His-tag Protein Purification Kit (Inclusion Body Protein)

Cat No: AKIT-His-NTA-Ni-1

Description

His-tag Protein Purification Kit (Inclusion body protein) is composed of His-tag protein agarose gel and optimized prefabricated buffer and 2 empty chromatographic column sets for the purification of His-tag inclusion body proteins. Eluted samples can be directly used for SDS-PAGE electrophoresis, and the final sample needs to be denaturated for specific purposes.

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)
Denatured cracking/bonding solution	120 mL
Denatured washing solution	120 mL
Denatured eluent	120 mL
Protein quick stain solution	50 mL
PMSF (100 ×)	1 mL
SDS-PAGE Sample Loading buffer (5 ×)	10 mL
Affinity chromatography column empty column set	2 sets

Operation Process

This kit is only suitable for inclusion body protein purification, please confirm the solubility of the protein before the experiment.

1. Sample preparation

Take Escherichia coli expression system, 1000 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) Resuspend the bacterial cells in pre-cooled 1 × PBS, and if necessary, add an appropriate amount of inhibitor such as protease inhibitor (PMSF) or other protease inhibitors.

Note: The added inhibitors should not affect the performance of **His-tag protein agargo gel (NTA-Ni)**, and the crushing solution should not contain EDTA, EGTA and other chelating agents, DTT, mercaptoethanol and other reducing agents.

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), separate the supernatant and precipitation, collect the precipitation, and the precipitation is the inclusion body.

5) The inclusion body was pre-cooled with 1×PBS, washed, centrifuged at 4°C for 20 min (12,000 x g), and repeated once.

6) Weigh 0.5 ~ 0.8 g inclusion weight and suspend it in 8 mL **denaturing cracking/binding solution**, place it on a rotating mixing machine, dissolve it for 1 ~ 2 h to fully dissolve the inclusion body (or 2-8 degrees, dissolve it overnight).

7) Centrifuge at 4°C for 20 min (12,000 x g) and collect the supernatant. It is recommended to filter the supernatant with 0.22 μm or 0.45 μm filter membrane after centrifugation. Supernatant waiting.

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 2 mL **denature-cracking/binding solution**. Repeat the preceding steps one more time.

3) Close the bottom outlet of the chromatographic column, add the prepared protein supernatant containing His-tag inclusion body 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 ~ 2h. (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 5 mL **denatured detergent** to the chromatographic column, collect the detergent, and place it at 2 ~ 8°C for subsequent detection. Repeat the preceding steps two times.

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

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.

Notes

1. After purification, the inclusion body protein can carry out the next protein renaturation operation.

2. Protein renaturation methods generally include dilution renaturation, dialysis renaturation, ultrafiltration renaturation, and column renaturation for selection.

3. Factors affecting the renaturation efficiency of inclusion bodies: the properties of the protein itself, temperature, protein concentration, pH of the renaturation buffer, the initial concentration and removal rate of the denaturant, redox potential, ionic strength, the presence or absence of cosolvents and other additives, etc. Comprehensive consideration is needed to ensure successful renaturation of inclusion bodies.

(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 clean the gel and suspend it in 20% ethanol of the same volume and store it at 2 ~ 8°C.

Problem Solving

Problem	**Reason**	**Solution**
There was no target protein in the eluent	The protein may be soluble, without inclusion body proteins	The lysate can be analyzed for inclusion body proteins by electrophoresis, and soluble proteins need to be purified as soluble proteins.
	There are problems with the	Sequencing check gene

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

	gene sequence	sequences.
	Underexpression	Optimize expression conditions.
	The target protein is weakly bound and washed off during the scrubbing step	The washing solution can be prepared to reduce the imidazole concentration or increase the pH.
	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.
		The nickel ions were exfoliated with EDTA solution of 10 ~ 100 mM and the target protein was obtained.
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.) after denaturation.
Protein precipitation occurs during binding	Concentration is too large	Moderately diluted protein.
	Protein aggregation	Add a stabilizer, such as

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		0.1% Triton X-100 or Tween20, to the sample and all buffers.
	Low operating temperature	Can be operated at room temperature.

Ordering Information

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