FlyLink™ Biotin Labeling Kit

Cat #: D-AKE120

Size: 100 µg x 3

Storage: Stored at 4°C for 6 months, protected from light

Assay Principle

Biotin is a water-soluble B-vitamin, with a molecular weight of 244 Da. It is composed of a ureido ring fused with a tetrahydrothiophene ring. A valeric acid substituent is attached to one of the carbon atoms of the tetrahydrothiophene ring. Biotin has high affinity with Avidin or Streptavidin, so the Biotin-Avidin-System is widely used in immune detection as a multistage signal amplification system, with high sensitivity and strong specificity.

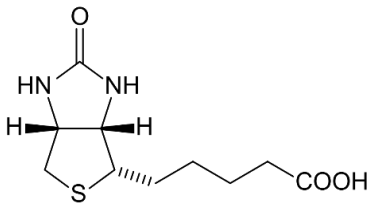

Fig.1: Biotin structural formula

FlyLink™ Biotin Labeling Kit is designed for preparing biotin conjugates directly from proteins, peptides, and other ligands that contain an active amino group. The Biotin provided in our kit has been pre-activated, and can be directly used for conjugation.

FlyLink™ Biotin Labeling Kit contains ready-to-use components to prepare Biotin labeled molecules.

Application examples: Directly labeled primary antibodies are advantageous as they eliminate the need for secondary reagents in immunoassay procedures, thus removing a tedious extra cycle of incubation and wash steps in applications such as ELISA and Western blotting.

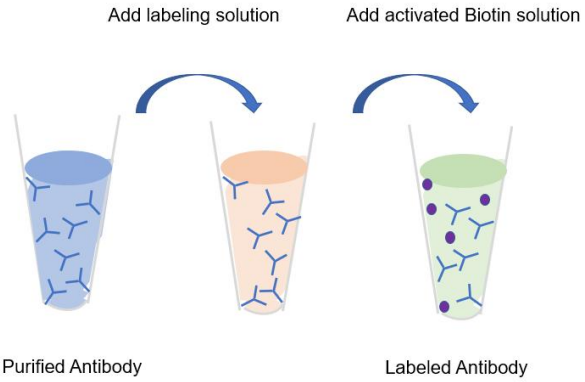

Fig.2: Schematic Diagram FlyLink™ Biotin Labeling Kit schematic diagram

Applicable samples: labeling biomolecules, especially proteins, peptides, and other ligands that contain a free amino group

Materials Supplied and Storage Conditions

Kit components	Size（ 100 μg*3）	Storage conditions
Activated Biotin Solution	7.5 μL	4°C, protected from light
Biotin Labeling Solution	15 μL	4°C
Purification Column (0.5 mL, 50KD)	3	RT
Booklet	1	RT

Materials Required but Not Supplied

·Prepared samples to be labelled

·Precision pipettes, disposable pipette tips

·Deionized water, PBS (pH 7.4)

Sample Preparation

The initial concentration of the sample to be labeled should be higher than 2 mg/mL. It is suggested that the protein

concentration is above 2.5 mg/mL. Otherwise, increasing protein concentration is required before the experiment. The components (or storage buffer) of the sample to be labeled should meet the following requirements:

(1) Do not contain amino components, otherwise it will affect the coupling effect.

(2) A small amount of BSA will not affect the coupling effect. it is suggested to use PBS as storage buffer.

(3) If the sample contains substances that may interfere with the labeling, it is suggested to replace the buffer with PBS. The specific method is as below.

Add the sample to the ultrafiltration tube and add 200-150 µL PBS. Centrifuge at 12,000 g, 4°C, 10 min, and discard the filtrate. Then PBS was added again, and centrifuge at 12,000 g, 4°C, 10 min. After centrifugation, the inner core of the ultrafiltration tube is taken out, placed it in a clean outer tube, and centrifuge at 4,000 g, 4°C, 2 min to collect the sample.

List of components (or storage buffer) requirements of the sample to be labeled:

pH	**6.5-8.0**
Amine Free Buffer	MES, PBS, HEPES
Chelating Agents (e. g. EDTA)	✓
Glycerol	< 50%
BSA	< 0.1%
Glycine	✗
Components Containing Amino	✗

Assay Procedure

Note: Please enlarge or reduce the volume of each component of the kit in the reaction system according to the actual sample volume.

1. Setting up your conjugation reactions

The recommended optimal labeling sample size and labeling system for different specifications are as follows:

Size	**Amount of Sample**	**Optimal Labeling System**
3×100 µg	3×100 µg	3×50-3×75 µL
1 mg	1 mg	500-750 µL

The following operation steps are based on the 3×100 μg specification. For other specifications, please adjust the dosage accordingly. If you need to adjust the volume, please keep the sample size unchanged and change the volume of Biotin labeling solution, Activated Biotin Solution and deionized water in the labeling system.

(1) Add 15 μL Biotin labeling solution to the sample solution to be labeled and mix gently with a pipette.

(2) Pipette 7.5 μL of Activated Biotin Solution to the reaction solution in step (1), add deionized water to 150 μL (Note: this volume is the labeled system), mix gently, and let stand at 37°C in the dark for 1 h.

Note: Step (1)/ (2) belong to the labeling steps.

(3) Add an appropriate amount of PBS (fixed volume to about 500 μL) to the reaction solution in step 2, mix gently, and move the solution to the purification column. Centrifuge at 12,000 g at 4°C for 10 min.

(4) Discard the filtrate and add an appropriate amount of PBS (fixed volume to about 500 μL) to the purification column. Centrifuge at 12,000 g for 10 min at 4°C.

(5) Take out the purification column, put it upside down in a clean centrifuge tube, centrifuge at 4,000 g for 2 min at 4°C. The solution collected in the centrifuge tube is the coupling product.

Note: Step (3)/ (4)/ (5) are the purification steps.

2. Storage of conjugates

The conjugate can be stable at 4°C in the dark for more than one month. For long-term preservation, the conjugate should be packed in small aliquots, placed at -20°C in the dark and added the same volume of glycerol. Avoid repeated freezing and thawing.

FAQ

Q1: After concentration, the sample concentration is still below 2 mg/mL?

A1: If the sample concentration is still less than 2 mg/mL after concentration, the volume of the labeling system can be adjusted appropriately, but the final concentration should be greater than 1 mg/mL. The volume of the Biotin labeling solution in step (1) should be 10% of the volume of the labeling system. In step (2), deionized water may not be added and the volume of the Activated Biotin Solution remains unchanged. You can also make appropriate adjustments based on your own experiments.

Q2: How to select the appropriate purification column for different molecular weight of the sample to be labeled?

A2: The purified column is suitable for samples with molecular weights ranging from 100 KD to 200 KD. If the samples with molecular weights higher than 200 KD or less than 100 KD, it should better be equipped with more suitable size of the purified column.

Q3: Is the molecular weight of the sample to be labeled similar to Biotin?

A3: If the molecular weight of the sample is similar to Biotin, after completing the labeling step corresponding to this instruction, add 30 µL 0.5 µM NH_4Cl and incubate at 37°C for 10 min to quench the free Biotin without the need for purification steps.

Q4: Do molecules with different molecular weights have different coupling effects?

A4: For molecules with a molecular weight less than 5 KD, the coupling effect is poor. You can increase the amount of molecules appropriately.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.