IPKine™ Anti-DDDDK Magnetic IP Kit

Cat #: KTI2014 **Size:** 20 T/100 T

	Anti-DDDDK Magnetic IP Kit		
REF	**Cat #:** KTI2014	LOT	**Lot #:** Refer to product label
	Capacity: ⩾ 0.6 mg Flag-Tag protein/mL Magnetic Beads		**Beads Concentration:** 20 mg/mL
	Applications: IP		
	Reactivity: Mammals, Bacteria		
	Storage: Store according to the recommended storage conditions of each component, stable for 12 months		

Assay Principle

Anti-DDDDK-Tag (Flag-Tag) Magnetic Beads are prepared by covalently coupling Anti-Flag-Tag Mouse Monoclonal Antibody to crosslinked Magnetic Beads, useful for the detection and capture of fusion proteins containing a Flag-Tag peptide sequence by commonly used immunoprecipitation procedures. The Optimized Anti-Flag Magnetic Beads have more efficient antigen binding capacity. According to the structure, biological function and subsequent application requirements of the target protein, this kit provides three elution methods, including competitive elution of peptide, acid elution and denatured elution. Especially by using Flag peptide elution and acid elution will not contain heavy chain and light chain of antibody, which could effectively avoid the interference of heavy chain and light chain of antibody in Western Blotting experiment after immunoprecipitation.

Materials Supplied and Storage Conditions

Kit components		Size		Storage conditions
		20 T	100 T	
Part 1 of 2	Non-Denaturing Lysis Buffer	20 mL	100 mL	4℃
	TBS (10×)	20 mL	100 mL	4℃
	Anti-Flag Magnetic Beads	0.4 mL	2 mL	4℃, Avoid freeze
	Mouse IgG Magnetic Beads	80 μL	400 μL	4℃, Avoid freeze
	Elution Buffer	2 mL	10 mL	4℃
	Neutralization Buffer	0.2 mL	1 mL	4℃
Part 2 of 2	3×Flag Peptide (25×)	80 μL	80 μL×5	-20℃
	SDS-PAGE Loading Buffer (5×)	1 mL	2 mL×2	-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)

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

Anti-Flag Magnetic Beads: Ready to use as supplied. Store at 4℃, Avoid frozen.

Mouse IgG Magnetic Beads: Ready to use as supplied. Store at 4℃, Avoid frozen.

Elution Buffer: Ready to use as supplied. Store at 4℃. Used for acid elution of non-denatured proteins.

Neutralization Buffer: Ready to use as supplied. Store at 4℃. Used for neutralize acid elution of non-denatured proteins.

Working 3×Flag Peptide: Add 1×TBS to 3×Flag Peptide (25×), the dilute factor is 25 times, then obtain Working 3×Flag Peptide, place it on ice to be used. Used for competitive elution of non-denatured proteins. Store at -20℃.

SDS-PAGE Loading Buffer (5×): Ready to use as supplied. Store at -20℃.

Note: (1) Protease inhibitors are not necessarily added, it is recommended that different types of protease inhibitors should be added to the Non-Denaturing Lysis Buffer according to the experimental requirements; (2) It is recommended to use the centrifugal tube with low adsorption for experiment, which can reduce the adhesion of magnetic beads to the centrifugal tube wall. Adding 0.01%-0.1%(V/V) non-ionic detergent (such as Triton X-100, Tween-20 or NP-40) to 1×TBS can also effectively reduce the adhesion of centrifugal tubes to magnetic beads.

Assay Procedure

A. Preparation of protein samples

Note：Prepare a certain amount of sample proteins, which were used as whole cell lysate (WCL) for subsequent Western Blotting detection.

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, 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 the supernatant.

2. Extract protein for Tissue Samples:

(1) Tissue Samples: Weigh 0.1 g of tissue and add 1 mL Non-Denaturing Lysis Buffer, Homogenize tissue with 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 the supernatant.

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 the supernatant.

Note: (1) The sample must contain Flag-Tag protein and its complex; (2) For Immunoprecipitation, fresh samples are preferred; (3) In Immunoprecipitation experiments, the affinity between different antigen and antibody is different, and the binding of antigen to antibody is also affected by lysis buffer and wash buffer. If the Non-Denaturing Lysis Buffer does not provide the best experimental results, it is recommended to optimize the operation details or screen and prepare suitable by lysis buffer and wash buffer for experiment

B. Preparation of magnetic beads

Note: Per 500 μL of protein sample add 20 μL Anti-Flag Magnetic Beads. Perform the following procedures, according to add 20 μL Anti-Flag Magnetic Beads

(1) Add Anti-Flag Magnetic Beads to a 1.5 mL centrifuge tube. Place the centrifuge tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant.

(2) Add 1 mL 1×TBS to re-suspend Anti-Flag Magnetic Beads, place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant, repeat 3 times. Add 20 μL 1×TBS to re-suspend Anti-Flag Magnetic Beads.

C. Immunoprecipitation

(1) Add 500 μL protein samples to the processed Anti-Flag Magnetic Beads, and incubate at room temperature for 1-2 h or overnight at 4℃ (It is recommended to use vertical rotating mixer with Low-speed rotation).

Note: a) It is recommended to add Mouse IgG Magnetic Beads for immunoprecipitation in some samples as negative control, which could exclude the non-specific binding of IgG to the target protein or other specific biological molecules; b) In the case of very high background after immunoprecipitation using Anti-Flag Magnetic Beads, it is recommended that Mouse IgG Magnetic Beads be used to preprocess samples to eliminate non-specific adsorption, then use Anti-Flag Magnetic Beads for immunoprecipitation.

(2) Place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant. **The supernatant can be transferred to a new centrifuge tube to test the effect of immunoprecipitation.**

(3) Add 1 mL 1×TBS, and re-suspend Anti-Flag Magnetic Beads, place the tube on a Magnetic Separation Rack, let stand for 10 s, remove the supernatant, repeat 3-5 times, until OD280 of the supernatant is lesser than 0.05.

(4) Elution

a) Denatured elution: This method is suitable for SDS-PAGE and Western Blotting analysis of elution samples. Add 100 μL (5 times volume of Beads) 1×SDS-PAGE Loading Buffer (Dilute the SDS-page Loading Buffer (5×) by 5 times with 1×TBS) 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) Competitive elution of peptide: This method can maintain their original biological activity, elution can be used for functional analysis. Add 100 μL (5 times volume of Beads) Working 3×Flag Peptide to the tube and mix well, incubate at 4℃ for 1-2 h (It is recommended to use vertical rotating mixer with Low-speed rotation), then centrifuge at 800 rpm for 2 min at 4℃, and collect the supernatant which is Flag-Tag protein and its complex to a new tube. In order to improve the elution efficiency, the incubation time can be Increased or repeat elution. Place Flag-Tag protein and its complex on ice to be used, or store at -20℃/-80℃ for long-term. **It is recommended to add 100 μL 1×SDS-PAGE Loading Buffer to beads precipitation to test the effect of immunoprecipitation and elution.**

c) Acid elution: This method can maintain their original biological activity, elution can be used for functional analysis. Add 100 μL (5 times volume of Beads) Elution Buffer to the tube and mix well, incubate at room temperature for 5-10 min (It is recommended to use vertical rotating mixer with Low-speed rotation), then centrifuge at 800 rpm for 2 min at 4℃, and collect the supernatant which is Flag-Tag protein and its complex to a new tube, and immediately add 10 μL Neutralization Buffer to adjust the pH to 7.0-8.0. In order to improve the elution efficiency, elution can be repeated, and combine the same samples. Place Flag-Tag protein

and its complex on ice to be used, or store at -20℃/-80℃ for long-term. **It is recommended to add 100 μL 1×SDS-PAGE Loading Buffer to beads precipitation to test the effect of immunoprecipitation and elution.**

Note: a) For a few samples, due to differences in target proteins, the binding of Flag-Tag and Anti-Flag antibody is very strong, and the effect of Acid elution and Competitive elution of peptide may be poor. Therefore, SDS-PAGE Loading Buffer denaturation elution method is recommended as a priority; b) Due to the difference of target protein, the elution efficiency of acid elution method also varies to some extent. If the requirement of elution efficiency is high, the pH value of acidic eluent can be adjusted appropriately between 2.5-3.1, and the pH value or quantity of corresponding neutralizing solution should be adjusted appropriately. For example, 100 μL Acid Elution Buffer (0.1 M Glycine-HCl, pH 2.8) and 15 μL Neutralizing Buffer (1 M Tris-HCl, pH 8.5).

Typical Data

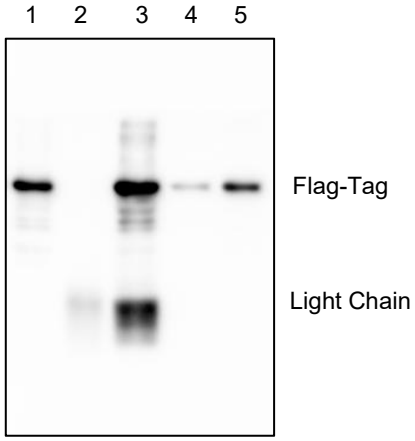

Figure. The immunoprecipitation effect of Anti-DDDDK Magnetic IP Kit used for Flag-Tag fusion protein.
Data provided for demonstration purposes only.

HEK293T cells were transfected with Flag-Tag plasmid, after 48 h, cells were lysed using Non-Denaturing Lysis Buffer after transfection, and then the sample was used for immunoprecipitation, Western Blotting primary antibody was Anti-DDDDK Tag Mouse Monoclonal Antibody (1B10) (Cat #: ABT2010), dilution factor is 1:5000; Secondary antibody was IPKine™ HRP, Goat Anti-Mouse IgG LCS (Cat #: A25112), dilution factor is 1:2000; SuperKine™ West Femto Maximum Sensitivity Substrate (Cat #: BMU102-EN) was used for development. Lane 1 was whole cell lysate (WCL); Lane 2 was the immunoprecipitation sample of Mouse IgG Magnetic Beads eluted by 1×SDS-PAGE Loading Buffer; Lane 3 was the immunoprecipitation sample of Anti-Flag Magnetic Beads eluted by 1×SDS-PAGE Loading Buffer. Lane 4 was the immunoprecipitation sample of Anti-Flag Magnetic Beads eluted by Elution Buffer; Lane 5 was the immunoprecipitation sample of Anti-Flag Magnetic Beads eluted by Working 3×Flag Peptide. By using peptide elution and acid elution, only contained Flag-Tag fusion protein, did not contain heavy and light chains of antibody.

FAQ

Problem	Cause	Suggested Solution
Very few or no tagged protein exists in the eluate	Protein is not completely eluted	Change elution methods
	No target protein expressed	Make sure the protein of interest contains the tagged protein by Western blotting or dot blotting analyses
	Very low protein expression level	1. Use larger volume of cell lysate 2. Optimize expression conditions to raise the protein expression level

	Washes are too stringent	Reduce the time and number of washes
	Incubation times are inadequate	Increase the incubation time
	Interfering substance is present in sample	Lysates containing high concentration of DTT, 2-mercaptoethanol, or other reducing agents may destroy antibody function, and must be avoided
	Detection system is inadequate	If Western blotting detection is used: 1. Check primary and secondary antibodies using proper controls to confirm binding and reactivity 2. Verify that the transfer was adequate by using prestained protein marker or staining the membrane with Ponceau S 3. Use fresh detection substrate or try a different detection system
Multiple protein bands found in the eluate	Proteins bind nonspecifically to the monoclonal antibody, insufficient washing on magnetic beads, or the microcentrifuge tubes	1. Pre-clear lysate with Mouse IgG Magnetic Beads to remove nonspecific binding proteins 2. After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before Magnetic separation
	Washes are insufficient	1. Increase the number of washes 2. Prolong duration of the washes, incubating each wash for at least 15 min 3. Choose other wash buffers. Increase the salt and/or detergent concentrations in the wash solutions 4. Centrifuge at lower speed to avoid nonspecific trapping of denatured proteins
	The protein is not stable at room temperature	Immunoprecipitation of the target protein at lower temperature, such as 4℃
	Protein degradation due to proteases activity during purification process	Add protease inhibitors to cell lysate

Recommended Products

Catalog No.	Product Name
KTD104-EN	Universal IP/Co-IP Toolkit (Magnetic Beads)
ABT2014	Magnetic Beads Conjugated Anti-DDDDK Tag Mouse Monoclonal Antibody (1B10)

Disclaimer

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