His-tag Protein Purification Gel, 45-135 μm-NTA-Co

Cat No: AG-His-4500-NTA-Co

Description

His-tag protein agarose gel (NTA-Co) can be used for the purification of histidine labeled (6xHis-tag) proteins from various expression sources, such as E. coli, yeast, insect cells, and mammalian cells. It is made from a highly cross-linked 4% agarose gel as a matrix chemically coupled to tetra-coordinated nitrogen triacetic acid (NTA). After chelating cobalt ions (Co^{2+}), a relatively stable planar quadrilateral structure can be formed, so that more sites can continue to coordinate with the imidazole ring on the histidine label to achieve the effect of binding the target protein.

Characteristics

Matrix	Highly crosslinked 4% agarose gel
Ligand	Triacetic acid (NTA)
Chelating metal ions	Co^{2+}
Particle size	45~135 μm
Concentration	The gel volume accounts for 50% of the suspension volume
Pressure flow rate	80 ~ 150 cm/h (0.3 MPa, 3 bar)
Carrying capacity	> 30 mg 6xHis protein
Storage buffer	1× PBS containing 20% ethanol
Shelf life	Stable storage at 2 ~ 8°C for two years

Operation process

1. Buffer preparation

Binding/balancing buffer, washing buffer and eluting buffer, please prepare or purchase our His-tag protein purification kit.

2. Sample preparation

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

1) Centrifuge at 4°C for 30 min (4000x g) to collect bacteria and discard supernatant.

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2) Re-suspend the bacteria with a pre-cooled **binding/balancing buffer**, adding 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 (NTA-Co)**. The crushing solution should not contain chelating agents such as EDTA and EGTA, reducing agents such as DTT and mercaptoethanol, and denaturants such as urea and guanidine hydrochloride.

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 (12,000x g), separate supernatant and precipitation, and filter for impurity removal. Retain samples of supernatant and precipitation for subsequent testing.

3. Purification of recombinant His-tag fusion protein

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

2) Absorb 2 mL of **His-tag protein agarose gel (NTA-Co)** and add it to the chromatographic column, and balance **His-tag protein agarose gel (NTA-Co)** with 10 mL **binding/balancing buffer**. 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 the mixer and incubate at room temperature for 1 ~ 2 h. (Can also be incubated at 2 ~ 8°C 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, and put it at 2 ~ 8°C as a flow through for subsequent detection.

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

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

Note: Gradient elution can also be used if the elution procedure is optimized.

Gradient elution: Different concentrations of imidazole were used for elution and eluent was collected.

7) SDS-PAGE detection

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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 put it on the shaking table, dyeing 10 ~ 30 min to observe the results.

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 ~ 8°C.

Problem solving

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	Increase the imidazole

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	effectively eluted from the gel	concentration.
		Use a 10-100 mM EDTA solution to strip cobalt ions and obtain the target protein.
The purified target protein is not pure	The laundry is not thorough	Increase the number of washes.
	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 (e.g., ion exchange, hydrophobic, etc.).
Protein precipitation occurs during binding	Concentration is too large	Dilute protein appropriately.
	Protein aggregation	Add a stabilizer, such as 0.1% Triton X-100 or Tween-20, to the sample and all buffers.
	Operating temperature is too high	Operate at 2-8°C.

Ordering Information

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