Biotin Labeling Kit - NH_2 (for 1 mg) Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/lk55.pdf

General Information

Biotin Labeling Kit-NH_2 is primarily used for the preparation of biotin-labeled antibody for enzyme immunoassay (EIA). NH_2-Reactive Biotin, a component of this kit, has a succinimidyl ester group, and can easily make a covalent bond with an amino group of the target protein or other macromolecules without any activation process. Filtration Tube included in this kit is used for sample protein in removing small molecules such as Tris buffer and amine compounds that interfere with the assay or labeling reaction. The labeling process is very simple. Add the NH_2-Reactive Biotin to protein solution on a filter membrane, and incubate at 37°C for 10 min. Excess biotin molecules can be removed by using a Filtration Tube. This kit contains necessary reagents for labeling, including the storage buffer for conjugates.

Kit Contents

- NH_2-Reactive Biotin ..... 1 tube
- WS Buffer ..... 13 ml x 1
- Reaction Buffer .....1.2 ml x 1
- Filtration Tube .....1 tube
- 15 ml Tube (for counterbalance) .....1 tube

Capacity

One sample labeling
- Sample requirement: Molecular weight > 50,000; amount: 1 mg as IgG

Storage Condition

Store at 0-5 °C. This kit is stable for 6 months at 0-5°C before opening.

Caution

Once a seal bag is opened, keep the unused NH_2-Reactive Biotin in the bag, seal tightly and store at -20°C. Store the other components at 0-5°C.

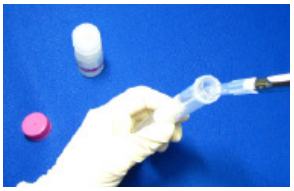
Required Equipment

- 200 µl and 1 ml adjustable pipettes
- Centrifuge and rotor for 15 ml tube
- Incubator (37°C)
- Tube (volume > 2 ml)
- DMSO

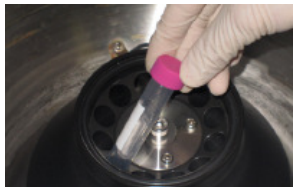
Precaution

- If the target protein solution contains other proteins with molecular weight larger than 10,000, such as serum albumin or gelatin, purify the protein solution, and use the purified target proteins for biotin labeling, because it might interfere the labeling reaction.
- If the protein solution contains small insoluble materials, centrifuge the solution, and use the supernatant for the labeling.
- The droplets which induced from the dry inhibitor of membrane, are occasionally found inside Filtration Tube while storing the kit at 0-5°C or after returning to room temperature. This phenomenon does not affect the performance.

General Protocol

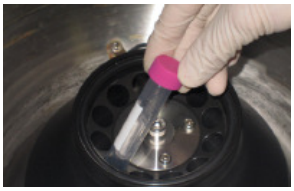


Step 1.
Add 1 ml WS Buffer and the sample solution containing 1 mg IgG[a] to a Filtration Tube. Prepare a 15 ml Tube.[b]

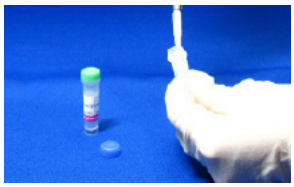


Step 2.
Pipette to mix and centrifuge at 6,000 x *g* for 30 min if using a fixed angle rotor.[c]

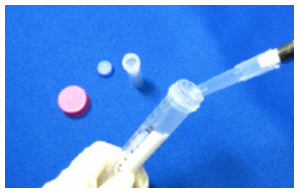
Step 3.
Add 1 ml WS Buffer to the Filtration Tube again.[b]



Step 4.
Centrifuge at 6,000 x *g* for 30 min again.[c]

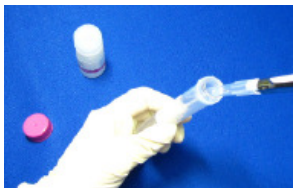


Step 5.
Add 100 µl DMSO to NH_2-Reactive Biotin, and dissolve it with pipetting.[d]

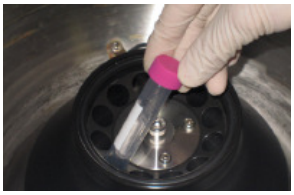


Step 6.
Add 900 µl Reaction Buffer to the Filtration Tube, and 80 µl NH_2-Reactive Biotin DMSO solution to the Filtration Tube.

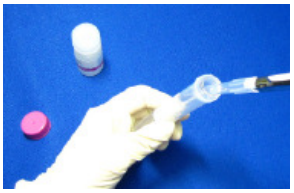
Step 7.
Pipette several times to mix, and incubate the tube at 37°C for 10 min.



Step 8.
Add 1 ml WS Buffer to the Filtration Tube and centrifuge at 6,000 x *g* for 30 min.[b][c] If the volume of the filtrate is 4 ml or more, discard the filtrate prior to centrifuge.



Step 9.
Add 2 ml WS Buffer to the Filtration Tube and centrifuge at 6,000 x *g* for 30 min.[b][c]
Repeat this step.



Step 10.
Add 2 ml WS Buffer and pipette 10-15 times to recover the conjugate. Transfer the solution to a tube (not included in this kit), and store the solution at 0 - 5 °C.[e]

a) The volume of sample solution should be 3 ml or less. If the volume of sample solution is larger than 3 ml, repeat step 1 and 2 until the total IgG accumulation becomes 1 mg. If the volume of the filtrate becomes 4 ml or more during the accumulation process, discard the filtrate prior to going to the next centrifuge step.

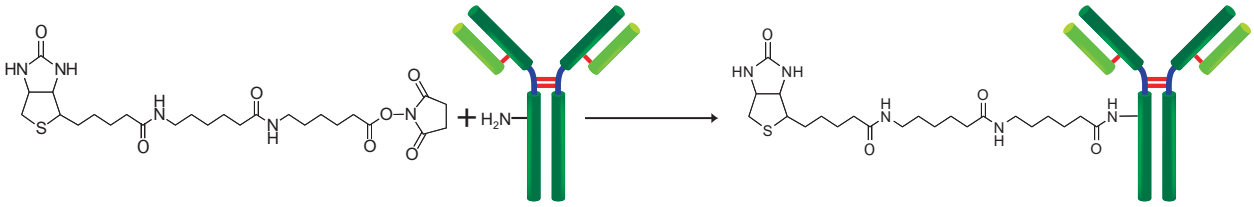
b) Measure the weight of the Filtration Tube. Prepare a same weight of 15 ml Tube with water. Use this 15 ml Tube for counter-balance.

c) Centrifuge at 4,000 x *g* if using a swinging bucket rotor. If more than 100 µl of the solution still remains on the membrane after the centrifugation, spin for another 10 min. If a maximum centrifugal force is less than 6,000 x *g*, additional spin time should be requied (ex. 2,000 x *g* for 50 - 60 min).

d) NH_2-Reactive Biotin is on the bottom of the tube. Add 100 µl DMSO to the bottom of the tube, and pipette several times to dissolve. NH_2-Reactive Biotin can be hydrolyzed by moisture in DMSO. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Biotin solution.

e) We recommend using WS Buffer to storage the conjugate. You can choose any kinds of buffers appropriate for your experiment.

Biotin Labeling Reaction



Q & A

- **Can I use this kit to label antibody which is commercially available?**
  Yes. However, if antibody solution contains other proteins such as serum albumin or gelatin, labeling reaction might be interfered by that protein. Purification of the antibody solution with affinity chromatography is necessary prior to use this kit. Contact us for the purification procedure, if you need.
- **Can I use this kit to label an oligonucleotides or oligopeptides?**
  No. Oligonucleotides and oligopeptides may be too small to retain on the membrane filter of the Filtration Tube.
- **Is it necessary to use WS Buffer to recover biotin-labeled protein?**
  We recommend using WS Buffer, however, you can also use your own buffer currently used instead of WS Buffer.
- **How long is the biotin-labeled protein stable?**
  Stability of conjugate depends on the protein itself. In the case of labeling for rabbit IgG is stable at 4°C for 2 months.
  However, for longer storage, add equal volume of glycerol to the sample solution and store at -20°C.
- **How many biotin molecules are introduced to protein?**
  The number of conjugated biotin depends on the protein. In the case of rabbit IgG, 7 to 10 biotin molecules conjugate to each protein molecule.
- **Is there any notice for treatment of living cells with the biotin-labeled protein?**
  We recommend using PBS including 2-10% FBS for preparation of cell suspension to maintain the best cell condition.
- **Does recovery buffer (WS Buffer) have harmful effect to living cells?**
  No. WS Buffer contains stabilizing agent (surfactant) that is controlled of its concentration without cytotoxicity.
  If you are concerned about the additive in WS Buffer, you can use your own buffer currently used instead of WS Buffer.

If you require assistance, please contact Dojindo customer service.

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