Universal IP/Co-IP Toolkit (Magnetic Beads/Anti Rabbit)

Cat #: KTI1020-EN Size: 20 T

	Universal IP/Co-IP Toolkit (Magnetic Beads/Anti Rabbit)		
REF	**Cat #:** KTI1020-EN	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria		
	Storage: Store according to the recommended storage conditions of each component		

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 (Magnetic Beads/Anti Rabbit); After designed and researched, the toolkit contains: ① The optimized natural lysate can meet the needs of IP/Co-IP at the same time; ② Compared with Protein A or Protein G Magnetic Beads, Protein A/G Magnetic Beads 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 mouse anti-rabbit IgG light chain secondary antibody) can perfectly eliminate heavy chain interference. Universal IP/Co-IP Toolkit (Magnetic Beads/Anti Rabbit), 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 (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 (Magnetic Beads/Anti Rabbit) (Part 1 of 2)	Non-Denaturing Lysis Buffer	25 mL	4℃
	10×Wash Buffer	20 mL	RT
	Protein A/G Magnetic Beads	0.5 mL	4℃
	Elution Buffer	2 mL	4℃
	Neutralization Buffer	0.2 mL	4℃
Universal IP/Co-IP Toolkit (Magnetic	100×Proteinase Inhibitor Cocktail	0.2 mL	-20℃
	Rabbit IgG (1 mg/mL)	30 μL	-20℃

Beads/Anti Rabbit) (Part 2 of 2)	IPKine™ HRP, Mouse Anti-Rabbit IgG LCS	30 μL	-20℃

Materials Required but Not Supplied

- Magnetic Separation Rack
- Vertical rotating mixer
- Freezing Centrifuge
- Precision Pipettes, Disposable Pipette Tips
- Deionized Water
- PBS Buffer
- Dounce homogenizer (for tissues)
- 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℃.

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

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.

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

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℃.

Rabbit IgG: Ready to use as supplied. Used as a negative control for Rabbit IP antibody. 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 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)).

3. Extract protein for Bacteria Samples:

(1) Collect bacteria by centrifugation (1,2000 rpm for 2 min at 4℃) and washed by PBS once.

(2) Per mL bacteria add 100-200 μL Non-Denaturing Lysis Buffer, Ultrasonic break in ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times).

(3) Centrifuge at 12,000 rpm for 10 min at 4℃, collect supernatant.

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 Protein A/G Magnetic Beads to a 1.5 mL centrifuge tube, place the tube on Magnetic Separation Rack, let stand for 10 s, remove the supernatant.

Note: Protein A/G Magnetic Beads must be fully suspended before use, that is, fully invert the mixture several times to mix well.

(2) Add 1 mL 1×Wash Buffer, and re-suspend Protein A/G Magnetic Beads, place the tube on Magnetic Separation Rack, let stand for 10 s, 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) Place the tube on Magnetic Separation Rack, let stand for 10 s, use supernatant for Immunoprecipitation.

C. Immunoprecipitation

(1) Add 20 μL Protein A/G Magnetic Beads to a 1.5 mL centrifuge tube, place the tube on Magnetic Separation Rack, let stand for 10 s, remove the supernatant.

(2) Add 1mL 1×Wash Buffer, and re-suspend Protein A/G Magnetic Beads, place the tube on Magnetic Separation Rack, let stand for 10 s, remove the supernatant, repeat 3 times.

(3) Add 0.2-2 μg antibodies, and re-suspend Protein A/G Magnetic Beads, shake and incubate at room temperature for 30 min. Place the tube on Magnetic Separation Rack, let stand for 10 s, collect the supernatant, use the precipitation for Step (4). **Optional, IgG negative control: Add 0.2-2 μL Rabbit IgG, and re-suspend Protein A/G Magnetic Beads, shake and incubate at room temperature for 30 min. Place the tube on Magnetic Separation Rack, let stand for 10 s, 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 Magnetic Beads 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 Magnetic Beads, place the tube on Magnetic Separation Rack, let stand for 10 s, 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) Place the tube on Magnetic Separation Rack, let stand for 10 s, collect the supernatant, use the precipitation for Step (7).

(7) Add 1 mL 1×Wash Buffer, and re-suspend Protein A/G Magnetic Beads, place the tube on Magnetic Separation Rack, let stand for 10 s, 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℃. Transfer the supernatant to a new tube, and immediately add Neutralization Buffer (1/10

Elution Buffer volume) 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 #: ABT2014, ABT2044, ABT2054) by Immunoprecipitation or Co-Immunoprecipitation. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

FAQ

1. What is the difference between this kit and KTD104-EN?

A: Compared with KTD104-EN, the amount of magnetic beads increased to 0.5 mL (about 25 T of experimental amount) provides sufficient amount of magnetic beads components;An anti-rabbit light chain-specific IP secondary antibody and a negative control of the same species IgG (Rabbit IgG) were provided to more accurately subdivide the experimental scene and improve the cost performance. Denaturing lysate is omitted, kit components are simplified, and availability is improved.

2. Protein A/G Magnetic Beads consist of 450 μL in 20 T kit. Is it suspension volume or microbead volume?

A: This is the volume of suspension liquid.

3. Can centrifugation be used instead of magnetic separation rack to separate magnetic beads and supernatant?

A: Magnetic separation can not be replaced by centrifugal separation, centrifugation can not achieve complete separation of magnetic beads and supernatant, will lead to magnetic beads and supernatant together by absorption and abandonment, resulting in the loss of magnetic beads.

Recommended Products

Catalog No.	Product Name	Recommended reason
KTP2070	PurKine™ Antibody Purification Kit (Protein A/G)	High throughput, high performance, high stability
A25022	IPKine™ HRP, Mouse Anti-Rabbit IgG LCS	Specifically identify the light chain of IgG and avoid the interference of heavy chain
A25122	IPKine™ HRP, Mouse Anti-Rabbit IgG HCS	Specifically identify the heavy chain of IgG and avoid the interference of light chain
ABT2014	Magnetic Beads Conjugated Anti-DDDDK Tag Mouse Monoclonal Antibody (1B10)	Featured DDDDK Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ABT2044	Magnetic Beads Conjugated Anti-HA Tag Mouse Monoclonal Antibody (4F6)	Featured HA Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ABT2064	Magnetic Beads Conjugated Anti-Myc Tag Mouse Monoclonal Antibody (2D5)	Featured Myc Tag Mouse Monoclonal Antibody, designed for immunoprecipitation (IP) of tagged protein
ATB2054	Magnetic Beads 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.