Universal IP/Co-IP Toolkit (Agarose)

Cat #: KTD105-EN **Size:** 20 T

	Universal IP/Co-IP Toolkit (Agarose)		
REF	**Cat #:** KTD105-EN	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells		
	Storage: Store according to the recommended storage conditions of each component, stable for 12 months		

Assay Principle

Immunoprecipitation (Immunoprecipitation, IP) is a small affinity purification method using specific antibodies fixed on solid-phase supports such as magnetic beads or agarose. As an important part of many proteomics-related studies, IP can be used to detect the existence of proteins, relative abundance, up-and-down expression of proteins, stability and interaction of proteins and so on. But they are tedious and time-consuming, with a wide variety of reagents, and poor stability and reliability of experimental results. Based on the pain points of the traditional IP/Co-IP experiment, Abbkine developed a Universal IP/Co-IP Toolkit (Agarose); After designed and researched, the toolkit contains: ① The optimized natural and denatured lysate can meet the needs of IP/Co-IP or WB at the same time; ② Compared with Protein A or Protein G Agarose, Protein A/G Agarose has a wider binding range, more efficient antibody binding capacity and lower protein non-specific adsorption rate, making the operation more time-saving, efficient and saving antibody consumption. ③ The ready-to-use IP negative control can eliminate the non-specific binding of IgG itself to the target protein or other specific biomolecules, ensure the specificity of IP antibodies, and make the experimental data more stable and reliable. ④ The unique IPkine™ second antibody (HRP labeled goat anti-mouse IgG light chain secondary antibody and HRP labeled mouse anti-rabbit IgG light chain secondary antibody) can perfectly eliminate heavy chain interference. Universal IP/Co-IP Toolkit (Agarose), as a standardized product of IP/Co-IP, can meet the IP/Co-IP needs of most users, and it's the best choice for IP/Co-IP detection experiments. In addition, the corresponding products (A25012, A25022) in the Toolkit can also be ordered separately.

Materials Supplied and Storage Conditions

Toolkit components	Kit components	Size	Storage conditions
Universal IP/Co-IP Toolkit (Agarose) (Part 1 of 2)	Non-Denaturing Lysis Buffer	25 mL	4℃
	Denaturing Lysis Buffer	25 mL	4℃
	10×Wash Buffer	20 mL	RT
	Protein A/G Agarose	0.45 mL	4℃
	Elution Buffer	2 mL	4℃

	Neutralization Buffer	0.2 mL	4℃
Universal IP/Co-IP Toolkit (Agarose) (Part 2 of 2)	100×Proteinase Inhibitor Cocktail	0.2 mL	-20℃
	Mouse IgG (1mg/mL)	30 μL	-20℃
	Rabbit IgG (1mg/mL)	30 μL	-20℃
	IPKine™ HRP, Goat Anti-Mouse IgG LCS	30 μL	-20℃
	IPKine™ HRP, Mouse Anti-Rabbit IgG LCS	30 μL	-20℃

Materials Required but Not Supplied

- Vertical rotating mixer
- Freezing Centrifuge
- Precision Pipettes, Disposable Pipette Tips
- Deionized Water
- PBS Buffer
- Dounce homogenizer (for tissue samples)
- SDS-PAGE Loading Buffer

Reagent Preparation

Non-Denaturing Lysis Buffer: Native protein lysis buffer, extract protein for IP samples. Ready to use as supplied. Place it on ice for use. Store at 4℃.

Denaturing Lysis Buffer: Denatured protein lysis buffer, extract protein for WB samples. Ready to use as supplied. Place it on ice for use. Store at 4℃.

1×Wash Buffer: Add Deionized Water to the 10× Buffer and dilute the 10×Wash Buffer to 1×Wash Buffer before use. Store at 4℃.

Protein A/G Agarose: Ready to use as supplied. Store at 4℃, Avoid frozen.收

Note: 10×Wash Buffer stored at 4℃ will produce precipitation, and can be preheated in 37℃ water bath for 10 min before use to dissolve precipitation.

Elution Buffer: Ready to use as supplied. Elute Non-Denaturing protein. Store at 4℃.

Neutralization Buffer: Ready to use as supplied. Neutralize Non-Denaturing eluted protein. Store at 4℃.

100×Proteinase Inhibitor Cocktail: Add 1×Proteinase Inhibitor Cocktail to Lysis Buffer before use. Store at -20℃.

Mouse IgG: Ready to use as supplied. Used as a negative control for Mouse IP antibody. Store at -20℃.

Rabbit IgG: Ready to use as supplied. Used as a negative control for Rabbit IP antibody. Store at -20℃.

IPKine™ HRP, Goat Anti-Mouse IgG LCS: HRP conjugated Goat Anti-Mouse IgG light chain specific secondary antibody. The recommended dilution ratio of antibody for WB is 1:1000-1:10,000 (optimal 1:2,000). Store at -20℃.

IPKine™ HRP, Mouse Anti-Rabbit IgG LCS: HRP conjugated Mouse Anti-Rabbit IgG light chain specific secondary antibody. The recommended dilution ratio of antibody for WB is 1:1000-1:10,000 (optimal 1:2,000). Store at -20℃.

Assay Procedure

I Immunoprecipitation

A. **Preparation of protein samples**

1. Extract protein for Cell Samples:

(1) Collect cells (Adherent cells: 80% to 90% of monolayer cells were grown in a 10 cm cell culture dish. Remove the medium and wash with PBS once; Suspended cells: Collect 5×10^6 cells by centrifugation and washed by PBS once)

(2) Add 0.5-1 mL ice-cold Non-Denaturing Lysis Buffer to cells (Add 1×Proteinase Inhibitor Cocktail to Non-Denaturing Lysis

Buffer), lytic cells at 4℃ for 5 min. During the process, the pipette is used to blow the mixture repeatedly, transfer cell suspension to a new tube.

(3) Centrifuge at 12,000 rpm for 10 min at 4℃, collect supernatant, and detect protein concentration by BCA Assay (It is is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay)).

2. Extract protein for Tissue Samples:

(1) Plant or Animal Tissue Samples: Weigh 0.1 g of tissue and add 1 mL Non-Denaturing Lysis Buffer (Add 1×Proteinase Inhibitor Cocktail to Non-Denaturing Lysis Buffer), Homogenize tissue with Liquid nitrogen or Dounce homogenizer. (If the protein concentration is low, reduce the volume of Non-Denaturing Lysis Buffer)

(2) Transfer the homogenate to a new tube, lytic samples at 4℃ for 5 min.

(3) Centrifuge at 12,000 rpm for 10 min at 4℃, collect supernatant, and detect protein concentration by BCA Assay. (It is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay))

Note: The optimal concentration of total protein was in the range of 0.5-1 μg/μL. Usually, the total protein concentration needs to be pre-adjusted according to the different expression levels of the target protein. Co-Immunoprecipitation (Co-IP) usually requires the use of fresh samples that have not been frozen. Although frozen protein samples can be used for immunoprecipitation, fresh samples are preferred.

B. Remove non-specifically binding (Optional):

(1) Add 20 μL (40 μL suspension) Protein A/G Agarose to a 1.5 mL centrifuge tube, Centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant.

Note: Protein A/G Agarose must be fully suspended before use, that is, fully invert the mixture several times to mix well. If Protein A/G Agarose agglomerated which is caused by the reduction of protective solution (0.1 M, pH 7.4 PBS containing 20% ethanol), it is recommended to add protective solution, and keep the same volume as Agarose. For per mL Protein A/G Agarose contains 0.5 mL Agarose and 0.5 mL protective solution, each sample need add 40 μL Protein A/G Agarose suspension.

(2) Add 1mL 1×Wash Buffer, and re-suspend Protein A/G Agarose, Centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant, repeat 3 times.

(3) Add 0.2-1 mL(0.2-1 mg) protein sample, shake and incubate at 4℃ for 30 min.(It is recommended to use vertical rotating mixer with Low-speed rotation)

(4) Centrifuge at 800 rpm for 2 min at 4℃, use supernatant for Immunoprecipitation.

Note: During centrifugation, the centrifugal speed should not be too high, which will damage Protein A/G Agarose. The centrifugal speed should be 800-2,000 rpm.

C. Immunoprecipitation

(1) Add 20 μL (40 μL suspension) Protein A/G Agarose to a 1.5 mL centrifuge tube, centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant.

(2) Add 1mL 1×Wash Buffer, and re-suspend Protein A/G Agarose, Centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant, repeat 3 times.

(3) Add 0.2-2 μg antibodies, and re-suspend Protein A/G Agarose, shake and incubate at room temperature for 30 min. Centrifuge at 800 rpm for 2 min, collect the supernatant, use the precipitation for Step (4). **Optional, IgG negative control: Add 0.2-2 μL Mouse IgG or Rabbit IgG, and re-suspend Protein A/G Agarose, shake and incubate at room temperature for 30 min. Centrifuge at 800 rpm for 2 min, collect the supernatant, use the precipitation for Step (4). This step could exclude the non-specific binding of IgG to the target protein or other specific biological molecules.**

Note: Protein A/G Agarose needs fluid to keep it flowing while use vertical rotating mixer, if it does not flow, add a certain volume of 1×Wash Buffer.

(4) Add 1 mL 1×Wash Buffer, and re-suspend Protein A/G Agarose, centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant, repeat 3 times.

(5) Add 0.2-1 mL(0.2-1 mg) protein sample or supernatant (supernatant which remove non-specifically binding), shake and incubate at room temperature for 1 h or at 4℃ overnight.

(6) Centrifuge at 800 rpm for 2 min at 4℃, collect the supernatant, use the precipitation for Step (7).

(7) Add 1 mL 1×Wash Buffer, and re-suspend Protein A/G Agarose, Centrifuge at 800 rpm for 2 min at 4℃, remove the supernatant, repeat 3 times.

(8) Elution of the Immune Complex.

a) Denatured elution: This method is suitable for SDS-PAGE and Western Blotting analysis of elution samples. Add 20-50 μL 1×SDS-PAGE Loading Buffer to the tube and mix well, incubate at 100℃ for 5 min, then Centrifuge at 800 rpm for 1 min, and collect the supernatant to a new tube for SDS-PAGE and Western Blotting analysis.

b) Non-Denatured elution: The eluted samples retained their original biological activity and could be used for subsequent functional analysis. Add 20-50 μL Elution Buffer to the tube and mix well, and incubate at room temperature for 10 min, then centrifuge at 800 rpm for 2 min at 4℃. Take the supernatant to collect the elution component, which is the target antigen. Transfer the supernatant to a new tube, and immediately add 1/10 Elution Buffer volume Neutralization Buffer to adjust the pH to 7.0-8.0, use the elution sample for subsequent functional analysis.

II Co-Immunoprecipitation

Refer to the steps of Immunoprecipitation.

Strawberry moment: The protein with high purity can be obtained with Universal IP/Co-IP Toolkit conveniently, and it also can be obtained with different tag mouse monoclonal antibody (Cat #: A02010AGB, A02040AGB, A02050AGB) by Immunoprecipitation or Co-Immunoprecipitation. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

Recommended Products

Catalog No.	Product Name	Recommended reason
KTP2070	PurKine™ Antibody Purification Kit (Protein A/G)	High throughput, high performance, high stability
A25012	IPKine™ HRP, Goat Anti-Mouse IgG LCS	Specifically identify the light chain of IgG and avoid the interference of heavy chain
A25022	IPKine™ HRP, Mouse Anti-Rabbit IgG LCS	Specifically identify the light chain of IgG and avoid the interference of heavy chain
A25112	IPKine™ HRP, Goat Anti-Mouse IgG HCS	Specifically identify the heavy chain of IgG and avoid the interference of light chain
A25122	IPKine™ HRP, Mouse Anti-Rabbit IgG HCS	Specifically identify the heavy chain of IgG and avoid the interference of light chain
ABT2013	Agarose Conjugated Anti-DDDDK Tag Mouse Monoclonal Antibody (1B10)	Featured DDDDK Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ABT2043	Agarose Conjugated Anti-HA Tag Mouse Monoclonal Antibody (4F6)	Featured HA Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ABT2063	Agarose Conjugated Anti-Myc Tag Mouse Monoclonal Antibody (2D5)	Featured Myc Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ABT2053	Agarose Conjugated Anti-His Tag Mouse Monoclonal Antibody (5C3)	Featured His Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.