ProtOn™

Biotin Labeling Kit

Cat. No. PLK-1202

Introduction:

The ProtOn™ Biotin Labeling Kit is designed for simple and fast labeling of proteins with biotin. The kit is ideally suited to label antibodies, hormones or other proteins.

The labeling reagent, Biotin (Long Arm) NHS, reacts with a primary amine on the protein, forming a covalent amide bond with a spacer arm between the biotin and the protein. Incorporation of the label is mainly accomplished through reaction with primary amines on lysine residues or terminal amino groups. The linkage between biotin and the protein produced with this reagent is stable, with no significant loss of the label during storage.

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Kit Components

- Labeling Reagent* 750 µg
- Dimethylsulfoxide (DMSO) 20 µl
- Reaction Buffer (0.1 M sodium phosphate, pH 8.0) 5 ml
- Stop Reagent (1 M ethanolamine) 10 µl
- Gel Filtration Slurry 5 ml
- Agarose Avidin (1:1 slurry)** 250 µl
- 1 ml Spin Columns 5 columns
- 2 ml Collection Tubes 5 tubes

** N-hydroxysuccinimidyl-6-(biotinamido) hexanoate (Biotin (Long Arm) NHS).*

*** This is an enhanced matrix with a higher binding capacity than Agarose Avidin D (Cat. No. A-2010).*

One kit contains sufficient reagents for five labeling reactions. Store at 4° C.

Protocol

Preparation of Solutions:

1. Labeling Reagent: Briefly centrifuge the Labeling Reagent tube. Remove cap and add 15 µl of dimethylsulfoxide and vortex. Once dissolved, the labeling reagent should be stable for up to 12 months when stored at -20 °C.

2. Protein solution: Dissolve the protein to be labeled in Reaction Buffer. The final concentration of protein should not be higher than 10 mg/ml. If the protein to be labeled is not purified but is present in a tissue culture supernatant, ascites fluid, etc., other constituents in that solution may also be labeled with biotin.

   If protein activity is inhibited by phosphate, the labeling reaction can be performed in 100 mM bicarbonate, HEPES, or borate buffers (pH 8.0). Do not use buffers that contain free amino groups, such as Tris or glycine.

Labeling up to 1 mg protein in 100 µl sample volume:

1. Add 2 µl of Labeling Reagent to 100 µl of protein solution (see *Note 1*).

2. Mix and incubate for 30 minutes at room temperature.

3. Stop the reaction by adding 2 µl of Stop Reagent.

4. Incubate the reaction for 5 minutes at room temperature.

Purification (See Note 2):

1. Resuspend the Gel Filtration Slurry before use.

2. Snap off the tip of a spin column and add 1 ml of the slurry into the column. Place the column into a collection tube and centrifuge for 1 minute at 500 x g.

3. Remove the spin column from the collection tube and discard the flow-through buffer. Place the spin column into a clean microcentrifuge tube.

4. Add the reaction mixture to the top of the gel in the spin column. Centrifuge the tube for 1 minute at 500 x g. The flow-through contains the biotin labeled protein.

Modified protocol for labeling up to 1 mg protein in sample volumes between 100 µl and 1 ml:

The preceding protocol is recommended for most labeling procedures. However, in some cases it may be necessary to carry out the labeling reaction in volumes greater than the recommended 100 µl. The following modified protocol is provided for labeling up to 1 mg protein in sample volumes between 100 µl and 1 ml.

1. Add 2 µl of Labeling Reagent (see *Note 1*) to up to 1 ml of protein sample in appropriate buffer (see "Preparation of Protein Solution").

2. Mix and incubate for 2 hours at room temperature.

3. Stop the reaction by adding 2 µl of Stop Reagent.

4. Incubate the reaction for 5 minutes at room temperature.

5. Remove excess labeling reagent by dialyzing the sample against 2 liters of appropriate buffer for 3 hours to overnight. (Use of spin columns is recommended only for reaction volumes of 100 µl - 120 µl.)

Notes:

Note 1) The optimal extent of biotin labeling of the protein will depend on the intended application. The concentration of Labeling Reagent used in this step is generally recommended for labeling most proteins, including immunoglobulins. However, since the number of available primary amino groups differs between proteins, the amount of labeling Reagent used may require optimization for individual applications. (See "Estimation of Percent Biotinylation".)

Note 2) A spin column is a fast and convenient purification method. However, for applications that may be affected by trace amounts of free biotin, or for proteins smaller than 25 kDa, purification can be accomplished by dialyzing the solution against 2 liters of PBS or other appropriate buffer for 3 hours to overnight.

Estimation of Percent Biotinylation

With an adequate amount of protein, the percent of all protein molecules containing biotins accessible to avidin can be determined by passing an aliquot of the purified labeled protein through Agarose Avidin and measuring the percentage of the protein sample bound to Agarose Avidin (based on O.D. 280 nm).

With smaller amounts of protein, the amount of sample required for accurate determination with this test generally precludes its use. However, the percent biotinylation of these samples can be approximated using the following test:

1. To 5-10 µl of labeled protein sample (at 1 mg/ml) in a microcentrifuge tube, add 50 µl of the resuspended Agarose Avidin slurry.

2. Incubate for 30 minutes, occasionally resuspending gently with a pipet tip. To prevent Agarose Avidin from adhering to the wall of the tube above the meniscus, do not vortex or mix vigorously.

3. Spin down the slurry by centrifuging for 2 minutes at 500 x g.

4. Carefully collect the supernatant without disturbing the settled Agarose Avidin. The supernatant contains unbiotinylated proteins (Solution A).

5. Add 50 µl of buffer to another aliquot of the labeled protein sample (Solution B).

6. Load serially diluted aliquots of Solution A and Solution B on an SDS-PAGE gel. After electrophoresis, stain the gel using a protein stain. The percent biotinylation can be approximated by comparing the relative intensities of the bands before and after incubation with Agarose Avidin. A successful labeling reaction should contain only a minor fraction of the protein band in Solution A.

The efficiency of biotinylation can also be checked using one of the following tests:

Test 1
Serially dilute an aliquot of the biotinylated protein. Similarly, make serial dilutions of a known biotinylated reference standard (e.g. biotinylated mouse IgG, Cat. No. BI-1003) over the same concentration range as the biotinylated protein. Dot these diluted samples onto a nitrocellulose membrane in a side-by-side fashion and compare the signal intensity of the dots after visualization with a streptavidin- or avidin-based detection system.

Test 2
If the protein has biological or enzymatic activity that is not affected by biotinylation, that activity can be used to estimate percent biotinylation.

Use the Agarose Avidin to remove biotinylated protein from solution, as described in Steps 1-6 above. Measure the enzymatic or biological activity in the supernatant. The percent biotinylation can be estimated by comparing the activity of the protein in the supernatant (A) with the activity of the protein before labeling (B) at the same concentration, using the following formula:

$$\text{percent biotinylation} = \frac{B - A}{B} \times 100$$

Test 3
To accurately determine the total number of biotins per protein, we recommend using the Quant*Tag™ Biotin Quantitation Kit (Cat. No. BDK-2000).

For a comprehensive catalog of antibodies and other immunohistochemical products please phone or visit our website:

www.vectorlabs.com

The ProtOn™ Biotin Labeling Kit is designed to be used for laboratory use only.