Sulfo-NHS-Biotin Kit (90-180 kDa)

Product description

Sulfo-NHS-Biotin Kit (90-180 kDa) is a kit that labels proteins such as antibodies with amino groups with biotin. Biotin molecules modified with active esters in the kit can specifically covalently label molecules such as amino-containing proteins to achieve biochemical modification of proteins and subsequent immunolabeling reactions. When this product is used to label proteins, especially immunoglobulin G (IgG), the Filtration Tube included in the kit can be used to remove inhibiting labeled reactants or low molecular weight compounds such as unreacted biotin, such as Tris, etc., to increase the labeling effect. Compared to conventional treatments such as dialysis and gel filtration, this kit results in higher recovery and higher purity of Biotin-labeled compounds.

This product is easy to operate, only ammonia-reactive biotin components need to be added to IgG and other protein antibody solutions, and incubated at 37°C for 10-15 min to achieve an average of 5-8 biotin molecular marker binding per IgG molecule, and the whole process can be completed in about 1 h. This product is only suitable for 90-180 kDa, 50-200 μg of protein molecular markers.

Components and storage conditions

Components	K1151-3 samples	K1151-10 Samples
Sulfo-NHS-LC-Biotin (A8003)	3 tubes	10 tubes
Wash Buffer	5 mL*1	5 mL*3
Reaction Buffer	500 μL	500 μL*4
Filtration Tube (30 kd)	3 tubes	10 tubes
Store Sulfo-NHS-LC-Biotin (A8003) at -20°C and the other components at 4°C for 12 months.		

Experimental manipulation

1. The components required for your own experiment but not included in the kit are as follows:

   10 μL versus 200 μL pipettes, 37°C incubator, DMSO, microcentrifuge, micro-EP tubes

2. Sulfo-NHS-LC-Biotin was centrifuged at 12,000 rpm for 30 s, then 50 μL of DMSO was added to mix well

and placed at room temperature. (*Note: Reconstitution before the start of the experiment, do not leave it at room temperature for too long, DMSO will freeze when placed on ice, and Biotin's repeated freeze-thaw will affect its labeling efficiency.)*)

3. Add 100 μL of Wash Buffer along with a sample solution containing 100 μg IgG to the Filtration Tube, followed by centrifugation at 8,000-10,000 g for 10 min. (*Note: When the amount of protein is close to 200 μg, it is recommended that the customer centrifuge for 5 min, then blow up the protein on the membrane with a pipette, and centrifuge again for 5 min to accelerate protein centrifugation.)*)
4. Add 100 μL of Reaction Buffer along with 8.8 μL of Sulfo-NHS-LC-Biotin dilution to the Filtration Tube and pipette to mix.
5. Place the Filtration Tube in a 37°C incubator and incubate for 10 min. (*Note: If higher labeling efficiency is required, the incubation time can be increased to 15 minutes, but usually 10 minutes is the ideal labeling effect.)*)
6. Add 100 μL of Wash Buffer to the Filtration Tube, centrifuge at 8,000-10,000 g for 10 min, and remove the filtrate.
7. Add 200 μL of Wash Buffer to the Filtration Tube, centrifuge at 8,000-10,000 g for 10 min, remove the filtrate, and repeat the operation once.
8. After adding 150 μL of Wash buffer to the Filtration Tube and pipetting 10-15 times, the solution is transferred to a new EP tube, and then 50 μL of it is pipetted from the EP tube to the Filtration Tube. After pipetting and mixing again, transfer the solution to the EP tube to obtain the recovered labeled product in the EP tube.
9. Store at 0-5°C in EP tubes. (*Note: It is recommended to use the Biotin product immediately, or to add 50% glycerin and store it at -20°C in the fridge to slow down the loss of biotin.*)

Notes

1. At present, the protein retention rate of this kit can reach 80%-95%, which can greatly facilitate the yield and effect of protein labeling.
2. This product can label proteins other than antibodies, but if the sample contains serum albumin or gelatin proteins, the labeling reaction may be disturbed, it is recommended to purify the sample before labeling it in this product box.
3. The amount of IgG required for labeling with this kit is 50 - 200 μg, and antibody labeling performance does not vary much in this range. If labeled with low levels of IgG, problems such as background elevation may occur.
4. The coexistence of other compounds in the sample solution may affect the Biotin labeling by confirming

which substance is included in the solution, e.g., if it contains polymers with a molecular weight of more than 10,000, even without amino groups, it may cause filter clogging and affect labeling results; If it contains high molecular weight compounds with amino groups that cannot be removed even with a Filtration Tube, such as BSA and gelatin, as these impurities are also labeled with biotin, it is recommended to perform a separate removal operation before the reaction.

5. Since the Filtration Tube filter tube included in the kit is an ultrafiltration filter with a molecular weight cut-off of 30 K, we recommend using 50,000 or larger proteins with margin. When labeling proteins with a molecular weight of 50,000 or less, they can be used by switching to an ultrafiltration filter with a smaller molecular weight cut-off.

6. This kit can label compounds with a molecular weight of 50,000 and above and amino groups (NH2) such as antibodies, proteins, etc. When considering the amount of labeled mg, it is recommended to use the Sulfo-NHS-LC-Biotin Kit (K1001). Oligonucleotides and oligopeptides are not recommended because their molecular weight is too small to retain them on the filter membrane.

7. Each IgG molecule can label an average of 7 to 10 biotin, and 5-8 biotin labeling is more common in experiments.

8. Live cells containing biotin-tagged proteins are recommended to prepare cell suspensions using PBS (containing 2-10% FBS) to keep cells in optimal condition.

9. The kit component Wash Buffer contains surfactants and other ingredients, and its concentration is controlled within the range of non-toxic to cells and harmless to living cells. If you are concerned that the additives in Wash Buffer may affect subsequent experiments, you can choose to use a commonly used buffer instead.

10. This product is for scientific purposes only.

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