(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS!)

Elabscience® HA(YPYDVPDYA) -tagged Protein Purification Kit

Product code: EA-TP-K002

Product specification: 1 T

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

Tel: 240-252-7368(USA)

Fax: 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please refer to the outer package label of the kit for the specific shelf life and use the kit within its shelf life.

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

Background Information

HA-Tag (YPYDVPDYA)is commonly used as eukaryotic protein recombinant expression marker. The HA tagged fusion protein purification kit is mainly composed of Anti-HA(YPYDVPDYA) affinity agarose, which is made by covalent coupling of high-quality HA antibody and Sepharose 4B agarose particles. It has the characteristics of high binding capacity of protein, high specificity, stability, and repeatability. It can be used for affinity purification of HA tagged fusion proteins.

Performance Index

1. Scope of application

Affinity purification of HA tag fusion protein.

HA tag can be located at the N-terminal, C-terminal or middle of the protein, such as N-terminal HA fusion protein (HA–Protein), C-terminal HA fusion protein (Protein–HA) and Met modified N-terminal HA fusion Protein (Met–HA–Protein).

2. Gel properties

Agarose gel granules, average size 50 μm.

3. Binding capacity

1mL Sepharose 4B agarose granules, covalently coupled with 6mg Anti-HA monoclonal antibody.

1mL affinity gel can purify at least 1.2mg HA fusion protein.

4. Repeatability

It can be used repeatedly for more than 5 times.

5. Components

1mL Anti-HA affinity gel, stored in 1mL PBS containing preservatives and 50% glycerol.

Product Components

Cat. No.	Component	Code	Specification	Storage
E-IR-IP001	Purification column	C	6mL×1	Room temperature, 12 months
EA-IP-002	Anti-HA（YPYDVPDYA）Affinity Agarose	G1	2mL	-20°C, 12 months
E-IR-IP004	Lysis buffer	L1	50mL	4°C, 12 months
E-IR-IP005	Acid prewashing buffer	E1	25mL	4°C, 12 months
E-PP-1263	HA peptide	E2	10mg	4°C, 12 months
E-IR-IP006	Acid elution buffer	E3	25mL	4°C, 12 months
E-IR-IP007	Neutralizing buffer	N1	25mL	4°C, 12 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	P10	50mL	4°C, 12 months
Manual	one copy			

Matters Needing Attention

1. Transportation and storage

This kit is transported under refrigerated conditions.

After receiving the goods, if it is not used temporarily, please take out the purification column **C** and store it at room temperature; Anti–HA affinity agarose **G1** was stored at - 20°C; the rest of the kit components are stored at 4 °C.

2. Precaution

The acid eluent in this kit is irritating to the skin. Please wear gloves and other personal protective equipment during the experiment.

3. Suggestions on reagent use

Dilute **P10** (PBS Buffer, pH7.4 (10 ×)) to 1x PBS with deionized water for use.

Store affinity agarose **G1** in gel preservation solution at - 20 °C after use.

4. Gel suspension and affinity gel

This kit provides affinity gel in the form of gel suspension. The content of affinity gel in gel suspension is 50%. Before use, gently re-suspend gel suspension, and then use it as required.

For example, 2mL gel suspension contains 1mL affinity gel.

5. Selection of acid eluent

According to literature, compared with the traditional Glycine HCL eluent, the Arginine HCL with pH 3.0 provided by this kit can reduce protein denaturation and extend the service life of affinity gel. You can also choose acid eluent according to the actual situation.

Self-Prepared Reagent

1. Competitive eluent

HA peptide **E2** is a light powder that needs to be centrifuged before opening the lid. It is recommended to add 2mL 1×PBS to 10mg polypeptide powder to make 5mg/mL storage solution, which should be stored at -20°C. Use 1×PBS to dilute to the required working concentration.

Note: The recommended working concentration is 2mg/ml-5mg/ml. You can also adjust the working concentration according to the properties of the target protein. Generally speaking, higher the concentration of the peptide, the stronger the elution ability.

2. 1×PBS

Dilute **P10** (PBS Buffer, pH7.4 (10×)) with deionized water in a ratio of 9:1 for use. For example, add 1mL of **P10** into 9mL of deionized water and mix it to 1× PBS. Prepare when needed.

3. Gel preservation solution

Mix glycerin with 1xPBS at a ratio of 1:1. Prepare when needed.

Note: It is recommended to add a certain concentration of preservatives in the gel preservation solution to prevent the bacterial growth.

Method of Application

Note: All steps must be performed on ice as much as possible to avoid degradation of target proteins. The amount of reagents used below is calculated by 1mL gel volume. You can also adjust the amount of reagents proportionally according to the specific amount of gel.

1. Preparation of Lysis buffer

1) Collecting cells

Blow the suspended cells and semi-adherent cells off the cell culture flask and transfer them into a centrifuge tube, centrifuge at 1000rpm for 5min, and discard the supernatant.

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

2) Re-suspend the cells with 1x PBS pre-cooled to 4 °C, centrifuge at 1000rpm for 3min, and discard the supernatant. Repeat.

3) Add the corresponding volume of cell lysis buffer **L1** according to the number of cells, and place on the ice for 10-20min after repeated blowing.

Note: Generally, 1mL of cell lysis buffer can process about 0.5–1 $\times 10^7$ cells. To avoid degradation of that target protein, you may add protease inhibitor.

4) Treat cell lysate with ultrasonic crusher until cell lysate is clear and no longer viscous. After 30 min on ice, centrifuge at 12000 rpm for 10 min at 4 °C. Take out the supernatant and freeze at −80 °C.

5) If the target protein is secreted and expressed, the above treatment is not required, the supernatant of the medium can be directly collected and the following steps can be performed after concentration。

2. Column installation and incubation

1) Gently re-suspend Anti-HA affinity agarose **G1**, mix evenly, and aspirate 2mL of gel suspension into purification column **C** with the pipette (cut off the tip head).

Note: If the sample volume is small, if there is only 1-2 mL cell lysate, it is recommended to use the immunoprecipitation kit (Cat. No.: EA-IP-K002).

2) After the liquid is drained, add 1xPBS 10 times the volume of gel to the gel column to wash the affinity gel.

3) After the liquid is drained, add the eukaryotic cell lysate containing the target protein. Seal the purification column with a pipe cap and a plug. During the sealing process, insert the pipe cap into the upper end of the purification column first, then turn the purification column over to make the drain port face up, gently tap the pipe wall to float the bubbles in the column, push the pipe cap to discharge the gas from the purification column, and finally seal the drain port with a plug. Check the sealed purification column to ensure no leakage, and incubate it overnight at 4°C in a shaker.

4) Collect cell lysate and temporarily store it at 4°C for possible re-purification.

5) After the cell lysate is drained, add 1×PBS of 5 times the volume of gel to wash the affinity gel that binds the target protein. Repeat this step 3 times.

6) Select competitive elution or acid elution according to protein properties. For specific methods, refer to **Problems and Suggestions**.

7) If the amount of target protein is large, the cell lysate collected in step **4)** can be purified again with the re-generated affinity gel. Multiple purification can improve the yield of target protein.

3. Competitive elution

1) Prepare HA peptide competitive eluent with 1xPBS, the final concentration is 2mg/mL, or adjust the working concentration according to the specific situation (see **Reagent Preparation**).

2) After the 1xPBS in the purification column is drained, add the acid prewashing buffer **E1** 5 times the volume of the precooled gel, to wash the affinity gel and remove the non-specific binding protein.

3) After the acid prewashing buffer **E1** in the purification column is drained, add HA peptide competitive eluent with the volume of 2 times of gel. According to the method described in the previous section, seal the purification column with pipe cap and plug to ensure no leakage, mix gently, incubate at 4 °C in shaking table for 2h, and collect the eluent. If necessary, HA peptide competitive eluent with twice the volume of gel can be added again for repeated elution.

Note: In order to improve the protein yield, the incubation time can be extended.

4) Identify protein purity through SDS-PAGE, and process and store protein as required.

4. Acid elution

1) Prepare at least 10 2mL eluent collection tubes, and add 50 μL neutralizing buffer **N1** to each tube.

2) After the 1×PBS in the purification column is drained, add the acid prewashing buffer **E1**, 5 times the volume of the precooled gel, to wash the affinity gel and remove the non-specific binding protein.

3) After the acid prewashing buffer **E1** in the purification column is drained, add 1mL of acid elution buffer **E3** for elution, and collect the eluent with the eluent collection tube prepared in step **1)**. Repeat this step for 10 times to obtain 10 1mL eluents.

Note: The service life of affinity gel will be shortened if it is placed in acidic environment for too long. The contact time between affinity gel and acid eluent should be shortened as much as possible, not more than 10min.

4) Combine the collected eluent.

Note: If the concentration of target protein is low, the concentration of target protein can be increased by concentrating the collected solution.

5) Identify protein purity through SDS-PAGE, and process and store protein as required.

5. Washing and regeneration of affinity gel

Note: Wash and regenerate affinity gel immediately after elution.

1) Wash with 1×PBS cleaning affinity gel with 10 times gel volume.
2) Wash affinity gel by acid elution buffer **E3** with 3 times the volume of gel.
3) Wash affinity gel 3 times by neutralizing buffer **N1** with 3 times the volume of gel affinity gel.
4) Detect the pH of the penetrating liquid, and if it is neutral, proceed to the next step; if it is still acidic, repeat steps **2**) and **3**).
5) Wash affinity gel with gel preservation solution (see **Reagent Preparation**) with twice the volume of gel.
6) Add gel preservation solution of 1 times the gel volume, mix well, and store at -20°C.

Problems and Suggestions

1. How to select competitive elution and acid elution?

1） Competitive elution

Competitive elution means that HA peptide competes with the HA tag on the protein to bind the antibody on the gel, so that the binding between the protein and the antibody is replaced and separated from the affinity gel, thus being eluted. The characteristics of this elution method are:

a) The mild elution conditions will not cause protein denaturation generally; it is beneficial to the preservation and repeated use of affinity gel.

b) As affinity adsorption is relatively slow to reach equilibrium, it often takes a long time.

c) Smaller elution volume.

d) With strong specificity, it can further eliminate the influence of non-specific adsorption, so as to obtain a high purity target protein. Basically, anti-HA antibodies will not be eluted.

2） Acid elution

a) Low cost.

b) The purification speed is fast.

c) Acidic pH will cause denaturation of some proteins, causing precipitation, degradation or inactivation of target proteins.

d) Sometimes, a small part of Anti-HA antibody will be eluted, causing non-specific signals.

e) Repeated acid elution will cause antibody shedding, destroy the physical and chemical properties of affinity gel, and reduce the use of affinity gel.

2. What are the common follow-up treatment methods for protein?

1） Dialysis and ultrafiltration

The dialysis method uses a semi permeable membrane to separate proteins with different molecular sizes. Ultrafiltration uses high pressure or centrifugation to force water and other small solute molecules pass through the semi-permeable membrane while the proteins remain in the ultrafiltration

tube. Both can achieve the purpose of fluid change. Pay attention to selecting dialysis bag and ultrafiltration tube with correct molecular weight when using.

2） Filtration and sterilization

Microporous membrane filter can be used to pass protein solution through 0.22 μm filter membrane, which can achieve the purpose of sterilization.

3） Quantitative detection of protein

The protein concentration can be detected by BCA method and detect protein purity by SDS-PAGE.

4） Detection of physical and chemical properties of protein

Protein binding activity can be detected by Western Blot (WB) and Immunoprecipitation (IP); Detection of protein structure by mass spectrometry (MS).

Declaration

1. This product is limited to the scientific use of a professional.
2. Please pay attention to safety matters, and follow the laboratory reagent operation specification.
3. The lysis solution provided by the kit is a formula repeatedly optimized for a long time and verified by a large number of experiments. When treating cells, it is recommended to use the lysis buffer provided with this kit, and the lysis buffer provided by other manufacturers may affect protein co-precipitation or the results of subsequent IP experiments.
4. The conditions recommended in this specification are general. The user can optimize the experimental conditions and select the most appropriate experimental protocol according to the properties of different target proteins.