His-tag Protein Purification Kit (Denaturant)

Introduction

The 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 eluent needed to purify His-tagged proteins under denaturing conditions, and contains 8 M urea for effective protein denaturation and solubilization. The denaturation conditions have good solubility, and the eluted samples can be directly detected by PAGE. The final purified protein of interest can be used for specific purposes after urea can be removed by dialysis.

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 (~55 kDa) is 12-18 mg per purification, and the specific maximum purification amount is related to 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.

Components and Storage

Components \ Size	10 mL	Storage
His-tag Purification Resin	10 mL	4℃
Denaturing Lysis Buffer	120 mL	4℃
Denaturing Wash Buffer	60 mL	4℃
Denaturing Elution Buffer	60 mL	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 proteins expressing the His-tagged recombinant protein were picked and inoculated into 3 mL or 10-20 mL of 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 OD_{600} 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 denaturing 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), 1 mL of the bacterial pellet was collected by centrifugation, the supernatant was discarded, and 100 μL of lysate (Denaturing Lysis Buffer) was added , resuspend the bacterial pellet well in the lysate, and perform a slight vortex (try to avoid 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 in the range of 25:1-5:1, for example, when the expression abundance is very high, 200 μL of lysate can be added per milliliter of bacterial precipitate, and when the expression abundance is very low, 40 μL of lysate can be added per milliliter of bacterial precipitate. This Denaturing Lysis Buffer ensures lysis of the vast majority of soluble and inclusion body proteins. If necessary, an appropriate amount of protease inhibitor cocktail (e.g., Protease Inhibitor Cocktail for bacterial cell extracts (100 X in DMSO) (Cat. #: K1024) protease inhibitor cocktail (for bacterial extraction) can be added to the lysate prior to lysing bacteria, or Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO) (Cat. #: K1007) or Protease Inhibitor Cocktail (EDTA-Free,100X in DMSO) (Cat. #: K4002) Protease inhibitor cocktail (general-purpose).

b. Ultrasonic lysis of bacteria on ice: ultrasonic power 200-300 W, 10 s each sonication, 10 s interval between each time, a total of 6 sonications. The specific method of ultrasonic treatment must be explored and optimized according to the specific model of ultrasound instrument.

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

d. Add 20 μL of well-mixed 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, depending on the schedule. Positive purification results have been tested directly with premixed His-tag Purification Resin, but if higher yields of tagged proteins are desired, His-tag Purifiction Resin can be equilibrated 2-3 times prior with one column volume of Denaturing Lysis Buffer(Refer to 3.e), the yield of the protein to be purified is likely to increase by about 5-20%.

e. Centrifuge (1,000 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.

f. Add 50 μL of Denaturing Lysis Buffer to resuspend the gel, centrifuge at 4°C (1000 g×10 s), take 20 μL of the supernatant for subsequent detection, and discard the remaining supernatant.

g. Repeat step 2 (f) for one more wash.

h. Add 20 μL of Denaturing Elution Buffer and gently resuspend the gel. Centrifuge at 4°C (1,000 g×10 s) and collect the supernatant and gel. The supernatant is the purified His-tagged protein of interest.

i. Repeat step 2 (h) twice. Approximately 60 μL of purified protein samples were collected by co-elution.

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

a. In step 1(e), for fresh or thawed bacterial pellets, 80 mL of Denaturing Lysis Buffer was added to the lysate per 1 L of expressing bacteria to resuspend the cells thoroughly.

b. Ultrasonic lysis of bacteria on ice: ultrasonic power 200-300 W, 2 s for each sonication, 2 s interval between each time, a total of 15-30 min for sonication.

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

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

***Note:** Ribonuclease A (RNase A) can be selected from Ribonuclease A (bovine pancreas) (Cat. #: A5983), Ribonuclease A (10 mg/mL) (Cat. #: K3001), or Ribonuclease A (100 mg/mL) (Cat. #: K3002). DNase I can be selected as DNase I (RNase-free) (Cat. #: K1088). Follow the manufacturer's instructions for specific usage methods.

d. 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.

e. Take an appropriate amount of His-tag Purification Resin, centrifuge at 4°C (1,000 g×10 s) to discard the stock solution, add a column volume of Denaturing Lysis Buffer to the gel to mix well to equilibrate the gel, centrifuge at 4°C (1000 g×10 s) to discard the liquid, repeat the equilibration 1-2 times, and discard the liquid.

f. Mix His-tag Purification Resin and bacterial lysate supernatant at a ratio (1:8) of 4 mL of bacterial lysate supernatant per 0.5 ml gel. Shake slowly at 4°C on a side swing shaker or horizontal shaker for 60 min.

g. Transfer the mixture of lysate and His-tag Purification Resin into an Empty Affinity Chromatography Column (3 mL).

***Note:** 1. Steps 3.f and 3.g can also be combined as follows: Empty Affinity Chromatography Column (3 mL) is loaded with His-tag Purification Resin first, then equilibrate with 1 column volume of Denaturing Lysis Buffer, add bacterial lysate to supernatant, and finally collect the flowthrough and repeat the column 3-5 times to fully bind the protein of interest. The method of mixing first and then loading the column (3.f and 3.g) is relatively cumbersome, but it is more conducive to the full binding of His-tagged recombinant proteins to nickel columns. 2. The Empty Affinity Chromatography Column (3 mL) can be added 3 mL at a time, and 80 mL of sample needs to be added multiple times to achieve complete binding; Other large empty column tubing formats such as 6 mL (Cat. #: PC2053), 12 mL (Cat. #: PC2054), 30 mL (Cat. #: PC2055), 60 mL (Cat. #: PC2056) can be used and other specifications of products.

h. The lid at the bottom of the cartridge was opened, gravity (no centrifugation required) was used to allow the liquid to flow out of the column, and approximately 20 μL of permeate was collected for subsequent analysis.

i. The column was washed 5 times, 0.5-1 mlof lysate was added each time, and about 20 μL of transcolumn-penetrating lysate was collected each time for subsequent analysis and detection. Coomassie Brilliant Blue can be used to quickly and easily measure the protein content in each wash and eluate during column washing and subsequent elution, so that the number of washes and eluents

can be increased or decreased.

***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. Wash the column 5 times again, add 0.5-1ml of Denaturing Wash Buffer each time, and collect about 20 μL of the column wash solution for subsequent analysis and detection.

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

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

Note

1. His-tag Purification Resin should not be frozen at -20°C or lower and kept moist during storage and purification.
2. During the use of His-tag Purification Resin, the concentration of buffer reagents such as Tris, HEPES, MOPS, etc. should not exceed 100 mM, 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 20 mM, and the concentration of calcium ions should not exceed 5 mM. Concentrations of sodium and magnesium ions can be as high as 2 M, guanidine hydrochloride concentrations can be as high as 6 M, urea concentrations can be as high as 8 M, 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. 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.
4. 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.
5. 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.
6. If chelating or reducing agents must be used for purification of the protein of interest, the His-tag Protein Purification Kit (Reductant and Chelator Resistant) (K4206) is recommended.
7. This product is for scientific research purposes only.

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