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

Elabscience® Anti-HA（YPYDVPDYA） FAST IP Kit

Product code: EA-IP-K002

Product specifications: 50 T

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

Tel: 240-252-7368(USA)

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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

Anti-HA-tag(YPYDVPDYA) fast IP kit is composed of Anti-HA affinity gel, Mouse IgG affinity gel, HA-Tag Rabbit pAb and Goat Anti-Rabbit IgG(peroxidase/HRP conjugated), which is used for fast, efficient and specific (Co)IP of HA tagged fusion protein.

Anti-HA affinity gel is made by covalently coupling high-quality HA-Tag Mouse mAb with agarose gel. It has the characteristics of high binding capacity of protein, high specificity and stability. Mouse IgG affinity gel is used as the control for CoIP experiment. The HA-Tag Rabbit pAb antibody has the advantages of high specificity, high affinity and high valence; Goat Anti-Rabbit IgG (peroxidase/HRP conjugated) is purified by cross-adsorption and only HA-Tag Rabbit pAb is identified, and there is no cross-reaction with the heavy-chain and light-chain of the mouse monoclonal antibody. The four components are strictly inspected and can be used independently; the combination of four sets has the advantages of fast, convenience and no interference band.

Performance Index

1. Scope of application

(Co)IP of HA tag fusion proteins.

The HA tag can be located at the N-terminal, C-terminal or middle of 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. Antibody properties

HA-Tag Mouse mAb: Mouse IgG2a subtype;

HA-Tag Rabbit pAb: Rabbit IgG.

3. Gel properties

Agarose gel granules, average size 50μm.

4. Binding capacity

0.5mL Sepharose 4B agarose granules, covalently coupled with 4 mg of Anti-HA mouse monoclonal antibody.

Product Components

Cat. No.	Component	Specification	Storage
E-IR-IP004	Lysis buffer	30mL	4°C, 12 months
EA-IP-002	Anti-HA（YPYDVPDYA）Affinity Agarose	2mL（0.5mL/mL）	-20°C, 12 months
EA-IP-100	Mouse IgG affinity agarose	2mL（0.5mL/mL）	-20°C, 12 months
E-AB-40523	HA-Tag Rabbit pAb	100μL（1mg/mL）	-20°C, 12 months
E-AB-1003	Goat Anti-RabbitIgG(peroxidase/HRP conjugated)	100μL（0.5mg/mL）	-20°C, 12 months
Manual	one copy		

* Note: Buffer is PBS containing 50% glycerol.

Matters Needing Attention

1. Transportation and storage

The kit is shipped under refrigerated conditions.

After receiving the goods, if it is not used temporarily, please take out the lysis solution and store it at 4℃; The rest ofthe kit components are stored at -20℃.

2. Gel suspension and affinity gel

The 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 of gel suspension contains 1mL affinity gel.

Self-Prepared Reagent

1. **Antibody diluent**

   1x PBST is used to prepare skim milk powder with the final concentration of 5%. Prepare when needed.

2. **1x PBST**

   Dilute 10x PBST (Cat.No. E-IR-R310) with deionized water in a ratio of 9:1 for use. For example, 1 mL of 10x PBST is mixed with 9mL of deionized water to obtain 1x PBST. Prepare when needed.

3. **1x PBS**

   Dilute 10x PBS (Cat.No. E-BC-R187) with deionized water in a ratio of 9:1 for use. For example, 1 mL of 10x PBS is mixed with 9mL of deionized water to obtain 1x PBS. Prepare when needed.

4. **Chemiluminescence Developer Solution (ECL) (Cat.No. E-IR-R307 or E-IR-R308)**

   The chemiluminescent substrate ECL solution A and ECL solution B are uniformly mixed in equal volume according to the ratio of 1:1. Prepare when needed.

Method of Application

Note: All steps must be performed on ice as much as possible to avoid degradation of the target protein.

1. Preparation of cell lysate

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 lysate 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 x10^7 cells. To avoid degradation of 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. 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, 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. (Co)IP HA-labeled protein

1) Pretreatment of affinity gel in experimental group.

Gently re-suspend Anti-HA affinity gel, mix evenly, and aspirate 40 μL gel suspension (containing about 20 μL affinity gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 10 times the gel volume (approximately 200μL) of 1xPBS, centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times

2) Pretreatment of affinity gel in control group.

Gently re-suspend Mouse IgG affinity gel, mix evenly, and aspirate 40 μL gel suspension (containing about 20 μL affinity gel) into the centrifuge tube with the pipette (cut off the tip head). Wash the affinity gel with 10 times the gel volume (approximately 200μL) of 1xPBS, centrifuge at 5000rpm for 30sec, discard the supernatant, and repeat the procedure three times.

Note: The following steps can be performed synchronously in the control group and the experimental group.

3) Add 50-200μL eukaryotic cell lysate containing target protein and incubate for 2h in a shaker at room temperature or overnight at 4°C.

4) Wash the affinity gel with 10 times the gel volume (approximately 200μL) of 1x PBS, centrifuge at 5000 rpm for 30 sec, discard the supernatant, and repeat the procedure three times.

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

6) Add 4 μL 5x loading buffer, boil for 5min, cool to room temperature and centrifuge.

7) Take the supernatant for SDS-PAGE test and for subsequent Western Blotting detection.

3. Western Blot detection of HA labeled proteins

1) Transfer the protein from the SDS-PAGE gel to the membrane using a WB transmembrane apparatus.

2) After the electrotransfer, take out the membranes and place in the membrane treatment solution for 1min, and then equilibrate at room temperature for 30min.

3) Add a proper amount of antibody diluent to block the non-specific binding sites on the membrane, and cover the membrane completely. Incubate the samples on a shaking table at 37 °C for 1 h.

4) Dilute the HA-Tag Rabbit pAb primary antibody with antibody diluent at a dilution ratio of 1:10000, and add it to the membrane ensuring complete membrane coverage. Incubate the membrane on a shaking table at 37 °C for 1 h.

5) Wash the membrane with PBST and incubate with shaking at 37 °C for 5 min. Repeat the procedure for four times.

6) Dilute the Goat Anti-Rabbit IgG (peroxidase/HRP conjugated) with antibody diluent at a dilution ratio of 1:10000, add it to the membrane ensuring complete coverage of the membrane, and incubate on a shaking table at 37 °C for 1 h.

7) Wash the membrane with PBST and incubate with shaking at 37 °C for 5 min. Repeat the procedure for four times.

8) Place the membrane on a clean surface, mix equal volume of ECL solution A and ECL solution B, evenly add to the membrane, and let it react for 1min in dark.

9) Take out the membrane, discard the ECL solution, and place in a cassette for development. Different exposure times can be selected according to the intensity of the background and the target band.

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 buffer 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 matching 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.