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# Anti-Flag Magnetic Beads (DYKDDDDK)

## Catalog No: tcmt1188

### Available Sizes

Size: 1ml

Size: 2ml

Size: 5ml

### Specifications

Application:
IP (immunoprecipitation)

Form:
Liquid , 200 nm particle size.

Storage Instruction:
Stored at 4°C, and is stable for up to 2 years. Do not centrifuge, dry or freeze the magnetic beads.

Tags:
DYKDDDDK

### References

•Signal Transduct Target Ther. 2020 Dec 26;5(1):296 •Mol Oncol. 2020 Dec 13 •J Med Virol. 2020 Aug 10

### Product Description

Anti-Flag magnetic beads are based on amino magnetic beads, with 200 nm particle size, covalently coupling with high quality mouse IgG1 monoclonal antibody that recognizes the Flag octapeptide sequence (DYKDDDDK).

During immunoprecipitation, only a small amount of magnetic beads are needed, and the non-specific binding is low.

Advantage as follows;

• Convenient and time saving.

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- Low non-specific binding.
- Minimal sample loss.
- Protein binding capacity up to 0.6 mg/mL.
- Stable, one bottle solution.

## Contains

| Cat. No. | Product Name | Package |
|---|---|---|
| tcmt1188 | Anti-Flag Magnetic Beads | 1mL |
| tcmt1188 | Anti-Flag Magnetic Beads | 1mL × 2 |
| tcmt1188 | Anti-Flag Magnetic Beads | 1 mL × 5 |

## General Information

Anti-Flag magnetic beads are used for immunoprecipitation (IP) of specific Flag-tagged proteins expressed in bacterial and mammalian cells and in vitro expression systems. Anti-Flag magnetic beads are based on amino magnetic beads, with 200 nm particle size, covalently coupled with high quality mouse $IgG_1$ monoclonal antibody that recognizes the Flag octapeptide sequence (DYKDDDDK). Magnetic beads are removed from the solution manually by using a magnetic stand or automatically by using an instrument. With high loading of Flag-tagged protein and high specificity, Anti-Flag Magnetic Beads are also suitable for Co-immunoprecipitation and purification of Flag-tagged protein.

## Characteristics

| | |
|---|---|
| Composition | Mouse $IgG_1$ monoclonal antibody covalently coupled to a blocked magnetic bead surface |
| Bead Diameter | 200 nm |
| Binding Capacity | >0.6 mg protein/mL of beads |
| Application | IP, Co-IP, Protein Purification |
| Recommended Dose | 10 µL for per 500 µL cell lysates |

## General Protocol

### Recommended Buffer

| | |
|---|---|
| Wash Buffer | TBS: 50 mM Tris-HCl, 150 mM NaCl, pH 7.4 |
| Elution Buffer A | 0.15 M Glycine, 0.5% Triton X-100 or Tween-20, pH 2.5-3.1 |
| Elution Buffer B | 1 mg/mL 3× Flag peptide, 50 mM Tris, 0.15 M NaCl, pH 7.4 |
| Neutralization Buffer | 1 M Tris-HCl, pH 8.0 |

### 1.Preparation of Magnetic Bead

1) Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times, do not vortex). Transfer 10 µL of Anti-Flag Magnetic Beads suspension into a new tube.

2) Add 500 µL of 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. Remove and discard the supernatant. Repeat this step for 2 times.

### 2. Protein Binding

1) Add 500 µL of cell lysate (the sample containing Flag-tagged protein) to the washed beads. For Ag binding, incubate for 2 hours at room temperature or overnight at 4°C while gently rotating the tube.

2) Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.

Note : Occasional aggregation of magnetic beads during the binding process doesn't affect experimental results.

### 3. Washing

Add 500 µL of Wash buffer to the Magbeads-Ag complex and mix gently. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant. Repeat this step for 4 times.

### 4. Elution & Detection

Three elution methods are recommended according to protein characteristics or further usage:

1) Elution with sample buffer for gel electrophoresis and immuoblotting.

Add 50 µL of 1× SDS-PAGE loading buffer to each tube and boil for 5 minutes. Cool and place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. Keep the supernatant containing the target antigen for SDS-PAGE analysis.

2) Elution with Elution Buffer A under acidic condition.

Add 50 µL of Elution Buffer A to each tube. Incubate with gentle shaking or on a rotator for 10 minutes at room temperature. Place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. Adding 25 µL of Neutralization Buffer for each 50 µL of eluate to neutralize the low pH, which may help preserve bioactivity of target protein.

3) Elution with Elution Buffer B under native condition.

Add 3-5 (v/v) volume of Elution Buffer B to each tube. Incubate with gentle shaking or on a rotator for 1 hour at room temperature or 2 hours at 4°C. Place the tube into a magnetic stand to collect the beads and transfer the supernatant to a new tube. For immediate use, store the eluates at 4°C, or store at -20°C for long term storage.

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## Precautions

1. The pH of Anti-Flag Magnetic Beads is 6-8.
2. Do not centrifuge, dry or freeze the magnetic beads. Centrifuging, drying or freezing will cause the beads to aggregate and lose binding affinity.
3. For the best results, determine optimal conditions for expression of Flag-tagged fusion protein before attempting immunoprecipitation.
4. To minimize protein degradation, protease inhibitor cocktails.
5. For the best experimental performance, it is recommended to use a magnetic Stand.
6. Do not use cell lysate containing dithiothreitol (DTT). DTT may cause the Anti-Flag antibody to leach from the beads.
7. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Trouble Shooting

| Problem | Possible Cause | Solution |
|---|---|---|
| High background | Nonspecific binding of protein 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 clear EP tube and then use magnetic separation or centrifugation |
| | Washing times were not sufficient | Increase the number and time of washes |
| Little or no Flag-tagged protein is detected | No or minimal tagged protein was expressed | Verify protein expression by SDS-PAGE or Western blot by using an Flag-tagged positive control |
| | | Increase the amount of lysate used for IP |
| | Tagged protein degraded | Prepare fresh lysate |
| | | Use appropriate protease inhibitors |
| | Incubation time was inadequate | Prolong the incubation time |
| | Interfering substance was contained | Do not use cell lysate containing dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents |
| | | Excessive detergent concentration may interfere with the antibody-antigen interaction |