



His-tag Protein Purification Kit (NTA-Ni Gel Method) PRODUCT DATA SHEET

His-tag Protein Purification Kit (NTA-Ni Gel Method)

Cat No: AKIT-His-NTA-Ni-2

Description

His-tag Protein Purification Kit (NTA-Ni Gel Method) is composed of His-tag protein agarose gel and optimized prefabricated buffer and 2 empty chromatographic column sets for purification of His-tag recombinant proteins expressed by fusion of various expression systems.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com **Phone:** +1 929-202-3014 **Email:** info@abvigenus.com

Kit Composition

His-tag protein agarose gel (NTA-Ni)	10 mL (50% v/v)
Binding/balancing buffer (2 ×)	100 mL
Washing buffer (5 ×)	100 mL
Elution buffer	100 mL
Protein quick stain solution	50 mL
SDS-PAGE Sample Loading buffer (5 ×)	10 mL
Affinity chromatography column empty column set	2 sets

Operation Process

Dilute the binding/balancing buffer (2 ×) and wash buffer (5 ×) to 1 × using ultra-pure water before the experiment.

1. Sample preparation

Take Escherichia coli expression system, 500 mL induced bacterial solution as an example.

- 1) Centrifuge at 4°C for 30 min (4000 x g) to collect bacteria and discard supernatant.
- 2) The bacteria are suspended with a pre-cooled **binding/balancing buffer (1 ×)**, with the addition of appropriate inhibitors, such as protease inhibitors (PMSF) or other protease inhibitors, if needed.



Note: The added inhibitors should not affect the performance of **His-tag protein agarose gel**, and the crushing solution should not contain EDTA, EGTA and other chelating agents, DTT, mercaptoethanol and other reducing agents, urea, guanidine hydrochloride and other denaturants.

3) Use ultrasonic crushing method to break the bacteria on the ice until the sample is broken completely.

Optional: If the lysate is too thick, RNase A (final concentration 10 µg/mL) and DNase I (final concentration 5 µg/mL) can be added and incubated on ice for 10 ~ 15 min.

4) Centrifuge at 4°C for 20 min (12,000 x g), separation of supernatant and precipitation, and filtration for impurity removal. Retain samples of supernatant and precipitation for subsequent testing.

2. Purification of recombinant His-tag fusion protein

1) Gently resuspension **His-tag protein agarose gel (NTA-Ni)**.

2) Absorb 2 mL of **His-tag protein agarose gel (NTA-Ni)** and add it to the chromatographic column, and balance **His-tag protein agarose gel (NTA-Ni)** with 10 mL **binding/balancing buffer (1 ×)**. Repeat the preceding steps one more time.

3) Close the bottom outlet of the chromatographic column, add the prepared supernatant containing His-tag protein to the chromatographic column, and then cover the upper inlet of the chromatographic column tightly, and it is recommended to seal with a sealing film. Place on a mixing machine and incubate at room temperature for 1 ~ 2 h. (Can also be incubated at 2 ~ 8°C for 2 ~ 4 h or overnight).

4) After the end of incubation, open the upper and lower inlet and outlet of the chromatographic column, and collect the supernatant after all the supernatant flows out of the chromatographic column, as a flow through, and place it at 2 ~ 8°C for subsequent detection.

5) Immediately add 10 mL **washing buffer (1 ×)** to the chromatographic column, collect the detergent and place it at 2 ~ 8°C for subsequent detection. Repeat the preceding steps four times.

6) Add 1 mL **eluent buffer** and collect the eluent with 1.5 mL Ep tube. Collect 5 to 10 tubes respectively.

7) SDS-PAGE test

The resulting sample (including run-off, washing solution and eluent) and the original sample were tested for purification using SDS-PAGE. Add an appropriate amount of **protein fast dyeing solution** to immerse the PAGE glue, and then shake it on a shaking table. The results can be observed after dyeing for 10 ~ 30 min.



Note: Before storage, the target protein should be dialyzed or ultrafiltration to remove impurities such as imidazole, and then packaged and frozen at -80°C.

(Optional) Gel Regeneration and Storage

Gel regeneration steps please refer to or directly buy our company His-tag protein purification regeneration kit.

After the gel is regenerated, it can be used immediately. If it is not used immediately, it is necessary to suspend the gel in an equal volume of 20% ethanol and store it at 2 ~ 8°C.

Problem Solving

Problem	Reason	Solution
There was no target protein in the eluent	The protein may be an inclusion body, but the supernatant has no protein	The lysate can be detected by electrophoresis to determine whether the supernatant contains the target protein, and the inclusion body protein needs to be purified according to the inclusion body protein.
	Underexpression	Optimize expression conditions.
	The target protein is weakly bound and washed off during the scrubbing step	Reduce imidazole concentration.
	The target protein is degraded by protease	Add appropriate protease inhibitors, such as PMSF, to the cleavage step or washing step.
	The target protein cannot be effectively eluted from the gel	Increase the imidazole concentration.
		10 ~ 100 mM EDTA solution was used to peel nickel ions

		and obtain the target protein.
The purified target protein is not pure	The laundry is not thorough	Increase washing times.
	The sample contained other histidine label proteins	The cleaning conditions were optimized by adjusting the concentration of imidazole. The elution components are then further purified by using other purification methods (such as ion exchange, hydrophobic, etc.).
Protein precipitation occurs during binding	Concentration is too large	Moderately diluted protein.
	Protein aggregation	Add a stabilizer, such as 0.1% Triton X-100 or Tween20, to the sample and all buffers.
	Operating temperature is too high	Operate at 2-8°C.

Ordering Information

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