

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

His-tag Protein Purification Kit (Gel Method)

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

Description

His-tag Protein Purification Kit (Gel Method) is composed of His-tag protein agarose gel and optimized prefabricated buffer and 2 empty chromatographic column sets for the 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 (IDA-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 \times) and wash buffer (5 \times) to 1 \times 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 agargo 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 μ g/mL) and DNase I (final concentration 5 μ g/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.

2) Absorb 2 mL of **His-tag protein agarose gel** and add it to the chromatographic column, and balance **His-tag protein agarose gel** 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 $1 \sim 2h$. (Can also be incubated at $2 \sim 8^{\circ}C$ for $2 \sim 4$ 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 $2 \sim 8^{\circ}$ C for subsequent detection.

5) Immediately add 10 mL washing buffer $(1 \times)$ 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 elution buffer 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 $10 \sim 30$ min.

Note: The target protein should be dialysis or ultrafiltration to remove imidazole and other impurities before storage, and then subpackaged and frozen to -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 not used immediately, it is necessary to suspend the gel in an equal volume of 20% ethanol and store it at $2 \sim 8^{\circ}$ C.

Prob	lem	So	lving
1100	CIII	50	6

Problem	Reason	Solution
There was no target protein	The protein may be an	The lysate can be detected
in the eluent	inclusion body, but the	by electrophoresis to
	supernatant has no protein	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	Reduce the imidazole
	bound and washed off	concentration.
	during the scrubbing step	
	The target protein is	Add appropriate protease
	degraded by protease	inhibitors, such as PMSF, to
		the cleavage step or washing
		step.
	The target protein cannot be	Increase the imidazole
	effectively eluted from the	concentration.
	gel	The nickel ions were
		exfoliated with EDTA
		solution of 10 $^{\sim}$ 100 mM and
		the target protein was



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

Ordering Information

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