

Immunoprecipitation Kit (With HRP-conjugated protein A)

Catalog Number: PK10007

Description

Immunoprecipitation (IP) is an experimental technique that can purify and separate target proteins by utilizing the affinity-binding properties of antigens and antibodies. The kit contains all reagents for immunoprecipitation and subsequent Western blotting detection. It provides the best solution for immunoprecipitation experiments by efficiently and rapidly separating the concentrated target proteins. Meanwhile, the kit also has the advantages of time saving, high efficiency, and low antibody dosage and so on.

About the Kit

- (1) IP lysis buffer can effectively extract total protein of cells or tissues for subsequent experiments.
- (2) rProtein A/G beads slurry is used to separate antigen-antibody complexes by precipitation.
- (3) Elution buffer with high salt and low pH is effectively for the dissociation of antigen-antibody complexes and rProtein A/G beads.
- (4) The use of spin columns makes the operation more convenient and faster, and reduces the loss of target proteins, while reducing the influence of non-specific proteins.
- (5) HRP-conjugated Protein A secondary antibody is used for the detection of Western blotting, which can effectively eliminate the influence of the light chain signal of the antibody and reduce the heavy chain signal of the antibody.
- (6) The kit can be widely used in immunoprecipitation (IP) and immunocoprecipitation (CoIP) experiments.

Components

Components	20 times	Storage
IP lysis buffer: 30 ml/bottle	1 bottle	6 months at RT
Incubation buffer: 20 ml/bottle	1 bottle	6 months at RT
100 × Protease inhibitor: 2 ml /vial	1 vial	6 months at -20 °C
20 × Washing buffer: 20 ml /bottle	1 bottle	6 months at RT
rProtein A/G beads slurry: 1 ml/vial	1 vial	6 months at 4 °C
Elution buffer: 2.5 ml/vial	1 vial	6 months at 4 °C
Alkali neutralization buffer: 250 ul/vial	1 vial	6 months at 4 °C
5 × Sample buffer: 800 ul/vial	1 vial	6 months at RT
HRP-conjugated Protein A: 200 ul/vial	1 vial	6 months at 4 °C
Spin columns: 1 ml	20 units	12 months at RT
Collection tubes: 2 ml	20 units	12 months at RT
End caps	20 units	12 months at RT

*It is recommended not to use the kit after expiration.

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Package

Questions and Answers

Questions 1: Target protein not obtained ?

Reason a: High concentration of detergent affects the antigen-antibody binding.

Solution a: Prepare high-concentration cell or tissue lysates and dilute with incubation buffer before use.

Reason b: Low expression or degradation of target protein in lysates.

Solution b: Increase the amount of total protein of lysates used; Verify target protein expression in the lysate by Western blotting; Replace with fresh protease inhibitors.

Reason c: The antibody does not bind to the corresponding antigen.

Solution c: Replace antibodies that recognize different epitopes and increase the amount of antibodies used.

Reason d: Antigen-antibody complexes are not precipitated by rProtein A/G beads.

Solution d: Antibodies belong to IgM subtype, and antigen-antibody complexes are precipitated using anti-IgM-conjugated microparticles.

Questions 2: Antibody signal of eluate affects the signal of target protein ?

Reason: The molecular weight of target protein is around 45-55 kDa.

Solution: Choose to use HRP-conjugated Mouse Anti-Rabbit IgG Light Chain Specific secondary antibody (when both capture and detection antibodies are rabbit antibodies); Two different species of antibodies were used for IP and subsequent Western blotting analysis respectively.

How to choose a

secondary antibody for immunoprecipitation

For technical support and original validation data for this product please contact
 T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or
 1(312) 455-8498 (outside USA)

E: proteintech@ptglab.com
 W: ptglab.com

Mouse McAb/PcAb

Capture Antibody Types for IP

Primary Antibody Types for WB

Secondary Antibody Types for WB

Mouse McAb

Mouse PcAb

This product is exclusively available under Proteintech Group brand and is not available to purchase from any other manufacturer.

	Rabbit antibody	HRP-conjugated anti-Rabbit IgG second ary antibody
Rabbit antibody	Mouse McAb	HRP-conjugated anti-Mouse IgG second ary antibody
	Mouse PcAb	
	Rabbit antibody	HRP-conjugated Protein A; HRP-conjuga ted anti-rabbit IgG light chain specific a ntibody

Cautions

- *The kit is only used for IP and CoIP experiments of cell or tissue lysates.
- *Unless otherwise specified, all operations were done on ice.
- *Avoid skin and eyes contact with the Alkali neutralizing solution.