Biotin Conjugation Kit

User Manual

Catalog # CRG1122

Biotin Conjugation Kit uses a simple and quick process for Biotin labeling/conjugation of antibodies. It can also be used to conjugate other proteins or peptides.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Biotin Conjugation Kit provides all the reagents required for labeling, which can label proteins containing primary amino-group (-NH2) molecules simply and effectively. Within a certain pH range, NHS-Biotin specifically reacts with primary amino groups (N-terminal and lysine residue side chains) to form a stable amide bond, so as to realize the coupling of NHS-Biotin with protein.

II. KIT COMPONENTS

Component	1 mg Size	5 mg Size	Storage
Reaction Buffer	1 ml x 1	5 ml x 1	4 °C
NHS-Biotin	Powder x 1	Powder x 5	4 °C
DMSO	100 µl x 1	500 µl x 1	RT
Quencher Reagent	1 ml x 1	5 ml x 1	4 °C
3kD Filtration Tube	1 set	5 set	--
User Manual	1 Manual	1 Manual	--

III. STORAGE AND STABILITY

The unopened kit can be stored at 2-8 °C for 1 year, and the dissolved NHS-Biotin can be stored at -20 °C or -80 °C for 1 week.

IV. WORKING SOLUTION PREPARATION

1. Reaction Buffer, DMSO, Quencher reagent

All reagents must be warmed to the room temperature before use.

2. NHS-Biotin

Centrifuge the tube before use. Add 30 µl DMSO into the tube, stand for 10 min until it is fully dissolved. The concentration of NHS-Biotin is 10 mM. The dissolved reagent can be stored at -20°C for 1-2 weeks.

3. Filtration Tube

Add 200 µl Reaction Buffer into the dry filter device, stand at room temperature for 10 min, and discard Reaction Buffer before adding the reagent to be labeled (the filter device should remain moist throughout the labeling process).

Buffer Components & Conditions

Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
Antibody concentration	0.5-2 mg/mL
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
BSA	No
Sodium Azide	<0.1%
Chelating agents (e.g. EDTA)	Yes
Glycerol	No
Sugars	Yes
Gelatin	<0.1%
Tris	<50 mM
Glycine	No
Thiomersal / Thimerosal	No
Merthiolate	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

V. ASSAY PROCEDURE

Equilibrate all materials and prepared reagents to room temperature prior to use.

Note: If the protein contains free amino groups (Tris, amino acids or other interferents), please centrifuge it 2-3 times (8,000 × g, 10 min) using the Filtration Tube to ensure that it is removed.

For 1 mg antibody

1. Add 200 µl of Reaction Buffer into each 1 ml of antibody (e.g. antibody concentration 1 mg/ml) to be labeled and mix gently. (Reduce the amount of Reaction Buffer at the same scale)

2. Add 30 µl of 10 mM NHS Biotin into the protein solution, gently blow and mix fully. Replace cap on the vial and leave standing for 30 minutes in the dark at 37°C hot air circulating oven.

3. After incubating, add 5 µl of Quencher Reagent for each 30 µl of 10 mM NHS Biotin used and mix gently (Reduce the amount of Quencher Reagent at the same scale). The conjugates can be used after 10 minutes.

4. Add an appropriate amount of 1×PBS into the above reaction solution, cover the cap after matching with the collection tube, and centrifuge for 10-30 min at the speed of 12,000 × g. Discard the liquid in the collection tube, replenish 1×PBS in the filter device, and repeat the centrifugal ultrafiltration operation for 2-3 times.

5. Invert the filter device in another collection tube and centrifuge at 1000 × g for 2 mins. Collect the solution in the collection tube.

VI. PROTEINS AND BIOMOLECULES GUIDE

Small proteins

The protocols recommend the amount of antibody to add, as this is the most common use of the kit. However, the amount can be adjusted to suit other proteins. The molar ratio of label to protein is important, as excessive amounts of the label may damage the protein or cause high backgrounds in the final assay. For example, if the protein to be labeled is 75 kDa (half the size of an antibody) the addition of 10 µg of protein would introduce twice the amount in molar terms as with 10 µg of antibody. A consequence of this may be the presence of unlabeled protein at the end of the reaction. Whether this is a concern will depend on the assay application, but the addition of less protein (5 µg in this case) would normally be a safer starting position.

Peptides/small molecules

Many peptides will have a free N-terminus i.e. with a primary amine on the first amino acid, which can participate in the reactions. In some cases, however, the N-terminus will be 'blocked' e.g. by acetyl groups, which may have been added during peptide synthesis to improve peptide stability in vivo (e.g. to prevent attack by aminopeptidases). A peptide that is both N-terminally blocked and lacks lysine residues cannot be conjugated using the Conjugation Kit.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com