Biotinylated Protein Pull-Down Kit (Magnetic Beads)

Introduction

The Biotinylated Protein Pull - Down Kit (Magnetic Beads) is a kit for studying protein-protein interactions and related biomolecule-to-molecule interactions. It uses the high affinity between biotin and streptavidin to combine biotin-labeled proteins, antibodies or other active biomolecules with streptavidin magnetic beads and elute, capture the target protein or other binding molecules that interact with biotin-labeled target proteins and biotin-labeled proteins, and carry out pull-down experiments for biotinylated protein complexes, and acidic conditions are important conditions for eluting target molecules or complexes.

The kit provides reagents such as streptavidin magnetic beads for binding biotin tags on biomolecules such as tagged proteins, blocking solutions, acid washes, and eluents. It can be used to study the interaction between a known protein (bait protein) and a target protein to be discovered (prey protein) or between a known protein and DNA, RNA, or other molecules, as well as to confirm the interaction between a known protein and a biomolecule such as a protein. If this kit is used for pull down and elution of biotinylated protein complexes, the experimental workflow will be easier and more convenient. The eluate can be used to identify the bound protein of interest or other biomolecules by Western, mass spectrometry, PCR, DNA sequencing, and other analyses.

Composition and storage

Components \ Size	25 T	Storage
Streptavidin Magnetic Beads	1.5 mL	4℃
Binding Buffer	50 mL	-20℃
Biotin	1 vial	-20℃
Biotin Blocking Buffer	10 mL	-20℃
Wash Buffer	50 mL	-20℃
Elution Buffer	10 mL	-20℃
Neutralization Buffer	1 mL	-20℃
Shipping: Blue ice	Shelf life: 12 months	

Protocol

1. Biotin Labeling Reactions and Purification:

a. Preparation of known proteins to be labeled: Take an appropriate amount of known protein to be labeled and dissolve it in 1X PBS at a final concentration of 0.2 - 2 mg/mL. 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. Labeling and purification of biotin: Operate by referring to the user manuals of the biotin rapid labeling kits, such as NHS-LC-Biotin Kit (K1003), Sulfo-NHS-LC-Biotin Kit (K1001), or NHS-LC-LC-Biotin Kit (K4401), etc., to perform biotin labeling and purification on the known proteins to be labeled.

***Note:** If using the Avi-tagged protein biotin labeling kit (BirA method) (K4402), follow the instructions for the kit;

c. Biotinylated proteins: Biotinylated proteins can be used directly after removing free biotin, and the recommended protein concentration after labeling is 0.5-2 mg/mL, if the concentration is too high or it is in a lyophilized powder state, it can be diluted with Binding Buffer.

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

a. Preparation of avidin magnetic beads: Thoroughly invert and mix the Streptavidin Magnetic Beads, 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 protein (step 1.c), mix by inverting and incubate for 30 minutes with rocker shaking. Typically, 50 μL of Streptavidin Magnetic Beads bind 10-50 μg, so the amount of protein 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 Streptavidin Magnetic Beads in 500 μL Wash Buffer and place on a magnet for 1 minute, carefully removing the supernatant without touching the bottom beads. Repeat this step 1 time. After washing, it can be used for subsequent blocking experiments. If the biotinylated complex is pulled down, it can be directly moved on to the subsequent elution step4.

3. Blocking of avidin bead complexes

a. Preparation of Biotin Blocking Solution: Firstly, take an appropriate amount of Biotin Blocking Buffer to completely dissolve 1 vial of biotin. Then, suck the liquid back into the Biotin Blocking Buffer tube and mix it with the remaining Biotin Blocking Buffer. The final mixed liquid is named Biotin Blocking Solution. It can be aliquoted and stored at -20°C, and it will remain valid for at least half a year.

b. Blocking: Add 250 μL of Biotin Blocking Solution to the washed (step 2e) tube, mix by inverting at room temperature and incubate for 5-10 min with rocker shaking.

c. Magnetic separation: Place on a magnetic stand for 1 minute to separate, carefully remove the supernatant without touching the bottom beads.

d. Wash: Resuspend the Streptavidin Magnetic Beads complex with 250 μL of Binding Buffer and place on a magnet for 1 minute, carefully removing the supernatant without touching the bottom beads. Repeat this step 2 times, for a total of 3 washes.

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 Streptavidin Magnetic Beads (step 4d) and mix by gentle pipetting.

***Note:** The recommended lysis buffers are 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 buffers or those produced by other companies, you need to ensure that the pH of the lysis buffer is within the range of 6 - 8. When lysing cells or tissues, please pay attention to adding appropriate protease inhibitors to avoid protein degradation.

b. Incubation: Incubate for at least 1 h on a 4ºC rocker shaker 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 also be performed at room temperature, but the incubation time at room temperature should not be too long.

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

d. Wash: Resuspend Streptavidin Magnetic Beads in 250 μL Wash Buffer, place on a magnetic stand for 1 minute, and carefully collect the supernatant for subsequent use. Do not touch the bottom bead. Repeat this step 2 times, for a total of 3 washes.

e. Preparation of the neutralization reaction solution: Prepare three portions of the required neutralization reaction solution in advance to neutralize the elution solution (3 × 5 μL). The recommended volume ratio for addition is Elution Buffer: Neutralization Buffer = 10: 1.

f. Elution: Add 50 μL of Elution Buffer to resuspend the Streptavidin Magnetic Beads (step 5.d). Then, gently shake on a shaker for 2 - 5 minutes to elute the captured target proteins and so on.

***Note:** Elution is a quick process, usually about 3 minutes to complete the initial elution, after elution beads should not be reused.

g. Magnetic separation: Place on a magnetic stand for 1 minute to collect the eluent, and the tip does not touch the bottom beads. Repeat step 4f-g 2 times, i.e., co-elute and separate 3 times, and the resulting eluent is the protein of interest. 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.

h. Neutralization reaction: After each elution separation, the eluate is added to the prepared neutralization reaction solution (step 4.e) and mixed thoroughly. The eluted samples were stored at 4ºC or -20ºC for long-term storage.

Notes

1. 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.
2. 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.
3. It is recommended to set up negative and positive control groups to rule out interference from non-specific adsorption of Streptavidin Magnetic Beads.
4. The type and size of the molecule to be bound, as well as the method and degree of biotin labeling, will affect the binding efficiency, and it is recommended to determine the number of beads for each specific application by serial dilution, and consider increasing the number of beads to 2-3 times the number of molecules to be bound to ensure adequate binding.
5. This product is for scientific research use only.

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