Anti-Flag magnetic beads

Description

Anti-Flag magnetic beads is based on hydroxyl magnetic beads covalently coupling with high quality recombinant mouse monoclonal antibody. With high loading of Flag-tagged protein (more than 1.1 mg protein/mL) and high specificity, it is recommended to use for co-immunoprecipitation and protein purification.

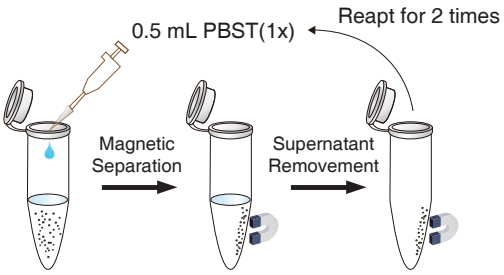
Components

Content	Cat# : B26101	Cat# : B26102
Magnetic beads	1 mL	5 mL

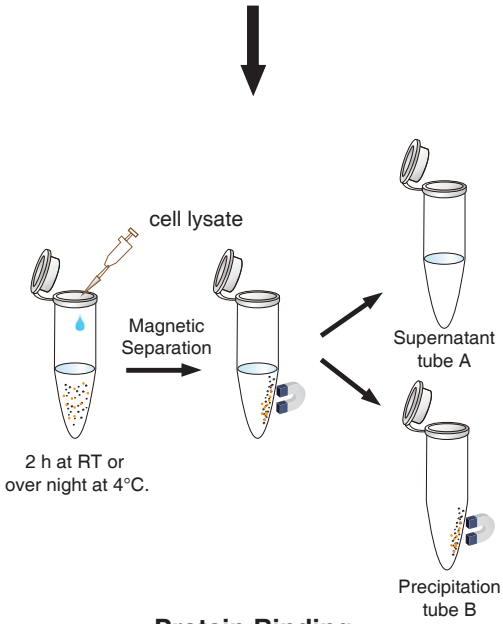
Storage

Store at 2-8°C for 2 years. DO NOT freeze or centrifuge Magnetic Beads.

Protocol



Magnetic Beads Preparation

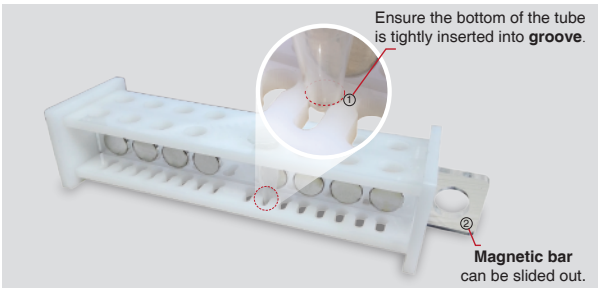


Protein Binding

Magnetic Beads Preparation

1. Suspend the Anti-Flag magnetic beads in the vial (pipet gently for 10 times, don't vortex). Transfer 10 µL (the amount may be scaled up or down as required) Anti-Flag Magnetic Beads suspension to a new tube.
2. Add 0.5 mL PBST(1x) buffer. pipet gently for 5 times Anti-Flag magnetic beads. Place the tube on the magnet to separate the beads from the solution for 10 s and remove the supernatant. Repeat this step for 2 times.

Note: Prepare all Magnetic Beads together in one large tube and then divide it into aliquots if samples are in batch.



Magnetic separator

Protein Binding

3. Add 500 µL of cell lysate to the washed magnetic beads. Gently rotate the tube for 2 h at room temperature or over night at 4°C.
4. Place the tube on the magnet to separate the beads from the solution for 10 s and then transfer the supernatant into a new tube for detecting whether Flag-tag protein is residual.

Note: During the binding process, it won't affect the result if magnetic beads occasionally cluster together.

※ **Please continue reading the protocol overleaf .**

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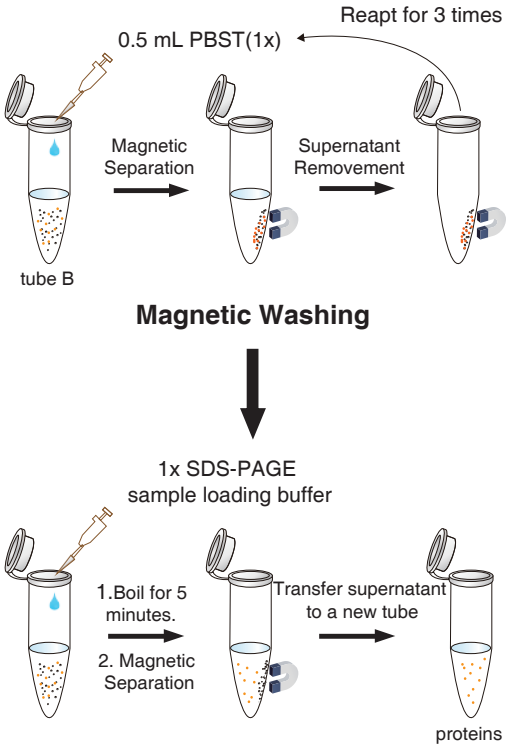

Magnetic Washing

5. Add 500ul PBST(1x) to the tube, resuspend the magnetic beads by pipeting gently. then rotate the tube for 5 mins. place the tube on the magnet to separate the beads from solution for 10 s and remove the supernatant.
6. Repeat step 5 for about 3 times until the OD280 of the supernatant reads is less than 0.05.

Note: Increase washing times if the OD280 of the supernatant reads >0.05.

Elution and Detectione

7. Choose elution methods according to the downstream application. For direct detection of target protein, add 50 µL 1x SDS-PAGE sample loading buffer into the magnetic beads of step 6. Boil it for 5 minutes. Place the tube on the magnet to separate the beads from the solution for 10 s and transfer the supernatant into a new tube for SDS-PAGE.

Trouble Shooting

Problems	Possible Reasons	Suggested Improvements
High background	Nonspecifically binding of proteins to the antibody, megnetic beads or EP tubes	Pre-clear lysate to remove nonspecific binding proteins. After suspending beads for the final wash, transfer the entire sample to a clean EP tube and then centrifugation.
	Washing times are not sufficient.	Increase the number of washes.
No signal is observed.	Flag tagged protein is not expressed in the sample.	Make sure the protein of interest contains the FLAG sequence. Prepare the fresh lysate. Use appropriate protease inhibitors.
	Incubation times are inadequate.	Increase the incubation times.
	Interfering substance is present in sample.	The lysate may contain high concentrations of dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents. Excessive detergent concentration may interfere with the antibody-antigen interaction.