

Anti-HA magnetic beads

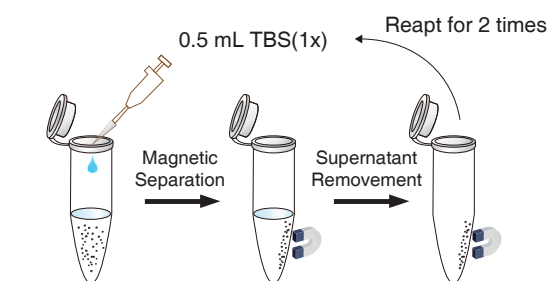
Description

Anti-HA magnetic beads kit is based on hydroxyl magnetic beads covalently coupling with high quality mouse IgG2b monoclonal antibody. With high loading of HA-tagged protein (more than 0.6 mg protein/mL) and high specificity, it is recommended to use for co-immunoprecipitation and protein purification.

Storage

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

Protocol

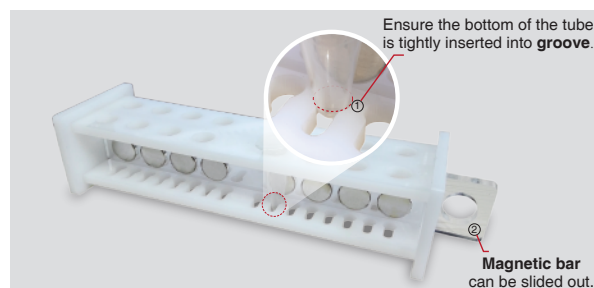


Magnetic Beads Preparation

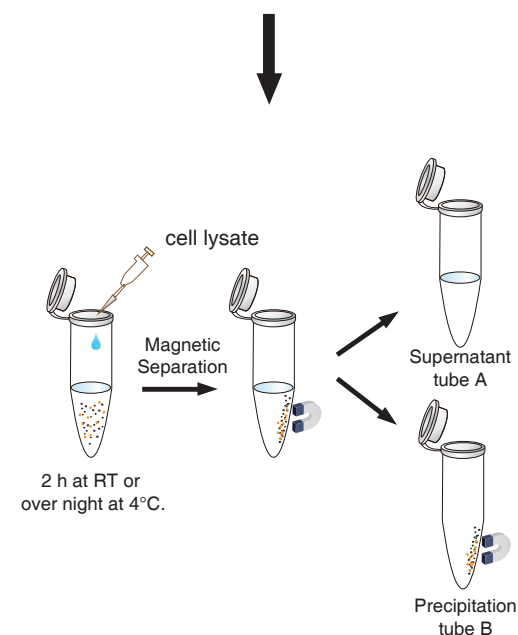
Magnetic Beads Preparation

1. Suspend the Anti-HA magnetic beads in the vial (pipet gently for 10 times, don't vortex). Transfer 10 μ L (the amount may be scaled up or down as required) Anti-HA Magnetic Beads suspension to a new tube.
2. Add 0.5 mL TBS buffer (50 mM Tris HCl, 150 mM NaCl, pH 7.4). Pipet gently for 5 times Anti-HA magnetic beads. Place the tube on the magnet to separate the beads from the solution for 10 sec 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

Protein Binding

3. Add 500 μ L 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 sec and then transfer the supernatant into a new tube for detecting whether HA-tag protein is residual.

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

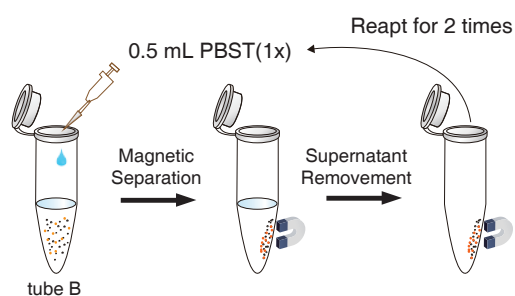
✘ **Please continue reading the protocol overleaf .**



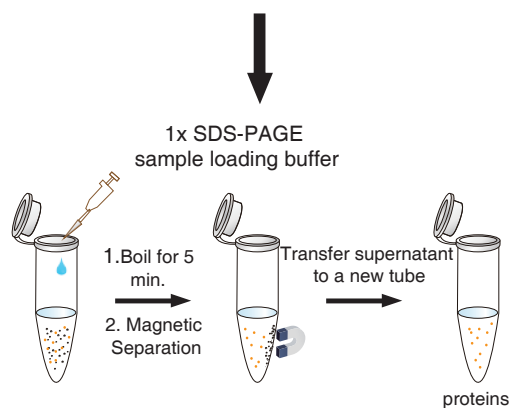
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Magnetic Washing



Elution and Detection

Magnetic Washing

5. Add 500 μ L PBST(1x) to the tube (NaCl 136.89 mM; KCl 2.67 mM; Na_2HPO_4 8.1 mM; KH_2PO_4 1.76 mM; 0.5% Tween20), resuspend the magnetic beads by pipeting gently. Then rotate the tube for 5 min. Place the tube on the magnet to separate the beads from solution for 10 sec and remove the supernatant.

6. Repeat step 5 for about 2 times. If the non-specific impurity proteins are left over, please extend the cleaning time, increase the cleaning times or properly enlarge the detergent content in the cleaning solution.

Elution and Detection

Choose different elution methods according to the downstream use. For IP, go to the step 7-8. For protein purification, go to step 9-10 for low pH elution.

Denaturing elution (suitable for IP experiments using Anti-HA beads):

7. For direct detection of target proteins, add 50 μ L 1x protein sample loading buffer in the precipitation mentioned above, boil for 5 min, chill to room temperature and then place the tube on the magnet to separate the beads from the solution for 10 sec.

8. Detect the supernatant by SDS-PAGE.

Low-pH elution (Suitable for protein purification by Anti-HA beads):

9. Add the 0.1 M glycine HCl (pH 3.0) elution buffer into the product of step 6, and incubate at shaker for 5 min (The elution time should be less than 20 min). Generally, the volume of elution buffer is 5 times of the gel.

10. Centrifugation the products obtained from the above step at 5000 rpm for 30 sec. Then transfer the elution product quickly into 1 M Tris (pH 8.0) for neutralization until the pH is near neutral.

Trouble Shooting

Problems	Possible Reasons	Suggested Improvements
High background	Nonspecifically binding of proteins to the antibody, magnetic 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.	HA tagged protein is not expressed in the sample.	Make sure the protein of interest contains the HA 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.



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