Manual

# BiotinPure™ Antibody Biotinylation Kit

KROMNIGON
Multiplex IHC made easy

**Size**:

Biotinylation of 25-100 µg antibody
5 reactions

**Time**:

1 hour

**Kit content and Storage:**

| | |
|---|---|
| Biotinylation Reagent (powder) | -20°C |
| DMSO (100 µl): | RT |
| 10x Reaction Buffer (1.0 ml): | RT |
| 10x Wash Buffer (1.5 ml): | RT |
| Spin columns (5 pcs) | RT |

**Not provided in the kit**

1.5 ml microcentrifuge tube
Antibodies (free of other proteins, such as BSA)

**Applications**

The BiotinPure® Antibody Biotinylation Kit is designed for use with StreptaClick® reagents. The biotinylated antibodies may also be used for other applications.

**General information**

The BiotinPure® Antibody Biotinylation Kit is a complete kit for easy biotinylation of antibodies at room temperature within 1 hour. The kit uses amine/ester chemistry to attach 2-3 biotins per antibody and includes a purification wash step that removes unreacted free biotin from the final biotinylated antibody solution.

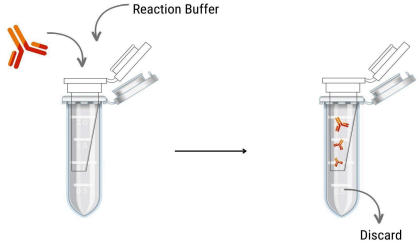
The antibodies to be biotinylated should be free from other proteins, such as BSA. To fit in the spin column, the minimal antibody concentration for a 100 µg reaction is 0.2 mg/ml. For antibodies with lower concentration – see Trouble shooting guide on page 2.

## (A) Preparation

1. Bring the Biotinylation reagent to room temperature, add 100 µl DMSO and vortex. If not all five reactions are used at once, aliquot unused Biotinylation Reagent and stored at -20°C.
2. Prepare 1xReaction Buffer and 1xWash Buffer by diluting 10xReaction Buffer and 10xWash Buffer 10 times with distilled water. For one biotinylation reaction you need approximately 1 ml 1xReaction Buffer and 1.5 ml 1xWash Buffer.

## (B) Biotin conjugation

3. Add 25-100 µg of antibody to the bottom of the provided spin column. Maximum volume = 500 µl.
4. Fill up with 1xReaction Buffer to a final volume of 500 µl.
5. Centrifuge the spin column at 12000 x *g* for 3 min. Discard the flow-through. The remaining antibody solution in the column should be <100 µl. If not, repeat the centrifuge step for 1-2 min.

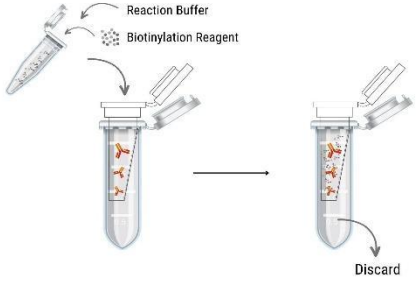



6. In a separate 1.5 ml microcentrifuge tube, prepare a biotinylation reaction solution by mixing 5 µl of the dissolved Biotinylation Reagent and 495 µl of 1xReaction Buffer.
7. Transfer the appropriate volume of biotinylation reaction solution (see Table 1) to the spin column from step 5 and mix immediately by pipetting up and down three times.

**Table 1. Volume of biotinylation reaction solution**

| Antibody amount | Volume of biotinylation reaction solution |
|---|---|
| 100 µg | 500 µL |
| 50 µg | 250 µL |
| 25 µg | 125 µL |

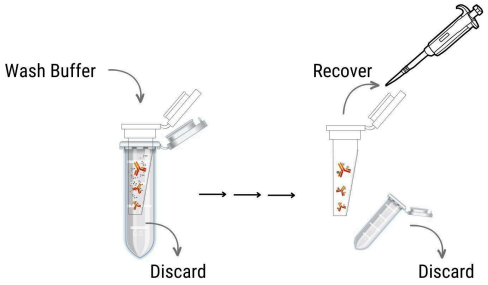
8. Incubate the mixture in the spin column for 30 min at RT.
9. Centrifuge the spin column at 12000 x *g* for 3 min. Discard the flow-through. The remaining antibody solution in the column should be <100 µl. If not, repeat the centrifuge step for 1-2 min.




## (C) Wash (removal of free biotin)

10. Add 500 µl 1x Wash Buffer to the spin column.
11. Centrifuge at 12000 x *g* for 3 min. Discard the flow-through.
12. Repeat steps 10-11 twice.
13. The remaining antibody solution in the column should be <100 µl. If a higher concentration of the biotinylated antibody is preferred, centrifuge at 12000 x *g* for an additional 1-3 min to reduce the volume in the spin column.

14. Discard the flow-through tube and recover the biotinylated antibody from the spin column using a pipette.




**Troubleshooting guide**

*Low concentration of starting antibody*

If your antibody concentration is lower than 0.2 mg/ml, perform steps 3 and 5 without adding more reaction buffer (step 4) until all your antibody solution is on the spin column. Then resume from step 4 and follow the rest of the protocol.

*Low yield of biotinylated antibody*

Verify the concentration and purity of your antibody.

*Biotinylated antibody not working well*

Check if the antibody works using a secondary antibody.

Alter the degree of biotinylation by adjusting the added volume of biotinylation reaction solution.