His-tag Protein Purification Magnetic Particles, 10-30 μm-NTA-Ni

Cat No: AM-His-1000-NTA-Ni

Description

His-tag protein agarose magnetic bead (NTA-Ni) is a new magnetic microsphere product designed for efficient and rapid purification of His-tag protein, which can directly purify high-purity target protein from biological samples in one step under the action of magnetic field, greatly simplifying the purification process and improving the purification efficiency. It is suitable for high throughput purification of His-tag protein for scientific research and industrial customers.

Compared with the traditional column chromatography method, the use of His-tag protein agarose magnetic beads does not need to control the sample flow rate, and does not need expensive chromatography equipment as well as centrifugal equipment. The combination of sample and magnetic beads and the separation of target protein and magnetic beads become very simple and fast, and it is easier to achieve high-throughput and automatic protein purification method.

Abvigen His-tag protein agarose magnetic beads (NTA-Ni) can be used for the purification of histidine label (6xHis-tag) proteins from various expression sources such as E. coli, yeast, insect cells, and mammalian cells. It is composed of a highly cross-linked 4% agarose magnetic bead as the substrate, and chemically coupled to a tetra-coordinated nitrogen triacetic acid (NTA). After chelating nickel ions (Ni^{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.

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Characteristics

Matrix	Highly cross-linked 4% magnetic agarose beads
Ligand	Triacetic acid (NTA)
Chelating metal ions	Ni^{2+}
Particle size	10 ~ 30 μm
Concentration	The magnetic bead volume accounts for 25% of the suspension volume
Pressure flow rate	80 ~ 150 cm/h (0.3 MPa, 3 bar)
Carrying capacity	> 20 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.

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 Agarose Magnetic Beads (NTA-Ni)**. 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

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1) **His-tag protein agarose magnetic beads (NTA-Ni)** were thoroughly mixed, 2 mL magnetic bead suspension was placed in a 50 mL centrifuge tube, and 10 mL **binding/balancing buffer** was added. After full mixing, magnetic separation was performed, supernatante was discarded, and the above steps were repeated once.

2) The prepared fusion protein supernatant containing His label was added to the treated magnetic beads, and after mixing, the centrifuge tube was placed on the mixing machine and incubated at room temperature for 1 ~ 2 h. (Can also be incubated at 2 ~ 8°C 2 ~ 4 h or overnight)

3) After incubation, the centrifugal tube is placed on the magnetic rack, magnetic separation, suction supernatant, as a flow through, placed at 2 ~ 8°C for follow-up testing. Add 15 mL **washing buffer** into the centrifuge tube, mix it in the mixing machine for 10 ~ 15 min, and then magnetic separation, absorb the supernatant (reserved for sampling and testing). Repeat the preceding steps three times.

4) **Conventional elution:** Add 1 mL **elution buffer**, blow and mix for 10 ~ 20 times, and collect Ep tubes with superclear to 1.5 mL through magnetic rack. Repeat the operation to collect 5 ~ 10 tubes of elution 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.

5) SDS-PAGE detection

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 before preservation to remove impurities such as imidazole. Then pack and freeze to -80°C.

(Optional) Regeneration and storage of magnetic beads

Magnetic bead regeneration steps please refer to or directly buy our company His label protein purification regeneration kit.

After the regeneration of the magnetic bead, it can be used immediately. If it is not used immediately, it is necessary to add equal volume 20% ethanol and store it at 2 ~ 8°C.

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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 effectively eluted from the magnetic bead	Increase the imidazole concentration.
		Use a 10 ~ 100 mM EDTA solution to strip nickel 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

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		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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