His-tag Protein Purification Kit

Cat No: AKIT-His-IDA-Ni

Description

His-tag Protein Purification Kit is composed of His-tag protein agarose magnetic bead and optimized prefabricated buffer and 50 mL magnetic rack 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 magnetic bead	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
Magnetic rack	50 mL double row four well

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 agargo magnetic bead**, 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) **His-tag protein agarose magnetic beads** were thoroughly mixed, 2 mL magnetic bead suspension was taken, placed in a 50 mL centrifuge tube, and 10 mL **binding/balancing buffer (1 ×)** was added, after fully mixed, magnetic separation was performed, supernant was discarded, and the above steps were repeated once.

2) The prepared fusion protein supernatant containing His-tag 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 for 2 ~ 4 h or overnight)

3) After incubation, the centrifuge tube is placed on the magnetic rack for magnetic separation, the supernatant is absorbed, and the supernatant is placed at 2 ~ 8°C as a flow through for subsequent testing. 15 mL **washing buffer (1 ×)** was added to the centrifuge tube and mixed in the mixer for 10 ~ 15 min, and then magnetic separation was performed to absorb the supernatant (reserved for sampling and testing). Repeat the preceding steps three times.

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

5) 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: The target protein should be dialysis or ultrafiltration to remove imidazole and other impurities before storage, and then subpackaged and frozen to -80°C.

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

(Optional) Megnetic Bead Regeneration and Storage

Megnetic bead regeneration steps please refer to or directly buy our company His-tag 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.

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 the 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.
		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 (e.g., 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

Website: www.abvigen.com

Phone: +1 929-202-3014

Email: info@abvigenus.com