(This kit is for in vitro research only, not for clinical diagnosis!)

Elabscience® His Tag (HHHHHH) Fusion Protein Purification Kit

Cat #：EA-TP-K005

Product specifications：10 Tests

Please read the instructions carefully before use. If you have any questions, please contact us via:

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Please refer to the outer packaging label of the kit for the specific shelf life. Please use the kit within the shelf life.

Please provide the product batch number (see the kit label) when contacting so that we can serve you more efficiently.

Background Information

This kit consists of His-tagged protein purification resin, cell lysis buffer, 500mM imidazole eluent and purification column. It is simple to operate and has excellent performance. It can quickly purify His-tagged products in a variety of expression systems.

Performance Index

1. Application Scope:

Purify His-tagged products from various expression systems.

2. Substrate Properties:

Highly cross-linked 6% agarose resin.

3. Particle size:

45-165μm.

4. Loading capacity:

>40mg 6×His-tagged protein/mL.

5. Maximum pressure:

0.3MPa，3bar.

6. Composition:

3mL of His tag purification resin in 1mL of 1×PBS containing 20% ethanol.

Kit Components

Item No.	Component	Component No.	Specification	Storage
E-IR-IP004	Lysis buffer	L1	50 mL	4℃, 12 months
E-IR-IP001	Purification column	C	1	Room temperature, 12 months
EA-TP-C02	His tag Protein purification resin	G1	20mL	4℃, 24 months
E-IR-IP009	500mM Imidazole elution buffer	E3	50 mL*2	4℃，6 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	P10	30 mL	4℃, 12 months
Manual	One			

Matters Needing Attention

1. Transportation and storage：

This kit is shipped under refrigerated conditions.

After receiving the goods, please take out the purification column C and store it at room temperature; the other components of the kit should be stored at 4°C.

Reagent Preparation

1. 1× PBS

Dilute P10 (PBS Buffer, pH7.4 (10×)) with deionized water at a ratio of 9:1 and set aside for use. For example: add 9 mL of deionized water to 1 mL of P10 and mix well to obtain 1× PBS. Ready for use.

2. Washing Solution

Dilute E3 (500mM imidazole eluent) with deionized water at a ratio of 9:1 and set aside for use. For example: add 1mL of E3 to 9mL of deionized water and mix well to obtain 50mM imidazole eluent. Ready for use.

Instructions

1. Sample processing

a. Prokaryotic expression sample preparation

1) Inoculate 60μL of bacterial strain into 200mL of resistant medium and culture at 37°C overnight.

2) Add fresh resistant medium to 800mL the next day, and continue culturing for 1-2 hours to maximize bacterial activity.

3) Add 200μL of 1M IPTG and induce at 37℃ for 3.5h.

4) Centrifuge to collect the bacterial cells at 4000rpm and 4℃, discard the supernatant, add 30mL 1×PBS to re-suspend the bacterial cells, and crush them with sonicator at 200W for 6 minutes in an ice bath.

Note: In order to avoid degradation of the target protein, you can add protease inhibitors (PMSF working concentration 0.1~1mmol/L).

5) After sonication, centrifuge at 8000rpm and 4℃ for 15min. Take the supernatant and proceed to the next step of purification.

6) If the target protein is secreted and expressed, the above treatment is not required. The culture supernatant can be directly collected and concentrated to proceed with the following steps. If the target protein content is high, it is recommended to dilute the sample with 1×PBS to a final target protein concentration of 10~100μg/mL.

b. Cell lysate preparation

1) Cell Collection

Suspension cells and semi-adherent cells can be blown off the cell culture flask and transferred into a centrifuge tube. Centrifuge at 1000 rpm for 5 minutes and discard the supernatant.

2) Gently scrape the adherent cells from the bottle wall with a cell scraper, transfer them together with the culture medium into a centrifuge tube, centrifuge at 1000 rpm for 5 minutes, and discard the supernatant.

3) Re-suspend the cells in 1×PBS pre-cooled to 4°C, centrifuge at 1000 rpm for 3 minutes, and discard the supernatant. Repeat once.

4) Add the corresponding volume of cell lysis buffer L1 according to the amount of cells, pipet repeatedly and place on ice for 10-20 minutes.

   Note: Generally, 1mL of cell lysis buffer can process about $0.5\sim1\times10^7$ cells. To avoid degradation of your target protein, you can add protease inhibitors.

5) Use a sonicator to treat the cell lysate until the cell lysate is transparent and no longer viscous. After placing on ice for 30 minutes, centrifuge at 12,000 rpm and 4°C for 10 minutes. The supernatant is taken as the protein sample. It is recommended to proceed to the next step of the experiment immediately. If time does not allow, store the protein sample at -80 ℃.

   Note: If there is no sonicator, you can also use a beveled pipe tip or syringe to blow repeatedly until the cell lysate is transparent and no longer viscous.

6) If the target protein is secreted and expressed, the above treatment is not required. The culture supernatant can be directly collected and concentrated before proceeding to the following steps. If the target protein content is high, it is recommended to dilute the sample with 1×PBS to a final target protein concentration of 10~100μg/mL.

2. Column installation and incubation

1) Gently mix the resin, use a pipette tip with the end cut off to add 2 mL of the suspension into the purification column, and rinse with deionized water 3-5 times of the column volume to remove the residual 20% ethanol.
2) After the liquid has drained out, add 1× PBS, 10 times the gel volume of to the gel column to clean the resin.
3) After the liquid has run out, add cell lysate containing the target protein. Seal the purification column with a pipe cap and a plug. During the sealing process, please insert the pipe cap into the upper end of the purification column first, then flip the purification column so that the discharge port is facing upwards. Gently tap the pipe wall to allow the bubbles in the column to float upwards, push the pipe cap to discharge the gas from the purification column, and finally seal the discharge port with a plug.
4) Collect the flow-through cell lysate and temporarily store it at 4°C for possible re-purification.

Note: The flow-through fluid can be collected and loaded onto the column 3 to 5 times to fully bind the target protein.

3. Impurity removal and elution

1) After the cell lysate has drained out, add 5 times the gel volume of washing solution to wash the resin bound to the target protein. Repeat this step 3 times.
2) Collect each washing solution, detect the protein residue in the washing solution by SDS-PAGE, stop washing after G250 detects no obvious color change, and prepare for the next elution step.

Note: If the protein purity is not high enough in the future, you can increase the number of column washes 2 to 3 times. During the column washing process, 20 μL of each flow-through fluid was collected for subsequent detection.

3) Elute 3 to 5 times with 500mM imidazole eluent, 0.5 times the column volume each time, and incubate at 4°C for 10 minutes. Collect the elution products and temporarily store them at 4°C until SDS-PAGE detection. They can be used immediately. Use or store at -20°C.

Declaration

1. This product is limited to scientific research by professionals.
2. Please pay attention to safety precautions and comply with laboratory reagent operating specifications.
3. The lysis buffer provided by this kit is a formula that has been repeatedly optimized over a long period of time and has been verified by a large number of experiments. When processing cells, it is recommended to use the lysis buffer provided by this kit. Lysis buffers provided by other manufacturers may affect the results of protein co-precipitation or subsequent IP experiments.
4. The conditions recommended in this manual are universal. Users can optimize experimental conditions and choose the most appropriate experimental plan based on the properties of different target proteins.