BioLinkedIn
LAB ON THE MAGNETICBEADS

Anti-Flag Magnetic Beads

Product Description

The Biolinkedin® Anti-Flag Magnetic Beads are used for the immunoprecipitation (IP) of specific Flag-tagged proteins expressed in human in vitro expression systems and bacterial and mammalian cell lysates. Anti- Flag magnetic beads are based on carboxyl magnetic beads, with 200 nm particle size, covalently coupling with high quality mouse IgG monoclonal antibody that recognizes the Flag-epitope tag (DYKDDDDK). For IP, the beads are added to a sample containing Flag-tagged proteins. The bound Flag-tagged proteins are dissociated from the beads using an elution buffer. The beads are removed from the solution manually using a magnetic stand or by automation using an instrument.

Product Features

Composition	mouse IgG monoclonal Ab
Magnetization	Superparamagnetic
Particle size	200 nm
Concentration	10 mg/mL
Binding Capacity	≥ 0.6 mg Flag-tagged fusion protei n/mL of beads
Application	IP，CoIP
Storage Condition	Store at 4°C for 2 years.

Protocol

1. Cell lysis

Cells may be lysed using any standard cell lysis protocol compatible with your starting material. We recommend the use of **Biolinkedin® IP Lysis/Wash Buffer**. Add protease inhibitor (such as PMSF at 1mM) if needed.

2. Preparation of Magnetic Beads

2.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

2.2 Transfer 25-50 µL of Anti-Flag Magnetic Beads into a 1.5 mL tube (Transfer amount may be adjusted as required).

2.3 Add 500 µL of IP Lysis/Wash Buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Herein after referred to as magnetic separation). Remove and discard the supernatant. Repeat this step twice.

3. Immunoprecipitation

3.1 Remove the tubes from the magnetic separator and add your sample containing Flag-tagged protein to the pre-washed magnetic beads and incubate at room temperature for 2h or overnight at 4°C with mixing.

3.2 Collect the beads with a magnetic stand, remove the unbound sample and collect for analysis.

3.3 Add 500µL of IP Lysis/Wash Buffer to the tube and gently mix. Collect the beads and discard the supernatant. Repeat wash twice.

4. Elution

Note: Select one of the elution protocols below. If the eluted Flag-tagged protein will be used for function applications or is sensitive to pH extremes, then elute the protein with Flag Peptide.

Gentle Elution Protocol

1. Prepare Flag Peptide at 0.2-1mg/mL in TBS.
2. Add 100 uL of 0.2-1mg/mL Flag Peptide to the beads, gently vortex to mix and incubate the sample at 4°C on a rotator for 2h-6h.
3. Separate the beads on a magnetic stand and save the supernatant containing the target antigen.
4. Repeat elution step once for higher recovery.

Chemical Elution Protocols

• Basic Elution

1. Add 100µL of 1x SDS-PAGE loading buffer to the tube.
2. Boil for 5 minutes on a dry bath.
3. Magnetically separate the beads and save the supernatant containing the target antigen.

• Acidic Elution

1. Add 100µL of 0.1M glycine, pH 3.0.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 minutes.
3. Magnetically separate the beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 20µL of Neutralization Buffer (1 M Tris pH 8.5) for each 100µL of eluate.

The final solution can be used as samples for denaturing SDS-PAGE.

Troubleshooting

Problem	Possible Cause	Solution
High background band	Non-specific binding of proteins to antibodies, insufficient washing on magnetic beads or EP tubes	The lysate was pretreated to remove non-specific proteins; before the last wash, the entire sample was transferred to a new EP tube and centrifuged.
	Not enough washing	Increase the time and number of washes
No protein band	No or minimal tagged protein was expressed	Verify protein expression; Prepare fresh lysate; Use the appropriate protease inhibitor.
	Insufficient incubation time	Increase incubation time
	Interfering substances in the sample	High concentrations of DTT, 2-mercaptoethanol or other reducing agents are present in the lysate.