



# Anti-DYKDDDDK (Flag) MagBeads

## Product description

Anti-DYKDDDDK (Flag) MagBeads are magnetic polymer beads. They are made of high quality murine monoclonal antibodies and carboxyl magnetic beads. They provide rapid magnetic responsiveness. Superparamagnetism, good dispersion, uniform particle size, very low non-specific binding and abundant binding sites. It can be used for Flag labeled protein immunoprecipitation (IP).

Anti-DYKDDDDK (Flag) MagBeads can bind to the Met modified N-flag fusion Protein (MET-flag-Protein), N-flag fusion protein (Flag-protein), C-terminal FLAG Fusion Protein (protein-flag).

## Components

Components No.	Name	20565ES76	20565ES03	20565ES08
20565	Anti-DYKDDDDK (Flag) MagBeads	500 $\mu$ L	1 mL	5 mL

## Specifications

Clone	1E6
Isotype	Mouse IgG2b
MagBeads Concentration	10 mg/mL
Protein binding capacity	$\geq$ 0.6 mg protein/mL
Storage buffer	ddH <sub>2</sub> O

## Storage

The products should be stably stored at 2~8°C for 1 year. Do not freeze!

## Instructions

### 1. Sample preparation

Note: The sample may be a bacterial fermentation broth or a cell lysate. It should not contain insoluble particles, which can be filtered by 0.22  $\mu$ m filter membrane or centrifuged by 10,000 $\times$ g for 15 min.

### 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).Transfer 25-50  $\mu$ L of Anti-Flag MagBeads into a 1.5mL tube(Transfer amount maybe adjusted as required).

2.2 Add 500  $\mu$ L of IP Lysis/Wash Buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the 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.

2.3 Repeat step 2.2 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 2 h 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.

##### 4.1 Gentle Elution

4.1.1 Prepare Flag Peptide at 0.2-1mg/mL in TBS.

4.1.2 Add 100 µL 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 2 h-6 h.

4.1.3 Separate the beads on a magnetic stand and save the supernatant containing the target antigen.

4.1.4 Repeat elution step once for higher recovery.

##### 4.2 Acidic Elution

4.2.1 Add 100 µL of 0.1 M glycine, pH3.0.

4.2.2 Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 minutes.

4.2.3 Magnetically separate the beads and save the supernatant containing the target antigen.

4.2.4 To neutralize the low pH, add 20 µL of Neutralization Buffer (1 M Tris pH 8.5) for each 100 µL of eluate. The final solution can be used as samples for denaturing SDS-PAGE.

##### 4.3 Basic Elution

4.3.1 Add 100 µL of 1xSDS-PAGE loading buffer to the tube.

4.3.2 Boil for 5 minutes on a dry bath.

4.3.3 Magnetically separate the beads and the supernatant was taken for SDS-PAGE detection..

#### Notes

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. For research use only.