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

Elabscience® Anti-His (HHHHHH)-tag FASTIP Kit

Anti-His(HHHHHH)-tag FAST IP Kit

Cat #: EA-IP-K004

Product specifications: 50 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

Anti-His tag (HHHHHH) FAST IP kit consists of four components: Anti-His Affinity Agarose, Mouse IgG Affinity Agarose, His-Tag Rabbit pAb and HRP-labeled goat anti-rabbit secondary antibody. For rapid, efficient and specific immuno(co)precipitation of His-tagged fusion proteins.

Anti-His Affinity Agarose is made by covalently conjugating high-quality His-Tag Mouse mAb to agarose gel. It has the characteristics of high loading capacity, high specificity and stable properties; Mouse IgG Affinity Agarose is used as CoIP experimental control with stable properties; His-Tag Rabbit pAb antibody has the advantages of high specificity, high affinity, and high titer. The HRP-labeled goat anti-rabbit secondary antibody was purified through cross adsorption and only recognizes His Tag Rabbit pAb, without cross reactivity with the heavy and light chains of mouse monoclonal antibodies. The four components have undergone strict quality inspection and can be used individually; the set of the four components has the advantages of speed, simplicity and no interference with the strips.

Performance Index

1. Application Scope:

Immuno(co)precipitation of His-tagged fusion proteins.

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

Suitable for secreted proteins.

2. Antibody properties:

His-Tag Mouse mAb: mouse IgG2a subtype; His-Tag Rabbit pAb: rabbit IgG.

3. Gel properties:

Agarose gel particles, average particle size 100μm.

4. Loading Capacity:

0.5mL Sepharose 4B agarose particles, covalently conjugated to 4mg Anti-His mouse monoclonal antibody. Can purify or precipitate at least 0.6mg His fusion protein.

Kit components

Cat. No.	Component	Specification/concentrat ion	Preservation method
E-IR-IP004	Lysis buffer	30mL	4℃, 12 months
EA-IP-008	Anti-His Tag Affinity Agarose Anti-HHHHHH Affinity Agarose	2mL（0.5mL/mL）	-20℃, 12 months
EA-IP-100	Mouse IgG Affinity Agarose Mouse IgG affinity agarose	2mL（0.5mL/mL）	-20℃, 12 months
E-AB-40506	His-Tag Rabbit pAb	100μL（1mg/mL）	-20℃, 12 months
E-AB-1003	Goat Anti Rabbit IgG (peroxide/HRP conjugated)	100μL（0.5mg/mL）	-20℃, 12 months
Manual	One		

*Note: The buffer is PBS containing 50% glycerol.

Matters Needing Attention

1. Transport and Storage:

This kit is shipped under refrigerated conditions.

After receiving the goods, if it is not used temporarily, please take out the lysis buffer and store it at 4℃; the remaining components of the kit should be stored at -20℃.

2. Gel suspension and affinity gel

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

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

Reagent Preparation

1. **Antibody diluent**

   Prepare skim milk powder with a final concentration of 5% in 1×PBST. Ready to use.

2. **1×PBST**

   Dilute 10×PBST (Cat. No.: E-IR-R310) with deionized water at a ratio of 9:1 and set aside. For example: add 9mL of deionized water to 1mL of 10×PBST, and mix evenly to obtain 1×PBST. Ready to use.

3. **1×PBS**

   Dilute 10×PBS (Cat. No.: E-BC-R187) with deionized water at a ratio of 9:1 and set aside. For example: add 9mL of deionized water to 1mL of 10×PBS, and mix evenly to obtain 1×PBST. Ready to use.

4. **Enhanced chemiluminescence developer (ECL) (Cat. No.: E-IR-R307 or E-IR-R308)**

   Mix the chemiluminescent substrate ECL solution A and ECL solution B in equal volumes at a ratio of 1:1. Ready to use.

Instructions

Note: All steps should be performed on ice if possible to avoid degradation of the target protein.

1. Cell lysate preparation

1) Cell Collection

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

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.

2) 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.

3) Add the corresponding volume of cell lysis buffer 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~ 1×10^7 cells. To avoid degradation of your target protein, you can add protease inhibitors.

4) 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 ℃.

5) If the target protein is secreted and expressed, there is no need for the above treatment. The culture supernatant can be collected directly, and after concentration, the following steps can be performed.

2. Immuno(co)precipitation of His-tagged proteins

1) Experimental group Affinity Agarose pre-treatment. Gently re-suspend the Anti-His affinity gel, mix evenly, and use a pipette tip with the end cut off to draw 40 μL of gel suspension (containing approximately 20 μL of affinity gel) into a centrifuge tube. Add 10 times the gel volume (about 200 μL) of 1×PBS to wash the affinity gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

2) Control group Affinity Agarose pre-treatment. Gently re-suspend the Mouse IgG affinity gel, mix evenly, and use a pipette tip with the end cut off to draw 40 μL of gel suspension (containing approximately 20 μL of affinity gel) into a centrifuge tube. Add 10 times the gel volume (about 200 μL) of 1×PBS to wash the affinity gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

Note: The following steps are performed simultaneously in the control group and experimental group.

3) Add 50-200 μL of eukaryotic cell lysate containing the target protein and incubate on a shaking table at room temperature for 2 hours or overnight at 4°C.

4) Add 10 times the gel volume (about 200 μL) of 1×PBS to wash the affinity gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

5) Add 5 times the gel volume (approximately 100 μL) of PBST pre-wash solution pre-cooled to 4°C to wash the affinity gel to remove non-specific binding proteins. Centrifuge at 5000 rpm for 30 seconds and discard the supernatant.

6) Add 4 μL of 5× loading buffer, boil for 5 minutes, cool to room temperature and centrifuge.

7) Take the supernatant and run SDS-PAGE in preparation for subsequent Western Blotting detection.

3. Western Blotting to detect His-tagged proteins

1) Transfer proteins from SDS-PAGE gel to membrane using WB transfer membrane apparatus.
2) After electroporation, remove the membrane and place it in the membrane treatment solution for 1 minute, take it out, and allow it to equilibrate at room temperature for 30 minutes.
3) Add an appropriate amount of antibody diluent to block the non-specific binding sites on the membrane until it completely covers the membrane, and incubate on a shaking table at 37°C for 1 hour.
4) Dilute His-Tag Rabbit pAb primary antibody with antibody diluent to a dilution of 1:10000, add it to the membrane to ensure complete coverage of the membrane, and incubate on a shaking table at 37°C for 1 hour.
5) Wash the membrane with PBST and incubate on a shaking table at 37°C for 5 minutes. Repeat this step 4 times.
6) Dilute HRP-labeled goat anti-rabbit secondary antibody with antibody diluent to a dilution of 1:10000, add it to the membrane to ensure complete coverage of the membrane, and incubate on a shaking table at 37°C for 1 hour.
7) Wash the membrane with PBST and incubate on a shaking table at 37°C for 5 minutes. Repeat this step 4 times.
8) Place the membrane flat on a clean surface, mix equal volumes ofECL solution A and ECL solution B and add evenly to the membrane, and let the reaction in the dark for 1 minute.
9) Take out the membrane, discard the ECL solution, and place it in a dark box for development. Different exposure times can be selected according to the intensity of the background and target bands.

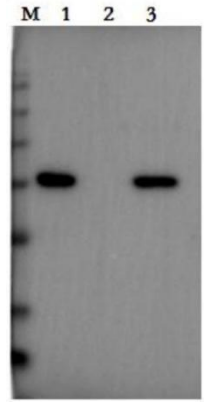


Figure 1: Anti-His (HHHHHH) Affinity Agarose IP effect diagram.

Lane M: Marker; Lane 1: Experimental group; Lane 2: Negative control group; Lane 3: Input group

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.