

Product Data Sheet

Product Name: Anti-Flag Magnetic Beads

Cat. No.: GK30004

Features

Applications	Contents: High-quality anti-Flag monoclonal antibody-conjugated magnetic beads. Bead diameter: 200 nm. Antibody isotype: Mouse IgG 1. Fusion protein binding capacity: >0.6 mg/mL. Applications: IP, Co-IP, Flag-tag protein purification. Recommended volume: 10 μ L of beads per 500 μ L of cell lysate.
Shipping	Blue ice transportation
Storage	Store at 4°C for 2 years, do not freeze
Usage	For Research Use Only! Not For Use in Humans.

Protocol

1. Magnetic Bead Pretreatment

Resuspend Anti-Flag Magnetic Beads. Place 10 μ L of beads in a 1.5 mL EP tube. Add 500 μ L of wash buffer, mix thoroughly, and separate on a magnetic rack. Discard the supernatant. Repeat the wash steps twice.

2. Sample Binding

Add 500 μ L of cell lysis buffer to the magnetic beads obtained above, mix thoroughly, and incubate on a shaker for 2 hours at room temperature or overnight at 4°C. Separate on a magnetic rack and discard the supernatant.

Note: During the binding process, the beads may clump or become flaky. This is normal and will not affect the experimental results.

3. Washing

Add 500 μ L of wash buffer to the magnetic beads obtained above, mix thoroughly, separate on a magnetic rack, and discard the supernatant. Repeat the wash steps three times. Continue washing until the OD280 of the washed supernatant is less than 0.05.

Note: If the OD280 of the supernatant is greater than 0.05, increase the number of washes as needed.

4. Elution

This manual provides three Flag-tagged protein elution protocols. The user can choose a different elution protocol based on the needs of subsequent analysis.

a. Denaturing Elution: Samples eluted with this method are suitable for SDS-PAGE analysis.

Steps: Separate the magnetic beads, discard the supernatant, add 50 μ L of 1 $\times$ SDS-PAGE Loading Buffer to the beads, mix thoroughly, and heat at 95°C for 5 minutes. Separate the beads, collect the supernatant into a new EP tube, and perform SDS-PAGE analysis.

b. Acidic Elution: Samples eluted with this method retain their original biological activity and can be

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used for subsequent functional analysis.

Steps: Separate the magnetic beads, discard the supernatant, and add 50 μ L of elution buffer to the beads. Mix well with buffer A and incubate at room temperature for 10 minutes. Separate the magnetic beads and collect the supernatant into a new EP tube.

Add 25 μ L of neutralization buffer per 50 μ L of eluate to adjust the pH of the eluted product to neutral. The sample will be used for later functional analysis.

c. Competitive elution method: The sample eluted with this method retains its original biological activity and can be used for later functional analysis.

Steps: Separate the magnetic beads, discard the supernatant, add 3-5 times the volume of elution buffer B to the magnetic beads, mix well, and incubate at room temperature for 1 hour or at 4°C for 1-2 hours. Separate the magnetic beads and collect the supernatant into a new EP tube. This is the target protein and the sample will be used for later functional analysis.

Precautions

1. The pH of this product is 6-8. Do not freeze.
2. Avoid centrifugation, drying, or freezing this product. Do not expose it to a magnetic field for extended periods, as this may cause the magnetic beads to aggregate. Handle gently during the procedure to avoid antibody shedding.
3. Before using this product for IP experiments, confirm the expression of the Flag-tagged fusion protein in the sample.
4. To minimize protein degradation, use protease inhibitor cocktails (Catalog Numbers: GK10014, GK10019).
5. Do not use cell lysate samples containing dithiothreitol (DTT), as DTT may cause the Flag antibody to detach from the magnetic beads.
6. This product is for professional research use only and is not intended for clinical diagnosis or treatment, or for use in food or medicine.
7. For your safety and well-being, please wear a lab coat and disposable gloves when handling this product.

Frequently Asked Questions

1. Is the test result band background too high?
 - A. This may be due to nonspecific binding of proteins to the antibody, magnetic beads, or EP tubes. We recommend pre-treating the lysate to remove nonspecifically bound proteins. Before the final wash, transfer the entire sample to a new EP tube and perform magnetic separation or centrifugation.
 - B. Inadequate washes can also result in high background bands. We recommend increasing the

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wash time and number of washes.

2. Is the test result bandless?

A. Failure to express the Flag-tagged fusion protein may result in bandless results. Recommended actions: a. Ensure the target protein is Flag-tagged; b. Prepare fresh lysate;

c. Use appropriate protease inhibitors.

B. Insufficient incubation time may result in bandless results. We recommend extending the incubation time.

C. The lysate contains high concentrations of interfering substances such as DTT, 2-mercaptoethanol, or other reducing agents. We recommend selecting an appropriate lysate.

Background

Anti-Flag Magnetic Beads are prepared by covalently coupling high-quality mouse IgG1 monoclonal antibodies to amino-magnetic beads. They have a high loading capacity (>0.6 mg protein/mL) for Flag (DYKDDDDK)-tagged fusion proteins and can be used for immunoprecipitation (IP), co-immunoprecipitation (Co-IP), and small-scale protein purification of Flag-tagged proteins from bacterial and mammalian cell lysates, as well as in vitro expression systems.

Storage : Stored at 4°C, and is stable for up to 2 years.

Do not centrifuge, dry or freeze the magnetic beads.

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