His label protein purification kit instructions

Item No. : P2012
Specification: 5T/10T
Storage: Store the reagent at room temperature and Ni-Agarose medium 2-8℃.

Product composition:

Components	Packaging (5T)	Packing (10T)	Save
Ni-Agarose media (never freeze!)	5mL	10mL	4 ℃
Non-denaturing lysate (**Binding Buffer A**)	70mL	140mL	Room temperature
Non-denaturing eluent: 500mm imidazole (Buffer 1)	70mL	140mL	Room temperature
Non-denaturing diluent (Buffer **2**)	85mL	170mL	Room temperature
Preserving liquid	50mL	100mL	Room temperature
Affinity chromatographic column empty column tube	5mL*5 of them	5mL*10	Room temperature

Components	Packaging (5T)	Packing (10T)	Save
Inclusion body solution (**Binding Buffer B**)	70mL	140mL	Room temperature
Inclusion body eluent: 500mm imidazole **(Buffer 3)**	70mL	140mL	Room temperature
Inclusion body diluent (Buffer **4**)	85mL	170mL	Room temperature

Product Description:

This kit is based on Ni affinity chromatography protein purification method for His label protein purification kit; It can be used under both denaturing and non-denaturing conditions, and can withstand high concentration denaturing agents (8M urea and 6M guanidine hydrochloride). It is suitable for purifying His labeled inclusion body proteins with high load (purification medium load is 20-30mg His labeled protein /mL medium), and the elution sample can be directly used for SDS-PAGE gel electrophoresis.

Prepare your own reagent:

1*PBS

Here's how:

First, install the column

1. After mixing Ni-Agarose medium well, take appropriate amount and add to the chromatographic column with a sieve plate pre-placed.

Note: The amount of medium needs to be determined by the protein yield of His label, please take appropriate amount of Ni-Agarose medium and add it to the chromatographic column as required. (10-20mL expression bacterial solution, it is recommended to add 500uL filler volume; 30-50mL expression bacterial solution, it is recommended to add a filler volume of 1mL; The total volume of filler is not recommended to exceed 1mL, to avoid column blockage).

2, wash the column with 4 times the column volume of 1*PBS. (or purified water is fine)

2. Sample treatment (50mL expression bacterial solution as an example)

Centrifuge at 12000rpm, 4℃, for 20min, collect 50mL expression bacterial solution, discard supernatant; Resuspension with 1*PBS, centrifugation at 4℃ for 20min, precipitation placed on ice for use or -80℃ for reserve.

1. 10mL of non-denatured cracking solution (Binding Buffer A) was precipitated and re-suspended for ultrasonic crushing. (It is recommended to add PSMF protease inhibitor)
2. Centrifuge at 12000rpm for 20min at 4℃.
3. Collect supernatant and precipitation respectively. His labeled soluble protein was in the supernatant, and the supernatant was over 0.45μM filter membrane to prevent blocking the column. His labeled inclusion body proteins were in the precipitation and placed on ice for later use or stored at -20℃.

Iii. Purification

A. Purification of His labeled soluble protein

1. Put the supernatant obtained in the previous step on the column, after the liquid is filled with the medium, put the lid on the bottom of the chromatographic column, let the bacterial lysate and medium combine at room temperature for 20min (if you want to obtain more target proteins, the bacterial lysate and medium can be combined at 4℃ overnight), remove the lid, and let the lysate flow out naturally under natural gravity.
2. The column was washed with Binding Buffer A (4 times the column volume). (The penetrating solution can be collected and preserved, and the unadsorbed His labeled proteins can be detected by SDS-PAGE electrophoresis).
3, configure eluents containing different concentrations of imidazole, and dilute the non-denaturing eluent (Buffer 1) with the non-denaturing diluent (Buffer 2) into eluents with different concentrations of imidazole to determine the most appropriate imidazole concentration of the washing solution for His label proteins. Wash and collect samples at 3-5 times column volume in order from low to high. SDS-PAGE electrophoresis detects the area where His labeled proteins are located. Recommended imidazole concentration gradients are: 30, 100, 200, 300, 500 mM.
4. After elution, wash the column with purified water of 10 times the column volume, balance with preservation solution of 5 times the column volume, and finally block the leak at the lower end of the chromatographic column, and store at 4℃ for next use.

B, His labeled inclusion body protein purification

1. The inclusion body was rehung twice with 1* PBS and washed. The inclusion body precipitates were re-suspended with 5mL of Inclusion body solution (Binding Buffer B) and placed in a shaker at 37℃ for 1 h to fully dissolve the inclusion bodies. (4℃ overnight is better; If there is undissolved precipitation, it is recommended to dissolve 6M guanidine hydrochloride)
2. Centrifuge at 12000rpm for 20min at 4℃ and pass the dissolved supernatant through a 0.45μM filter.
3. Put the filter liquid on the column, after the liquid is filled with the medium, cover the bottom end of the chromatographic column, let the bacterial lysate and medium combine at room temperature for 20min (if you want to obtain more target proteins, the bacterial lysate and medium can be combined at 4℃ overnight), remove the lid, and let the lysate flow out naturally under natural gravity.
4. Wash the column with 3-5 times the column volume inclusion body washing solution (Buffer 3). (The penetrating solution can be collected and preserved, and the unadsorbed His labeled proteins can be detected by SDS-PAGE electrophoresis)
5, configure eluents containing different concentrations of imidazole, and dilute the inclusion body eluent (Buffer 3) into different concentrations of imidazole with the inclusion body diluent (Buffer 4) to determine the optimal imidazole concentration of the washing solution for His label proteins. In the order from low to high
3-5 times column volume wash and collect samples, SDS-PAGE electrophoresis detects the area where His labeled proteins are located. Recommended imidazole concentration gradients are: 30, 100, 200, 300, 500mM.
4. After elution, wash the column with purified water of 10 times the column volume, balance with preservation solution of 5 times the column volume, and finally plug
The leak at the lower end of the chromatographic column is stored at 4℃ for next use.

Packing cleaning: see product "P2010 Nickel-agarose gel 6FF", the cleaning and regeneration methods are the same.

Precautions:

1. Ni-Agarose medium mixed before use and stored in a 4℃ refrigerator.
2. Protease inhibitors are purchased separately on demand, item number P6735.

Related products:

P2010	*Nickel-Agarose gel 6FF*
P6735	*Protease Inhibitor mixture (His-Tag protein purification, 100X)*
YA2410	*Ni NTA Sepharose 6FF*
P1300	*Coomassie Brilliant Blue Quick Dyeing Solution*
PR1910	*Rainbow 180 Broad Spectrum Protein Marker (11-180KD)*
P1200	*SDS-PAGE gel preparation kit*