KAMIYA BIOMEDICAL COMPANY

# Biotin-SH Labeling Kit

**For the rapid Biotin labeling of proteins**

Cat. No. KT-331

**For Research Use Only.**

# PRODUCT INFORMATION

## Biotin-SH Labeling Kit
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### PRODUCT
Biotin-SH Labeling Kit is primarily used for the preparation of biotin-labeled IgG for enzyme immunoassays (EIA). SH-reactive Biotin (a component of this kit) has a maleimide group (NHS) that reacts with a sulfhydryl group of IgG and other macromolecules. This kit contains all of the necessary reagents for the labeling. Reducing agent in this kit creates free sulfhydryl groups in the IgG molecule without loss of antibody affinity. The labeling process is simple. After the reducing reaction, add SH-reactive Biotin to a IgG solution on a filter membrane and incubate for 30 minutes at 37˚C. On the average, 5-8 biotin molecules conjugate to each IgG molecule. Excess biotin molecules can be removed using a Filtration Tube included in the kit.

### COMPONENTS
- SH-reactive Biotin 3 tubes
- Reducing Agent 3 tubes
- WS Buffer 4 mL
- Reaction Buffer 1 mL
- Filtration Tubes 3 tubes.

### Materials or equipment required but not provided
- 0.5 mL microtubes.
- 10 µL and 200 µL adjustable pipettes.
- Microcentrifuge
- DMSO
- 37˚C Incubator

### SAMPLE REQUIREMENT
Proteins: Molecular weight >50, 000; amount: <200 µg (IgG)

### PROCEDURE
### Labeling of IgG
1. Add 100 µL of WS Buffer and the sample solution containing 100 µg of IgG to the Filtration Tube.
2. Centrifuge at 8,000-10,000 g for 10 minutes.
3. Add 150 µL of WS Buffer to a tube of Reducing Agent and dissolve by pipetting.
4. Transfer 100 µL of the Reducing Agent solution onto the membrane of the Filtration Tube and pipette to dissolve the IgG on the membrane.
5. Incubate the tube at 37˚C for 30 minutes. Add 100 µL of Reaction Buffer and centrifuge at 8,000-10,000 g for 10 minutes.
6. Add 10 µL DMSO to the SH-reactive Biotin and dissolve it by pipetting.
7. Add 100 µL Reaction Buffer and 8 mL of the Biotin solution to the Filtration Tube. Mix with the solution by pipetting.
8. Incubate the tube at 37˚C for 30 minutes. Add 100 µL of WS Buffer to the tube. Centrifuge at 8,000-10,000 g for 10 minutes.
9. Add 200 µL of WS Buffer to the membrane where the IgG is concentrate and centrifuge at 8,000-10,000 g for 10 minutes. Repeat this step.
10. Add 200 µL WS Buffer and pipette 10 to 15 times to recover the conjugate. Transfer the solution to a 0.5 mL tube and store the solution at 4˚C.

## Precautions

IgG or biotin–conjugated IgG is always on the filter membrane of the filtration tube during the labeling process. If the IgG solution contains proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling with this kit. IgG solution can be purified by IgG purifications kits (not included in this kit). If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for labeling.

The recommended amount of IgG is 100 μg in a volume of 100 μL or less. If the antibody concentration is lower than 1 mg/mL, repeat step 1 and 2 until the total IgG accumulation becomes 100 μg. If the volume of the filtrate becomes 400 μL or more during the process, discard the filtrate prior to going on to the next centrifuge step.

If solution still remains on the membrane after centrifugation, spin another 5 minutes or increase the centrifuge speed.

The SH-reactive Biotin is found in the bottom of the tube. To dissolve, add 10 μL of DMSO to the bottom of the tube and pipette several times.

If the amount of IgG is 200 μg add the entire tube of Biotin solution.

You do not have to use the WS Buffer to recover the biotin-conjugated IgG. You can choose a buffer that is appropriate for your experiment.

## STORAGE

Store all components at 4˚C. Stable for 1 year at 4˚C with protection from moisture.

## FAQ

Q. Can I use this kit for other proteins?
A. Yes, if the molecular weight is greater than 50,000.

Q. Do I have to use a filtration tube prior to labeling the protein?
A. There is no need to use the Filtration Tube if the following conditions are met.
a) The protein has disulfides groups and its solution does not contain small molecules with SH groups or disulfide groups. The concentration of the protein is 10 mg/mL, or about 70 μM. Mix 10 μL of the sample solution with 90 μL of Reducing Agent and follow the protocol starting at Step 7.

Q. How long is the biotin-labeled protein stable?
A. If you store the biotin-labeled protein at 4˚C, it is stable for 2 months. For longer storage add 100% volume glycerol, aliquot and store at -20˚C. However, please note that stability depends on the protein itself.

Q. What is the minimal amount of IgG that can be labeled with this kit?
A. The minimal amount is 10 μg, simply follow the protocol. The labeling ratio remains the same for 10-200 μg IgG.

Q. How can I determine the number of biotin per protein?
A. The average number of biotin per IgG should be in the 5-8 range. If you need to determine the precise number of biotin molecules per protein use a HABA assay. A protocol for a HABA assay is as follows:

Reagent Solution:
200 μM HABA (4-hydroxyazobenzene-2-carboxylic acid) solution prepared with PBS, pH 7.4……1 mL
0.5 mg avidin/mL solution prepared in PBS, pH 7.4……1 mL
Diluted sample solution (55 μL biotinlylated protein solution + 110 μL PBS, pH 7.4)
25 μM biotin prepared with a mixed solution (2 volumes of PBS, pH 7.4 + 1 volume of WS buffer)……200 μL
Prepare various concentration solutions (12.5 μM, 6.25 μM, 3.13 μM, 1.56 μM) with serial dilution……200 μL each

1. Mix HABA solution and avidin solution in a plastic tube.
2. Add 100 μL of the HABA-avidin solution to 15 wells for multiple assays (n=3)
3. Add 50 μL biotin solution (12.5 μM, 6.25 μM, 3.13 μM and 1.56 μM) to 3 wells each and add 50 μL of diluted sample solution to the rest of the 3 wells.

4. Read the O.D. at 405 nm with a reference at 492 nm, and prepare a calibration curve using the O.D. of various concentrations of biotin solutions. Read the O.D. at 280 nm and determine the protein concentration (e.g. molar absorptivity of IgG at 280 nm:216,000)
5. Determine the concentration of biotin in the sample solution and calculate the number of biotin molecule per protein.

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