



KAMIYA BIOMEDICAL COMPANY

Biotin-NH₂ Labeling Kit

For the rapid Biotin labeling of proteins

Cat. No. KT-221

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

Biotin-NH₂ Labeling Kit is primarily used for the preparation of biotin-labeled IgG for enzyme immunoassays (EIA). NH₂-reactive Biotin (a component of this kit) has a succinimidyl group (NHS) that reacts with an amino group of IgG and other macromolecules. This kit contains all of the necessary reagents for labeling. The labeling process is simple. Add NH₂-reactive Biotin to a IgG solution on a filter membrane and incubate for 10 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 kit included in the kit.

COMPONENTS

- NH₂-reactive Biotin 3 tubes
- Reaction Buffer 0.5 mL
- WS Buffer 4 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 Sample

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 10 µL DMSO to the NH₂-reactive Biotin and dissolve by pipetting.
4. Add 100 µL Reaction Buffer and 8 µL of the Biotin solution to the Filtration Tube. Mix with the solution by pipetting.
5. Incubate the tube at 37°C for 10 minutes.
6. Add 100 µL of WS Buffer to the tube. Centrifuge at 8,000-10,000 g for 10 minutes. Discard the filtrate.
7. Add 200 µL of WS Buffer to the tube. Centrifuge at 8,000-10,000 g for 10 minutes. Repeat the step.
8. Add 200 µL of WS Buffer to the membrane where the IgG is concentrate 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 volume of IgG solution should be less than 100 µL. 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 NH₂-reactive Biotin is found in the bottom of the tube. To dissolve, add 10 μ L of DMSO to the bottom of the tube and pipette several times.

If the amount of IgG is 200 μ g 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 the 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. If the protein solution does not contain small molecules with amino groups and the concentration of the protein is 10 mg/mL, or about 70 μ M, there is no need to use the filtration tube. Mix 10 μ L of the sample solution with 90 μ L of Reaction Buffer and 8 μ L NH₂-reactive Biotin solution and follow the protocol starting at Step 4.

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 μ g, simply follow the protocol. The labeling ratio remains the same for 10-100 μ g 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 μ M HABA (4-hydroxyazobenzene-2-carboxylic acid) solution prepared with PBS, pH7.4.....1 mL
 0.5 mg avidin/mL solution prepared in PBS, pH 7.4.....1 mL
 Diluted sample solution (55 μ L biotinylated protein solution + 100 μ L PBS, pH 7.4)
 25 μ M biotin prepared with a mixed solution (2 volumes of PBS, pH 7.4 + 1 volume of WS buffer).....200 μ L
 Prepare various concentration solutions (12.5 μ M, 6.25 μ M, 3.13 μ M, 1.56 μ M) with serial dilution..... 200 μ L each

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