Biotinylated Protein Pull-Down Kit (Magnetic Beads)

B1511291

Storage 2-8℃,-20℃. For detailed storage conditions, please refer to the Kit Contents.

Introduction

The Biotinylated Protein Pull-Down Kit (Magnetic Beads) produced by Aladdin is specifically designed for studying biomolecular interactions. It enables efficient capture of target proteins (prey proteins) that interact with biotin-labeled proteins (bait proteins) and can also be used for the pull-down and elution of biotin-labeled protein complexes.

The kit includes binding buffer, TBS buffer, streptavidin magnetic beads, acid elution buffer, neutralization buffer, and denaturing loading buffer for elution, requiring no additional preparation and offering straightforward operation. The beads exhibit high specificity for binding biotin-labeled samples. When used with the provided reagents, they enable efficient elution while ensuring stable experimental performance and reliable results. This kit meets the needs of routine scientific research, facilitating efficient studies in protein-protein interactions, signal transduction, and related areas.

Kit Contents

B1511291	Component	20 T	Storage conditions	Quantity Per Test
B1511291A	Binding Buffer	50 mL	2-8°C	500 μL
B1511291B	TBS(10×)	10 mL	2-8°C	50 μL
B1511291C	Streptavidin Magnetic Beads	1 mL	2-8°C	40 μL
B1511291D	Elution Buffer	5 mL	2-8°C	50 μL
B1511291E	Neutralization Buffer	1 mL	2-8°C	5 μL
B1511291F	SDS-PAGE Loading Buffer(5×)	1 mL	-20°C	20 μL

Instruction for use

1. Biotinylation Reaction and Purification:

(1) Take an appropriate amount of the target protein to be labeled and dissolve it in 1× PBS to a final concentration of 0.2-2 mg/mL. If the solution contains primary amines (e.g., Tris or Glycine) or ammonium ions, it is highly recommended to perform desalting using a desalting column to ensure the accuracy and effectiveness of subsequent experiments.

(2) For biotin labeling and purification, it is recommended to refer to the instructions of the Aladdin Biotin Antibody Labeling Kit (Sulfo-NHS-LC-Biotin) (Product No. B1491943).

Note: If using the Avi-tag Protein Biotinylation Kit (BirA Method) (Product No. B1505408), please follow the instructions provided with that kit.

2. Binding of Streptavidin Magnetic Beads to biotin-labeled protein and other samples:

(1) Preparation of Streptavidin Magnetic Beads: Thoroughly invert and mix the Streptavidin Magnetic Beads. Transfer the required volume to a new 1.5 ml centrifuge tube. Typically, add 40 µl of the well-mixed Streptavidin Magnetic Beads per sample.

(2) Add Binding Buffer to a final volume of approximately 0.5 ml. Gently resuspend the Streptavidin Magnetic Beads by pipetting. Place the tube on a magnetic stand for 30 seconds, then carefully remove the supernatant (avoiding contact with the beads) to complete one wash step. Repeat the wash step once more as described, and finally remove the supernatant.

(3) Sample Binding: Add 50–200 µl of the biotin-labeled and purified sample. Typically, 50 µl of Streptavidin Magnetic Beads can bind 10–50 µg of protein, and it is not recommended to exceed 50 µg. Invert to mix and incubate with rocking on a tilting shaker for 2 hours.

(4) After incubation, place the tube on a magnetic stand for 1 minute. Retain the Streptavidin Magnetic Beads and collect the supernatant. The amount of known protein sample bound to the beads can be roughly determined by measuring the protein concentration in the supernatant or by SDS-PAGE analysis.

(5) Washing: Resuspend the Streptavidin Magnetic Beads in 500 µl of 1× TBS. Place the tube on a magnetic stand for 1 minute, then carefully remove the supernatant, avoiding touching the beads at the bottom. Repeat this step once. For pull-down of biotin-labeled complexes, proceed directly to the subsequent elution step.

3. Pull-Down of Target Proteins:

(1) Add 50–400 µl of sample lysate (or other samples containing the target protein) to the Streptavidin Magnetic Beads, and mix gently by pipetting.

Note: Select an appropriate lysis buffer for preparing cell or tissue lysates. It is recommended to use Aladdin's RIPA Lysis Buffer (Strong) (R1506976), RIPA Lysis Buffer (Medium) (R1505849), or RIPA Lysis Buffer (Weak) (R1505850) for sample preparation. If using self-prepared or other commercially available lysis buffers, ensure the pH is between 6 and 8. When lysing cells or tissues, be sure to add appropriate protease inhibitors to prevent protein degradation.

(2) Incubate at 4°C on a rocking shaker for at least 2 hours or overnight. Do not vortex.

Note: Higher binding capacity may require longer incubation times, which should be optimized for each protein. If the target protein is stable and the lysis buffer is compatible, incubation can also be performed at room temperature, but the duration should not be excessively long.

(3) After incubation, place the tube on a magnetic stand for 1 minute, and collect the supernatant for subsequent analysis. When aspirating the supernatant, avoid touching the beads at the bottom.

(4) Resuspend the Streptavidin Magnetic Beads in 500 µl of 1× TBS, place the tube on a magnetic stand for 1 minute, and carefully collect the supernatant for later use. Avoid touching the beads at the bottom. Repeat this step twice, for a total of three washes.

4. Elution of Complexes:

(1) Denaturing Elution: This method is suitable for samples to be analyzed by SDS-PAGE. Add 100 μL of 1× SDS-PAGE loading buffer to the washed Streptavidin Magnetic Beads containing the complexes and mix thoroughly. Heat at 95 °C for 10 minutes. After cooling, place the tube on a magnetic stand for 1 minute. Collect the supernatant for SDS-PAGE analysis.

(2) Native (Non-Denaturing) Elution: Resuspend the Streptavidin Magnetic Beads in 50 μL of Elution Buffer and gently shake on a rocking shaker for 5–10 minutes to elute the captured target proteins. Place the tube on a magnetic stand for 1 minute and collect the eluate, being careful not to touch the beads with the pipette tip. Repeat this elution step twice, for a total of three elutions. The collected eluates contain the target proteins. The first elution typically yields the highest concentration of complexes, while the last yields the lowest. Depending on experimental needs, either a single elution or a pooled sample may be used for downstream assays. Immediately add Neutralization Buffer to the eluate at a recommended volume ratio of Elution Buffer : Neutralization Buffer = 10 : 1. The eluted samples can be stored at 4 °C for short-term use or at –20 °C for long-term storage.

Notes: When Elution Buffer is used, streptavidin detachment may occur; therefore, the incubation time should not exceed 10 minutes.

Elution Buffer disrupts most antibody-antigen interactions. To ensure better elution efficiency, the beads may be pre-washed once with 300 μL of 0.1 % Tween-20 in water. If higher elution efficiency is required, acidic elution buffer (8 M Guanidine·HCl, pH 1.5) can be used. In this case, the pH or amount of the neutralization solution should be adjusted accordingly—for example, 100 μL of acidic elution buffer (8 M Guanidine·HCl, pH 1.5) may be neutralized with 15 μL of neutralization buffer (1 M Tris-HCl, pH 9.5).

Matters needing attention

1. Streptavidin Magnetic Beads should be maintained at pH 6–8 Avoid high-speed centrifugation and drying. Do not leave the beads in a magnetic field for extended periods, as this may cause bead aggregation.
2. Before use, thoroughly resuspend the Streptavidin Magnetic Beads by gently inverting the container several times to ensure uniform mixing. Handle gently; do not vortex or shake vigorously to prevent protein denaturation.
3. It is recommended to include negative and positive control groups to account for any nonspecific adsorption by the Streptavidin Magnetic Beads.
4. Binding efficiency can be influenced by factors such as the type and size of the molecule to be bound, as well as the method and extent of biotinylation. It is advisable to determine the optimal bead amount for each specific application using a gradient dilution approach. Additionally, consider using a 2 to 3 fold molar excess of beads relative to the target molecule to ensure sufficient binding.