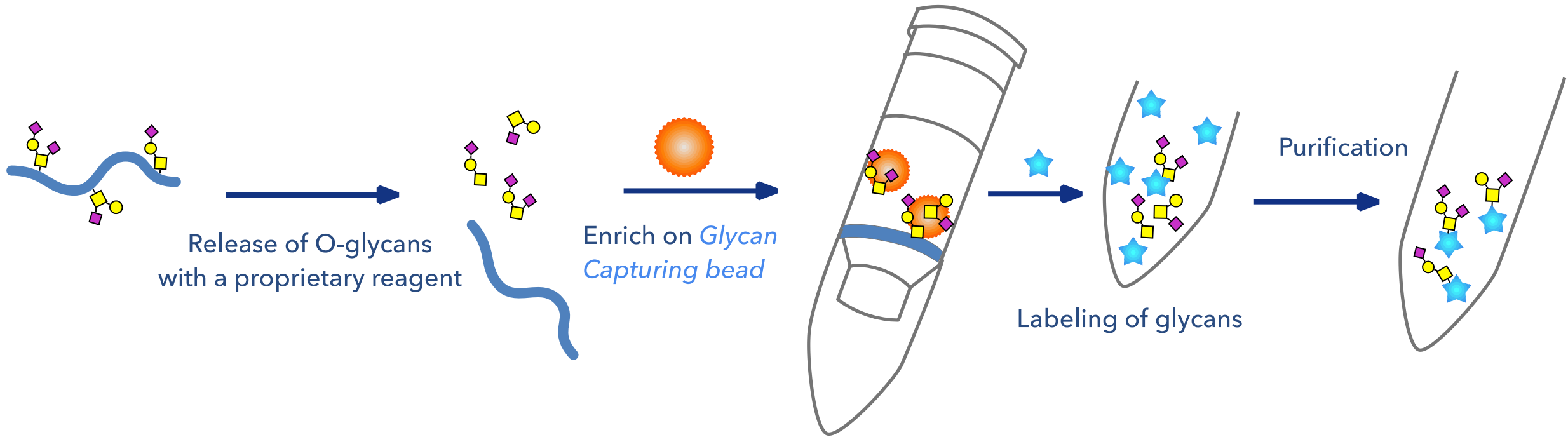
22. Set the Column **8** in a fresh 1.5-mL microcentrifuge tube.
23. Add 50  $\mu\text{L}$  of pure water to the Column **8**, and centrifuge at 3,000 x g for 1 min.
24. Discard the Column **8**, and mix well the recovery using a Vortex mixer followed by a spin down.
25. Analyze an appropriate amount of the recovered glycans using an LC system. If needed, dry down the elution. and reconstitute in an appropriate amount of solvent.



# Workflow of EZGlyco O-Glycan Prep Kit for HPLC and LC-MS-ready sample preparation in around 5 hours



# EZGlyco O-Glycan Prep Kit



HPLC and LC-MS-ready sample preparation of O-glycans in around 5 hours