His Pull-Down Kit (Agarose Beads)

H1506146

Storage: -20°C, 2-8°C and room temperature (For detailed storage conditions, please refer to the Kit Components).

Shipping: Shipped with ice packs. Upon receipt, please promptly store the product under the recommended storage conditions.

Introduction

The His Pull-Down Kit is designed for efficient co-sedimentation of tagged bait proteins and target proteins. It enables the isolation and purification of unknown captured proteins interacting with His-tagged bait proteins. The principle is straightforward: His residues are immobilized on agarose beads to form His-agarose resin. The bait protein is expressed as a His-fusion protein, which then binds to the His-agarose resin. If a captured protein that interacts with the bait protein is present in the sample, a complex of "agarose resin-His-bait protein-captured protein" will be formed, allowing the isolation and detection of the interacting captured protein.

This product utilizes high-quality His-tag protein agarose resin. The resin is prepared by immobilizing IDA ligands onto a high-rigidity agarose matrix via an ether linkage with a 9-atom spacer arm, followed by chelation with Ni^{2+} ions. The high-rigidity agarose matrix supports high flow rates and low backpressure. Its optimized pore structure also provides higher binding capacity compared to conventional agarose matrices. The kit features easy operation, strong binding capacity, and wide applicability. The storage temperature for components includes -20°C, 4°C and room temperature; for detailed storage information, please refer to Component Table 1.

Table1. Kit Components

H1506146	Components	Appearance	5T	25T	Storage	Quantity Per Test
H1506146A	Ni-IDA Agarose Resin	Pale blue homogeneous suspension	0.2 mL	1 mL	2-8°C, Do not freeze	40 μL
H1506146B	1×Lysis Buffer	Colorless clear liquid	5 mL	25 mL	2-8°C	150 μL per 250 μg sample
H1506146C	4M imidazole	Colorless Clear liquid	1 mL	5 mL	2-8°C	On request
H1506146D	10×Wash Buffer	Colorless Clear liquid	10 mL	50 mL	RT	0.5 mL
H1506146E	Protease Inhibitor Cocktail (100×)	Colorless clear liquid (RT)	0.05 mL	0.25 mL	-20°C	On request

Note: For conventional immunoprecipitation experiments, use 40 μL of chromatography media per 250 μg of sample.

Table 2. Performance of Ni-IDA Agarose Resin

Parameters	Specifications
Matrix	High-rigidity agarose
Ligand	$-N(CH_2COOH)_2$
Particle Size Range	75 μm (40-120 μm)
Ni^{2+} ion capacity	>40 μmol Ni^{2+}/mL
Binding capacity	25-35 mg His-tagged protein/mL wet gel (40 KD)
Compatibility	Avoid chelating agents (EDTA, citric acid, etc.) and strong reducing agents (DTT, etc.)
Storage	4-30°C in 20% ethanol

Procedure (Unless otherwise specified, all procedures should be performed at 4°C)

1 Reagent Preparation

Additional Required Materials:

(1) Reagents to be prepared by the user:

a) Primary Antibody: His tag antibody.

b) Secondary Antibodies: Goat Anti-Mouse IgG H&L (HRP), Goat Anti-Rabbit IgG H&L (HRP).

c) Other Reagents: BST, running buffer, transfer buffer, reducing SDS-PAGE gel, as well as the bait protein and target protein used for protein-protein interaction assays.

(2) Required Equipment:

Electrophoresis Apparatus, Transfer Apparatus, Imaging System

The above reagents, if required, can be ordered from Aladdin: His tag (C-terminal) Mouse mAb (Ab155835), Recombinant His tag Antibody (Ab086830), Goat Anti-Rabbit IgG H&L (HRP) (Ab176443), Goat Anti-Mouse IgG H&L (HRP) (Ab179001).

2 Solution Preparation

You may use the buffers provided in the kit, or prepare different buffer systems according to actual needs. It is recommended to filter all buffers through a 0.22 μm or 0.45 μm membrane filter before use. Buffers should be stored at 4°C. If any reagent appears cloudy, discard it immediately.

(1) Preparation of Elution Buffer: Prepare 1 mL of 250 mM imidazole solution. Specifically, add 62.5 μL of 4 M imidazole to 937.5 μL of 1×Wash Buffer.

(2) Prepare an appropriate amount of inhibitor-containing lysis buffer based on the proportion of using 100-200 μL of inhibitor-containing lysis buffer for lysis and 300-600 μL of inhibitor-containing lysis buffer for washing per 0.5-1 million cells. Mix Lysis Buffer and Protease Inhibitor Cocktail (100×) at a ratio of 100:1. For example, add 10 μL of Protease Inhibitor Cocktail (100×) to 1 mL of lysis Buffer to obtain 1 mL of inhibitor-containing lysis buffer

(Lysis Buffer with Protease Inhibitor Cocktail). The prepared inhibitor-containing lysis buffer should be placed on ice or at 4°C.

Note 1: Lysis buffer containing inhibitors should be prepared fresh immediately before use and is not suitable for frozen storage and later use.

Note 2: This lysis buffer is suitable for mammalian cells.

(3) Preparation of 1×Wash Buffer: Dilute the 10×Wash Buffer with deionized water at a ratio of 9:1 before use. For example: add 9 mL of deionized water to 1 mL of 10×Wash Buffer, mix well to obtain 1×Wash Buffer.

(4) Ni-IDA Agarose Resin Washing: Gently resuspend the Ni-IDA Agarose Resin to form a homogeneous gel suspension. Using 40 μL of well-mixed gel suspension per sample (all subsequent affinity precipitation steps use 40 μL gel suspension per sample as an example), transfer an appropriate amount of Ni-IDA agarose resin to a clean centrifuge tube. Add 0.5 mL of 1×wash buffer, centrifuge at 1250×g for 30 seconds at 4°C, discard the supernatant, and repeat this step twice.

Note: It is more convenient to aspirate the gel suspension using a wide-bore tip (e.g., by cutting off the end of a pipette tip with scissors).

3 Preparation of Bait Protein Samples (**Note**: Perform all sample lysis steps at 4°C or on ice)

(1) For His-tagged fusion protein expressed in *Escherichia coli*:

Grow and transform *E. coli* according to standard protocols. Transfer 5 mL of IPTG-induced *E. coli* culture to a sterile centrifuge tube. Centrifuge at 5000×g for 5 minutes and discard the culture supernatant. Resuspend the pellet in 1 mL of PBS buffer per 5 mL of original culture volume, and mix by pipetting or vortex. Transfer 1 mL of cell suspension to a 1.5 mL microcentrifuge tube. Centrifuge at 5000×g for 5 minutes and discard the supernatant. Resuspend the pellet in 200 μL of ice-cold PBS buffer per 5 mL of original culture volume, and mix by pipetting or vortex. Protease inhibitors may be added as desired; for optimal results, use a protease inhibitor cocktail during cell lysate preparation. Add 200 μL of pull-down lysis buffer per 5 mL of original culture volume, and immediately invert the tube until thoroughly mixed. Incubate on ice for approximately 30 minutes, inverting the tube periodically. Centrifuge at 12000×g for 5 minutes to clarify the crude *E. coli* lysate. Transfer the supernatant to a new microcentrifuge tube, store on ice, and label as “bait lysate”.

(2) For secreted His-tagged fusion protein:

Collect the culture supernatant and determine the bait protein concentration. If the bait protein concentration is high, dilute it to a final concentration of 500 μg/mL with 1×PBS for subsequent experiments.

(3) For pre-purified His-tagged fusion protein

Determine the bait protein concentration. If the bait protein concentration is high, dilute it to a final concentration of 500 μg/mL with 1×PBS for subsequent experiments.

4 Pull-Down Assay

(1) Immobilization of Bait Protein.

a) Add the bait protein obtained in Step 3 to the pre-washed gel, mix gently, and incubate on a shaker at 4°C for 3 h. Reserve a portion of the bait protein sample as an input control for later detection.

b) Separation by centrifugation. After incubation, centrifuge at 1250×g for 30 seconds at 4°C, transfer the supernatant to a new centrifuge tube, and store for analysis.

c) Transfer the complex in the centrifuge tube to a spin column, centrifuge at 1250×g for 30 seconds at 4°C, transfer the flow-through to a new centrifuge tube, and label as "bait flow-through" for later detection.

Note: For cell or bacterial lysate, add at least 500 µL of His-tagged fusion protein lysis buffer. For purified His-tagged fusion protein, use an adequate volume to ensure the addition of approximately 100-150 µg of bait protein.

d) Add 500 µL of 1×PBS to the spin column, invert gently several times to wash the gel, centrifuge at 1250×g for 30 seconds at 4°C, and discard the eluate. Repeat 4 times to obtain the bait protein-gel complex.

(2) Capture of Target Protein.

a) Add 800 µL of the prepared prey protein sample to the bait protein–gel complex. Incubate with gentle shaking on a shaker at 4°C for at least 3 hours or overnight. Reserve a portion of the prey protein sample as a prey input for later detection.

Note: Longer incubation times may be required to ensure sufficient binding between bait and prey proteins, and can be adjusted according to experimental conditions.

b) After incubation, centrifuge at 1250×g for 30 seconds at 4°C, transfer the flow-through to a new centrifuge tube, and label as "prey flow-through" for later detection.

c) Add 500 µL of 1×PBS to the spin column, invert gently several times to wash the gel, centrifuge at 1250×g for 30 seconds at 4°C, and discard the eluate. Repeat 4 times to obtain the bait-prey protein-gel complex.

5. Elution of Bait-Prey Protein Complex:

Elution can be performed using one of the following three methods, depending on the characteristics of the tagged protein and requirements of subsequent experiments.

(1) Non-Denaturing Elution

Pipette 100 µL of the prepared elution buffer into the washed resin, mix the resin and elution buffer thoroughly, then centrifuge and collect the eluate. If elution is inefficient, increase the glutathione concentration or perform multiple elution.

Note: If elution efficiency is unsatisfactory, the glutathione concentration may be increased or multiple elution steps may be performed.

(2) Peptide competitive elution

Add 100 µL of His-peptide elution buffer to every 40 µL of the original gel volume. Incubate the mixture on a vertical rotator for 5-10 minutes, then centrifuge at 1250×g and 4°C for 30 seconds. Aspirate the supernatant to obtain the eluted fraction, which contains the His-tagged protein and its complexes.

Note: If elution efficiency is unsatisfactory, the His-peptide concentration can be increased or

multiple rounds of elution can be performed. Once the gel beads are fully settled, transfer the supernatant to a new centrifuge tube for Western Blot analysis.

(3) Elution with SDS-PAGE Loading Buffer

For every 40 μL of original beads volume, add 50 μL of 1×PBS to resuspend the beads, followed by 50 μL of 2×SDS Loading Buffer. Mix gently by flicking the tube, then heat at 95-100°C for 5-10 minutes. Centrifuge at 1250×g for 30 seconds. Collect the supernatant for SDS-PAGE electrophoresis or Western Blot analysis.

Note: The recommended loading volume for WB is 20 μL or less. The remaining sample can be stored at -20°C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
No Bait Protein in Elution Fraction	Protein may be in inclusion bodies, not in the supernatant.	Check if the supernatant contains the target protein by analyzing the lysate via electrophoresis. Proteins in inclusion bodies require purification methods specific for inclusion bodies.
	Expression level is too low	Optimize expression conditions
	His tag is expressed but inactive	Optimize expression conditions
Non-specific bands are prominent	Insufficient washing during western blot	Increase the number of wash steps
Target Protein Not Captured	Insufficient amount of target protein in the sample	Verify protein expression or lysis efficiency by SDS-PAGE or Western Blot. Increase the amount of target protein to the recommended level
	Protein-protein interaction is too weak or non-existent	Optimize the Lysis/Was Buffer; Add co-factors required for the protein interaction.
	Protein degradation	Add protease inhibitors; For temperature-sensitive proteins, perform all operations at 4°C or on ice.

Important Notes

1. This product is intended for scientific research use by professionals only.
2. Unless otherwise specified, all steps are recommended to be performed at 4°C to minimize potential protein degradation. If cell lysis is insufficient, sonication may be used after adding the lysis buffer.
3. Ni-IDA Agarose Resin should be stored in the storage solution to prevent drying. Before

use, mix thoroughly by gently inverting the tube several times to ensure uniform suspension of the beads. Avoid vigorous vortex or shaking to prevent antibody denaturation.

4. Agarose Resin that has been boiled lose their binding capacity and should not be reused.
5. Reagent volumes can be adjusted according to experimental needs. It is recommended to perform preliminary experiments to validate immunoprecipitation conditions or optimize lysis parameters.
6. Please follow safety guidelines and adhere to laboratory reagent handling protocols.
7. The lysis buffer included in this kit already contains protease inhibitors. If specific needs arise, other appropriate inhibitor cocktails can be used.
8. After harvesting, protein samples should be purified as soon as possible and kept at 4°C or on ice at all times to minimize protein degradation and denaturation.
9. It is recommended to include positive and negative control groups during immunoprecipitation or purification procedures.