# Desthiobiotinylation and Pull-Down Kit (Magnetic Beads)

## Introduction

Desthiiobiotin is a monocyclic sulfur-free biotin analogue with nearly the same specificity as biotin when bound to streptavidin, but with relatively low affinity, with dissociation constants (Kd) of $10^{-11}$ M and $10^{-15}$, respectively M。Due to this property, the use of biotin eluents enables the competitive displacement of thiobiotylin-bound desulfurin-bound biotin markers under mild conditions. This release characteristic of dethiobiotin has many advantages, on the one hand, it helps to reduce the co-purification of endogenous biotin molecules, and on the other hand, it does not require harsh separation conditions to dissociate the complex, thus effectively avoiding the destruction of intermolecular binding such as target proteins. Therefore, when native or recombinant proteins are not suitable for expression of fusion tags, or when the target protein needs to be isolated in a condition as close to its native state as possible, desthiobiotin labeling and pull-down technology are undoubtedly the ideal choice.

The Desthiobiotinylation and Pull-Down Kit (Magnetic Beads) is a kit for studying protein-protein interactions and related biomolecule-to-molecule interactions. The principle is to dethiobiotin label the protein, antibody, or other active biomolecules of interest, and then bind to streptavidin magnetic beads to capture the target protein or other binding molecules that interact with it, and to efficiently elute the bound target protein or other binding molecule under mild conditions.

This kit provides desthiobiotin labeling reagents as well as purification of desthiobiotin labeled protein components. Purification components include desalting columns, streptavidin magnetic beads, and reagents such as biotin eluent. Kits can be used to study the interaction of a known protein with a protein of interest to be discovered, or between a known protein and DNA, RNA, or other molecules, as well as to confirm known protein-to-protein interactions with biomolecules such as proteins. This is especially true when native or recombinant proteins are not suitable for expression of fusion tags or where the protein of interest needs to be isolated under native conditions.

## Composition and Storage

| Components \ Size | 5 T | Storage |
|---|---|---|
| **Sulfo-NHS-LC-Desthiobiotin** | 1 vial | -20℃ |
| **Desalting Column (6-8 mL)** | 5 Sets | 4℃ |
| **Streptavidin Magnetic Beads** | 2 mL | 4℃ |

| | | |
|---|---|---|
| **Elution Buffer** | 8 mL | -20℃ |
| **Biotin** | 1 vial | -20℃ |
| **Shipping: Blue ice** | **Shelf life: 12 months** | |

## Protocol

1. Desthiobiotin labeling reaction

   a. Preparation of the protein to be labeled: Take an appropriate amount of the known protein to be labeled, dissolve it in 1X PBS (final concentration 0.2 - 2 mg/mL), and place it in a clean 1.5 mL centrifuge tube. If primary amines (e.g., Tris or Glycine) or ammonium ions are present in solution, desalting columns are highly recommended to ensure the accuracy and validity of subsequent experiments.

   b. Preparation of desthiobiotin solution (10 mM): Dissolve 1.5 mg of Sulfo-NHS-LC-Desthiobiotin in 280 µL of anhydrous DMSO to obtain 10 mM Sulfo-NHS-LC-Desthiobiotin. If it is not to be used immediately, it can be aliquoted and stored at -20 °C, and it will remain valid within two months.

   c. Prepare the appropriate amount of desthiobiotin: Add the required volume of desthiobiotin solution (1.b) to the prepared protein to be labeled according to the mass size of the protein to be labeled and refer to the table below. Normally, the molar amount of the desthiolibiotin solution is 5-25 times that of the protein sample (excess multiple), but in the actual reaction, it is generally recommended to have an excess multiple of 15 times the molar amount of the desthiolibiotin solution. For example, if a protein sample is known to be IgG, the volume is 1 mL, the concentration is 1 mg/mL, the molar molecular weight is 150,000, and the molar excess factor is 15. Then the volume of desthiobiotin solution required = [ (1 mL×1 mg/mL/150,000)×15/10 mmol]× $10^6$ µL/L = 10 µL.

| Steps | Calculation formula |
|---|---|
| **a.** Determine the quality of the sample | Sample volume (mL) × Sample concentration (mg/ml) = Sample mass (mg) |
| **b.** Convert the sample mass to moles | Sample mass (mg) / Sample molar molecular weight (g/mol) = Sample number of moles (mmol) |
| **c.** Determine the molar amount of Sulfo-NHS-LC-Desthiobiotin in the reaction. | Sample number of moles (mmol) × Excess multiple = Molar number of Sulfo-NHS-LC-Desthiobiotin (mmol) |
| d. Determine the volume of 10 mM Sulfo-NHS-LC-Desthiobiotin required. | (The molar number of Sulfo-NHS-LC-Desthiobiotin (mmol) / 10 mM) × $10^6$ µL/L = The volume of 10 mM Sulfo-NHS-LC-Desthiobiotin (µL) |

***Note:** The total volume of the labeling reaction (the sum of the volume of the known protein sample and desthiobiotin solution) needs to be between 500-1500 µL to allow for efficient removal of unreacted desthiobiotin and other salt ions using a desalination column.

   d. Proteobiotin labeling: 30-60 minutes at room temperature or 2 hours at 4°C, the reaction process is recommended to be carried out on a rocker shaker (side swing shaker).

2. Samples were purified after the thiobiotin labeling reaction

a. Pre-equilibration of the desalting column: Unscrew the cap of the 50 mL centrifuge tube containing the desalting column and remove the desalting column, removing the lower plug of the desalting column. Add 1X PBS to the desalting column for approximately 5 mL each time, and after all the PBS in the column tube has entered the desalting column, pour 1X PBS again to fill the column tube, and repeat this step 6-8 times.

***Note:** 1. Centrifugation causes the filler to compact to form an upward slope, but it is not affected and can continue to be used. 2. If you don't use it temporarily, you can plug the upper and lower plugs and add 1X PBS.

b. Loading: Slowly add the biotinylated protein (1.d) with a pipette close to the center of the upper spacerd) to draw the packing material in the desalting column into the sample.

***Note:** 1. Do not exceed 1 mL of sample volume, as this will reduce sample recovery and will result in inadequate desalting. 2. The method of sample injection has a direct impact on sample recovery, requiring the pipette tip to be inserted into the empty tube of the desalination column and the sample is added close to the center of the resin. 3. If the sample volume < 1000 μL, add an additional 100 μL of ultrapure water after the resin has been drawn into the sample to increase sample recovery. 4. When using the desalting column, it should be noted that its maximum carrying capacity is a single 2 mg protein, which can achieve better desalting and separation of small molecules.

c. Elution: Place the desalting column into the original 50 mL centrifuge tube (2.a) and centrifuge at 1,000×g for 2 minutes, and the flow-through solution contains a purified desulfurbiotinyl-labeled known protein sample, which can be directly used for subsequent binding and elution experiments. For storage, store dry at -20°C.

***Note:** It should be noted that the desalination column is not recommended for reuse

3. Binding of samples such as avidin magnetic beads and biotinylated proteins

a. Preparation of avidin magnetic beads: Invert and mix Streptavidin Magnetic Beads thoroughly, and then transfer an appropriate volume to a new 1.5 mL centrifuge tube. Generally, 50 μL of the well-mixed Streptavidin Magnetic Beads need to be added to each sample.

b. Wash of avidin magnetic beads: Add Binding Buffer to a final volume of approximately 0.5 mL and resuspend Streptavidin Magnetic Beads by gently pipetting. Place on a magnetic stand for 30 seconds to remove the supernatant (avoid touching the beads) and complete a wash step. Repeat the wash 1 time.

c. Sample binding: Add 50-200 μL of biotin-labeled and purified sample (2.c), invert the mix and rocker shake and incubate for 30 min. Typically, 50 μL of Streptavidin Magnetic Beads binds 10-50 μg, so the sample size should not exceed 50 μg.

d. Magnetic separation: After the incubation, place on a magnetic stand for 1 min to separate, retain the Streptavidin Magnetic Beads and collect the supernatant. The amount of a known protein sample bound to Streptavidin Magnetic Beads can be roughly determined by measuring the protein concentration in the supernatant or by performing SDS-PAGE analysis.

e. Wash: Resuspend avidin magnetic beads in 500 μL of 1X PBS and place on a magnetic stand for 1 minute, carefully removing the supernatant without the tip touching the bottom beads. Repeat this step 1 time. The washed avidin beads can be used for subsequent experiments.

4. Pull-down of target proteins, etc., and elution of complexes

a. Add 50-400 μL of sample lysate (or other sample containing the protein of interest) to the washed avidin beads (3.e) and mix by gentle pipetting.

***Note:** 1. For the lysis buffer, it is recommended to use RIPA Lysis Buffer (Strong) (K1020), RIPA Lysis Buffer (Medium) (K1121), RIPA Lysis Buffer (Weak) (K1122), and Cell lysis buffer for WB and IP without inhibitors (K1124). If you use self-prepared lysis buffer or that produced by other companies, you need to ensure that the pH of the lysis buffer is between 6 and 8. When lysing cells or tissues, please add appropriate protease inhibitors to avoid protein degradation. 2. If the lysis buffer is expected to contain natural biotin, new Streptavidin Magnetic Beads can be used to remove the biotin or biotin-containing proteins in it, so as to reduce the non-specific adsorption and background value in the Pull-Down experiment.

b. Incubate at 4°C with rocker shaking for at least 1 h without vortexing.

***Note:** Higher binding amounts require longer incubation times, and the incubation time for each protein needs to be explored on its own. If the protein of interest is stable and the lysate is stable, the incubation can be performed at room temperature.

c. Magnetic separation: After the incubation, place on a magnetic stand for 1 min to collect the supernatant for subsequent analysis. When pipetting the supernatant, do not touch the bottom bead.

d. Wash: Resuspend avidin magnetic beads in 100 μL of 1X PBS and place on a magnetic stand for 1 minute, carefully collect the supernatant for subsequent use. Do not touch the bottom bead. Repeat this step 2 times.

e. Elution:

a) Preparation of biotin elution solution: First, take an appropriate amount of Elution Buffer to fully dissolve 1 vial of biotin. Then, suck the liquid back into the Elution Buffer tube and mix it with the remaining Elution Buffer to form the Elution working solution. It can be aliquoted and stored at -20 °C later, and it will remain valid for at least half a year.

b) Elution: Resuspend Streptavidin Magnetic Beads with 50 μL of Elution Buffer and elute for 30 minutes with slow shaking on a 37°C shaker to elute complexes formed by known proteins labeled with dethiolibiotin-labeled proteins such as target proteins.

***Note:** 1. Temperature (37°C) is critical for sample elution; You can use a 37°C shaker commonly used in the laboratory for bacterial culture, and shake it slowly. 2. If a small volume (at least 50 uL) of the dethioliated biotinylated ligand is combined with avidin magnetic beads, and the beads are found to sink to the bottom after elution for 30 minutes in a low-speed 37°C constant temperature shaker, they can be artificially mixed and incubated again for 15 minutes, during which time manual mixing is repeated to increase the elution efficiency.

f. Place on a magnetic stand for 1 minute to collect the eluate without the tip touching the bottom beads. Repeat step 4.e-f for 2 times, i.e., a total of 3 elutions, and the resulting eluate is the complex of the

labeled known protein and the target protein. The concentration of the complex obtained from the first elution is the highest, and the lowest of the last elution can be carried out according to the experimental needs.

## Notes

1. You will need to bring your own PBS, DMSO, and some consumables, such as 1.5 mL EP tubes.
2. Streptavidin Magnetic Beads should be maintained at pH 6-8 to avoid high-speed centrifugation and drying; Do not leave the beads in a magnetic field for a long time, as this may cause the beads to clump.
3. Streptavidin Magnetic Beads should be properly and fully resuspended before use, that is, inverted several times to mix the magnetic beads evenly, and the mixing operation should be gentle, and it should not be violently vortex shake to avoid protein denaturation.
4. Storage and use conditions of magnetic beads: Streptavidin Magnetic Beads should be maintained at pH 6 - 8, avoid high-speed centrifugation and drying; Before use, it should be properly and fully resuspended (upside down several times), and the mixing operation should be gentle, and it should not be violently vortex and shake to avoid protein denaturation; Do not place the beads in a magnetic field for a long time, as this may cause the beads to clump; It is valid for at least half a year at 4ºC and one year at -20ºC.
5. Labeling reaction related: the solution of labeling known proteins should not contain primary amine groups or amine ions, and it is recommended to use PBS to solubilize the protein; In order to improve the labeling effect, the concentration of the protein to be labeled with desulfurized biotin should not be too low; Sulfo - NHS - LC - Desthiobiotin is susceptible to moisture hydrolysis inactivation, pay attention to keep it dry when storing, after formulating the mother liquor with DMSO, store it in aliquots at -20 °C, effective within two months.
6. This product is for scientific research use only.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com