His-tag Protein Purification Kit (Non-Denaturant)

Introduction

His-tagged protein purification kit is a tool for the extraction of His-tagged recombinant proteins from cells or cell lysates. These kits typically contain affinity chromatography media, such as Ni-Agarose or Ni-NTA resins, that specifically bind to histidine residues on the His-tag. When using the His-tagged protein purification kit, the protein sample is passed through an affinity chromatography medium, the target protein is adsorbed, while the other proteins are not bound, and the target protein can be eluted off by changing buffer conditions after the impurities are removed by washing.

This product is a denaturable His-tagged protein purification kit, which contains the relevant reagents required for His-tagged proteins and the empty column tube of the affinity chromatography column, which brings great convenience to the purification of His-tagged proteins. This kit provides the lysate, wash, and eluate needed to purify His-tagged proteins under native conditions.

This product is sufficient for 10 purifications of His-tagged recombinant proteins, with a wet weight of approximately 1 gram per bacterial pellet. The maximum amount of protein purified per time (~55 kD) is 12-18 mg, and the specific maximum amount of purification depends on the molecular weight of the protein. Purification of a small amount of His-tagged protein can be performed in 500 sessions without the use of an affinity column empty column tube.

Compositions and storage

Size / Components	10 mL	Storage
His-tag Purification Resin	10 mL	4℃
Native Lysis Buffer	120 mL	4℃
Native Wash Buffer	60 mL	4℃
Native Elution Buffer	60 mL	4℃
Lysozyme	60 mg	4℃
Empty Affinity Chromatography Column (3 mL)	10 Sets	4℃
Shipping: Blue Ice	Shelf life: 12 months	

Protocol

The following operation takes the expression and purification of His-tagged recombinant protein by E.

coli as an example, and the expression samples in other systems can be used as a reference.

1. Inducible expression of His-tagged recombinant protein in Escherichia coli (taking the IPTG inducible expression system as an example):

   a. Monoclonal expressing the His-tagged recombinant protein was picked and inoculated into 3 mL or 10-20 mL LB medium with appropriate antibiotics overnight.

   b. According to the ratio of 1:20, inoculate the overnight bacterial solution into LB culture medium pre-warmed to 37 °C and containing appropriate antibiotics. For example, 5 mL of overnight culture was inoculated into 100 mL of LB culture pre-warmed to 37°C with appropriate antibiotics. The specific culture volume depends on the amount of protein that needs to be purified, and the initial identification culture is 3-10 mL. For routine expression purification, 100-200 mL of culture can usually be considered; For preparative purification, culture volumes can be up to 1 L or larger. If you want to achieve better expression, it is recommended to inoculate the overnight culture at a ratio of 1:100, but it will take longer to reach the corresponding OD value for subsequent cultures.

   c. Routinely incubate at 37°C for about 30-60 minutes or more until the OD_{600} reaches 0.5-0.7 and the OD600 is preferably close to 0.6.

   d. IPTG was added to a final concentration of 1 mM and the culture was continued for 4-5 h.

***Note:** A small amount of the solution can be removed before the addition of IPTG and incubated for 4-5 hours as an uninduced control, or a small amount of the solution can be taken directly before the addition of IPTG as an uninduced control. For the induction of expression of a particular protein, the optimal IPTG concentration, induction temperature, and induction time need to be determined experimentally.

   e. Collect the bacterial solution into a centrifuge tube, centrifuge at 4,000 g at 4°C for 20 min or 15,000 g at 4°C for 1 min, discard the supernatant, and collect the pellet. It can then proceed to the bacterial lysis step or can be frozen at -20°C or -80°C for later use. Cryopreserved bacteria should be thawed on ice for 15 minutes before use.

2. Mini-purification of His-tagged proteins under native conditions

This method is often used for the rapid analysis and identification of small samples, laying the foundation for subsequent large-scale preparation.

   a. Following step 1(e), centrifuge 1 mL of the bacterial pellet and discard the supernatant, add 100 µL of Native Lysis Buffer, and resuspend the bacterial pellet in the lysate sufficientlys, allowing for a slight vortex (to minimize bubbles). If necessary, add the appropriate amount of protease inhibitor cocktail to the lysate before lysing the bacteria.

***Note:** Depending on the abundance of His-tagged recombinant protein expression, the volume ratio of the bacterial solution to the lysate can be adjusted appropriately in the range of 25:1-5:1. When the expression abundance is very high, 200 µl of lysate can be added per milliliter of bacterial solution pellet; At very low expression abundances, 40 µl of lysate can be added per milliliter of

bacterial solution pellet. The preparation method of the relevant solution is attached. This Native Lysis Buffer ensures that the vast majority of soluble and inclusion body proteins are lysed and can be used directly for SDS-PAGE. If necessary, add the appropriate amount of protease inhibitor cocktail to the lysate before lysing the bacteria.

b. Add Lysozyme to 1 mg/mL and mix gently, trying to avoid bubbles, in an ice water bath or on ice for 30 minutes.

***Note:** Lysozyme can be formulated as a 100 mg/mL mother liquor with Native Lysis Buffer, which is added before use. After making the mother liquor, it can be stored at -20°C after appropriate aliquots.

c. Gently vortex a few times to fully lyse the bacteria and try to avoid bubbles.

d. Centrifuge at 4°C (15,000 g×10 min), take 10 μL of the retained supernatant sample for subsequent testing, and collect the remaining supernatant into a new clean centrifuge tube.

e. Add 20 μL of mixed 50% His-tag Purification Resin and shake slowly for 30 minutes at 4°C on a shaker to fully bind the His-tagged protein of interest.

***Note:** Slow shaking for 30 minutes can already ensure that the protein is fully bound, but it can be shaken slowly for longer or even overnight according to the needs of the schedule. It has been tested to achieve good purification results with 50% His-tag Purification Resin. However, if you want to achieve higher yields of tagged proteins, you can equilibrate His-tag Purification Resin 2-3 times beforehand with one column volume of Native Lysis Buffer. After equilibration, depending on the protein to be purified, the yield of the protein to be purified may be increased by about 5-20%.

f. Centrifuge (1000 g ×10 s) at 4°C to precipitate the gel, take 20 μL of the supernatant for subsequent detection, and discard the rest of the supernatant.

g. Add 100 μL Native Lysis Buffer resuspension gel, centrifuge at 4℃ (1000 g × 10 s), take 20 μL supernatant retention sample for subsequent detection, and discard the rest.。

h. Repeat step 2 (g) with one more wash.

i. Add 20 μL Native Elimination Buffer and gently re suspend the gel. Centrifuge at 4℃ (1000 g × 10 s), collect the supernatant and gel. The supernatant is the purified target protein with His tag.

j. Repeat step i twice. Approximately 60 μL of purified protein samples were collected by co-elution.

3. Bulk purification of His-tagged proteins under native conditions

a. Following step 1(e), for fresh or thawed bacterial pellets, add 4 mL (2-5 mL) of Native Lysis Buffer per gram of bacterial pellet wet weight to resuspend the cells thoroughly. If necessary, add the appropriate amount of protease inhibitor cocktail to the lysate before lysing the bacteria.

***Note:** **The** dosage of subsequent reagents is 1 gram of bacterial weight and 4 ml of lysate.

b. Lysozyme was added to a final concentration of 1 mg/mL and mixed well, and placed in an ice water bath or on ice for 30 minutes.

***Note:** Lysozyme can be prepared as a 100 mg/mL mother liquor with lysate to be added immediately prior to use. After making the mother liquor, it can be stored at -20°C after appropriate aliquots.

c. Sonication of bacteria on ice. The ultrasonic power was 200-300 W, and each sonication was 10 s, with an interval of 10 s, and a total of 6 sonications.

***Note:** The specific method of sonication must be explored and optimized according to the specific model of ultrasound instrument.

d. If the lysate is very viscous after sonication, RNase A to 10 μg/ml and DNase I to 5 μg/mL can be added and placed on ice for 10-15 minutes. Alternatively, a syringe with an appropriate thinner needle can be used and repeated several times to cut viscous genomic DNA, etc.

e. Centrifuge at 10,000 g at 4°C for 20-30 min, collect bacterial lysate supernatant and place in an ice water bath or on ice. 20 μL of supernatant can be taken for subsequent testing.

***Note:** The supernatant must be clear, i.e., free of any insoluble matter, in order to proceed to the next step of purification. If insoluble impurities are mixed in the supernatant, the purity of the protein obtained in subsequent purification will be seriously affected.

f. Take 1 mL of evenly mixed 50% His tag Purification Resin, centrifugate at 4℃ (1000 g ×10 s) to discard the storage solution, add 0.5 mL of Native Lysis Buffer to the gel, mix well to balance the gel, centrifugate at 4℃ (1000 g ×10 s) to discard the liquid, and then repeat the balance 1-2 times to discard the liquid. Add about 4 mL of bacterial lysate supernatant and slowly shake on a side or horizontal shaker at 4℃ for 60 minutes.

g. Load the mixture of lysate and His-tag Purification Resin into the affinity column empty column tube provided with the kit. Note: You can also take 1ml of 50% BeyoGold™ His-tag Purification Resin (denaturing resistant dosage form) mixed evenly first, then equilibrate with 0.5ml of non-denaturing lysate for 2-3 times and then add about 4ml of bacterial lysate to supernatant, and then you can collect the permeate and repeat the column 3-5 times to fully bind the target protein. It is more troublesome to mix first and then load the column, but it is more conducive to the full binding of the Recombinant Protein with His tag to the nickel column, especially when the His-tag is partially blocked by the protein itself or the concentration of the His-tagged recombinant protein is very low.

h. The lid at the bottom of the cartridge was opened, and the liquid in the column was drained out under the action of gravity, and about 20 μL of the flow-through fluid was collected for subsequent analysis.

i. The column was washed 5 times, and 0.5-1 mL of Native Wash Buffer was added each time, and approximately 20 μL of the through-column wash solution was collected each time for subsequent analysis and detection. The Bradford method can be used to quickly and easily measure the protein content in each wash and eluate during column washing and the next step of elution, allowing for consideration of increasing or decreasing the number of washes and elutions.

***Note:** If the purity of the protein obtained is not high enough, the number of column washes can be increased by 2-3 times.

j. Elute the protein of interest 5-10 times with 0.5 ml Native Elution Buffer. Collect each eluate separately into a different centrifuge tube. The resulting eluate is a sample of the purified His-tagged protein.

Notes

1. Do not freeze His tag Purification Resin at -20℃ or lower, and keep it moist during storage and purification processes.
2. During the use of His-tag Purification Resin, the concentration of buffer reagents such as Tris, HEPES, MOPS, etc. should not exceed 100mM, the concentration of SDS and sarkosyl should not exceed 0.3%, the concentration of Triton, Tween, NP-40 should not exceed 2%, the concentration of sodium deoxycholate and CHAPS should not exceed 1%, the concentration of histidine should not exceed 20mM, and the concentration of calcium ions should not exceed 5mM. Sodium and magnesium ion concentrations can be as high as 2M, guanidine hydrochloride concentrations can be as high as 6M, urea concentrations can be as high as 8M, and glycerol concentrations can be as high as 50%. This product cannot tolerate reducing agents, chelating agents, and strong acids and bases. The compatibility of other reagents not mentioned can be referred to the above reagents, but it has yet to be verified experimentally.
3. In many cases, native conditions should be preferred for cleavage of the protein of interest. If it is found that the cleavage effect of the target protein under non-denaturing conditions is not good, but the expression level of the target protein can reach the expected level, it is advisable to first consider adjusting the induced expression conditions of the target protein, such as adjusting the concentration of inducers such as IPTG and the temperature during induction. If the lysis effect is still not good under non-denaturing conditions after adjusting the induction conditions, it is advisable to consider selecting denaturing conditions for lysis, washing, and elution.
4. If centrifugation does not completely remove the insoluble matter of the protein sample, the sample solution can be filtered through a 0.45 μm filter.
5. Purification of protein samples should be completed as soon as possible after collection and should always be placed at 4°C or in an ice bath to slow down protein degradation. To effectively inhibit protein degradation, an appropriate amount of protease inhibitor cocktail can be added to the lysate.
6. If the conditions provided in this manual do not achieve the desired purification results, try changing the concentration and/or pH of imidazole in the wash and eluent to achieve the best results.
7. If the purification of the target protein requires the use of chelating or reducing agents, it is recommended to use the His tag Protein Purification Kit (Reductant and Chelator Resistant) (K4206).
8. This product is for scientific research purposes 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