Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

IPKine™ ProteinA/G Magnetic Beads

Cat #: BMR2080 Size: 1 mL/5 mL

	ProteinA/G Magnetic Beads		
REF	**Cat #:** BMR2080	LOT	**Lot #:** Refer to product label
	Applications: IP, Co-IP		
	Coupled protein: Recombinant Protein A/G		**Capacity:** > 500 μg Rabbit IgG/mL
	Storage Buffer: PBS, 0.01% Tween-20, 0.02% NaN_3		**Beads Concentration:** 10 mg/mL
	Storage: Stored at 4℃ for 24 months, avoid frozen		

Assay Principle

Protein A and G are popular choices for Immunoprecipitation and antibody purification, because they are both stable and target selective. IgG class antibodies from multiple species bind to protein A and/or G, allowing antibody to be captured on protein-agarose microspheres. Protein A and G bind IgG subtypes with varying affinities, determined by species and the properties of the heavy chain. IPKine™ ProteinA/G Magnetic Beads provides a simple, rapid, and efficient method for Immunoprecipitation and Co-Immunoprecipitation.

Materials Supplied and Storage Conditions

Component	Size		Storage conditions
	1 mL	5 mL	
ProteinA/G Magnetic Beads	1 mL	5 mL	4℃, avoid frozen

Materials Required but Not Supplied

- Magnetic separation rack
- Vertical rotating mixer
- Freezing Centrifuge
- Precision pipettes, disposable pipette tips
- PBS
- Dounce homogenizer (for tissues)
- Normal IgG of the same species as antibody
- SDS-PAGE Loading Buffer

Reagent Preparation

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

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22 or 0.45 μm filter before use. For most proteins, the following recommended buffers can be used:

Lysis Buffer： 50mM Tris-HCl, 0.15 M NaCl, 1% IGEPAL-CA630, 1mM PMSF, pH7.4

Wash Buffer： 0.15 M NaCl, 20 mM Na_2HPO4, pH 7.0

Elution Buffer： 0.1 M Glycine, pH 3.0

Neutralization Buffer： 1 M Tris-HCl, pH 8.5

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 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 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 Lysis Buffer, Homogenize tissue with Liquid nitrogen or Dounce homogenizer.

(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 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 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 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, when the volume is less than 500 μL, fill with Wash

Buffer, 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 μg Normal IgG of the same species as antibody, 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.**

4. Add 1 mL 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 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.

(1) 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 place the tube on Magnetic Separation Rack, let stand for 10 s, and collect the supernatant to a new tube for SDS-PAGE and Western Blotting analysis.

(2) 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 place the tube on Magnetic Separation Rack, let stand for 10 s. 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.

Attached table

Species	Subtypes	Protein A	Protein G	Protein A/G
Human	IgA	Varible	—	++
	IgD	—	—	—
	IgE	—	—	—
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	—	++++	++++
	IgG4	++++	++++	++++
	IgM	Varible	—	—
Avian egg yolk	IgY	—	—	—
Cow		++	++++	++++
Dog		++++	++	++++
Goat		—	++++	++++
Guinea pig	IgG1	++++	++	++++
Hamster	IgG2	++++	++	++++

Horse	Total IgG	++	++++	++++
Koala		—	+	
Llama		—	+	
Monkey (rhesus)		++++	++++	++++
Mouse	IgG1	+	++++	++
	IgG2a	++++	++++	++++
	IgG2b	+++	+++	+++
	IgG3	++	+++	+++
	IgM	Varible	—	—
Pig		+++	+++	++++
Rabbit	Total IgG	++++	+++	++++
Rat	IgG1	—	+	++
	IgG2a	—	++++	++++
	IgG2b	—	++	++
	IgG3	+	++	++
Sheep	Total IgG	+/-	++	++

Table 1. The binding capacity of Protein A, Protein G, and Protein A/G to different antibodies, ++++=Strong combination ability; ++=Medium binding ability; —=Weak or no binding ability

Recommended Products

Catalog No.	Product Name
KTD104	Universal IP/Co-IP Toolkit (Magnetic Beads)
KTI1010	Universal IP/Co-IP Toolkit (Magnetic Beads/Anti-Mouse)
KTI1020	Universal IP/Co-IP Toolkit (Magnetic Beads/Anti-Rabbit)

Disclaimer

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