#M20118

# Anti-DYKDDDDK-Tag mAb (Magnetic Beads) (Same as Sigma's Anti-FLAG?)

□ 100μl
□ 500μl
□ 5000μl

**Orders** ■ 400-6123-828
orders@ab-mart.com
**Web** ■ www.ab-mart.com.cn

**Magnetic bead diameter** 200-300nm
**Conjugated antibody** Anti-flag monoclonal antibody

## Package:

Volume:1ml;10mg magnetic beads, supplied as a 10% suspension ( w/v ) in 10mM sodium phosphate, 150mM sodium chloride, pH 7.4, and 0.01% (v/v) Proclin 300.
The beads are recommended to be stored at 2~8℃. The product is stable for 24 months at 2~8℃. Freezing the magnetic beads will irreversibly damage the bead structure.
Isolation of fusion proteins containing flag tag sequence

## Applications:

### Product Description

Anti-Flag Magnetic Beads are composed of the murine derived, Anti-Flag monoclonal antibody attached to super paramagnetic iron impregnated, plastic beads with an average diameter of 200-300nm. The antibody binds to fusion proteins containing the Flag peptide sequence, which can be located anywhere in the fusion proteins.
The Anti-Flag Magnetic Beads are useful for detection and capture of fusion proteins containing a Flag Peptide sequence by commonly used immunoprecipitation procedures. The magnetic properties allow for very rapid separation of the beads from a suspension, significantly accelerating manipulations , such as repetitive washings or processing of multiple samples performed in multi-well plates. This leads to faster experimentation, better reproducibility, and more accurate quantitation of the proteins of interest.

**Binding capacity:** 1mg of Flag fusion protein per 1ml of packed magnetic beads.
**Specificity:** ≥ 90% specificity towards Flag fusion proteins from mammalian and bacterial cell extracts.

### Product Description

Note: For antigens and protein: protein complexes requiring a special lysis buffer composed of a different percentage of detergent, it is recommended to pretest the beads before use.
The Anti-Flag Magnetic beads are resistant to the many detergents at the following concentrations: 5.0% Tween-20, 5.0% Triton X-100, 0.1% Igepal CA-630, 0.1% Chaps, and 0.2% digitonin. It can also be used with 1.0 M NaCl or 1.0 M urea. See the Reagent Compatibility Table for use with additional chemicals.
The following procedure is an example of a single immunoprecipitation reaction. For multiple immunoprecipitation reactions, calculate the volume of reagents needed according to the number of samples to be processed. For immunoprecipitation reactions, it is recommended to use 40 μl of the 10mg/ml bead suspension per reaction. Smaller amounts of beads (~10 μl volume, which binds 1 μg Flag fusion protein) can be used.Note: Two control reactions are recommended for the procedure.
The first control is immunoprecipitation with the Polytag fusion protein (positive control) and the second is a reagent blank with no protein (negative control).
4.1.Thoroughly resuspend the beads by gentle inversion. Make sure the bottle of Anti-Flag Magnetic Beads is a uniform suspension. Remove an appropriate volume for use (see Table 1).
4.2.Place tube in the appropriate magnetic separator to collect the beads. Aspirate and discard storage buffer.
4.3.Wash the beads twice with 10beads volume of TBS (50 mM Tris HCl, 150 mM NaCl, pH 7.4) buffer. Be sure most of the wash buffer is removed and no bead is discarded.
Note: For multiple immunoprecipitation samples, wash the total volume of beads needed for all samples together. After washing, resuspend the beads in TBS buffer and divide the beads according to the number of samples tested. Place tube in the appropriate magnetic separator to collect the beads. Remove and discard TBS buffer.
4.4.Add 200~1000 μl of cell lysate to the washed beads. If necessary, bring the final volume to 1 ml by adding lysis buffer (50 mM Tris HCl, pH 7.4, with 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100). The volume of cell l ysate to be used depends on the expression level of Flag fusion protein in the transfected cells. For the positive control, add 1 ml of TBS buffer and 4 μl of 100ng/μl Polytag fusion protein (~400 ng) to the washed beads. For the negative control, add 1 ml of lysis buffer only with no protein. The amount of Polytag fusion protein to be precipitated depends on the detection method. 400 ng of protein is sufficient for an activity assay or for an immunoblot analysis. ForSDS-PAGE analysis with Coomassie blue or silver staining detection, use 2 μg of Polytag fusion protein.
4.5.Agitate or shake (a roller shaker is recommended) all samples and controls gently for 2 hours. In order to increase the binding efficiency, the binding step may be extended overnight.
4.6.Place tubes in the appropriate magnetic separator to collect the beads and remove the supernatant with a narrow-end pipette tip.
4.7.Wash the beads three times with a total of 20 beads volumes of TBS-washing buffer(50mM Tris HCl, 150mM NaCl, 250μM EDTA, 0.25% Triton X-100, 0.25% Na deoxycholate, 0.025% SDS, pH 7.4). Make sure all the supernatant is removed.
4.8.Elution of the Flag fusion proteins – Three elution methods are recommended according to protein characteristics or further usage:
Protein elution under native conditions by competition with 3× Flag peptide. The elution efficiency is very high using this method.
Elution under acidic conditions with 0.1 M glycine HCl, pH 3.0. This is a fast and efficient elution method. Neutralization of the eluted protein with wash buffer may help preserve its activity.
Elution with sample buffer for gel electrophoresis and immunoblotting.

### Elution With 3×Flag peptide

5.1.Prepare 3×Flag elution solution. Dissolve 3×Flag peptide in 0.5 M Tris HCl, pH 7.5, with 1 M NaCl at a concentration of 25 μg/μl. Dilute 5-fold with water to prepare a 3×Flag stock solution containing 5 μg/μl of 3×Flag peptide. For elution, add 3 μl of 5 μg/μl 3×Flag peptide stock solution to 100 μl of TBS buffer (150 ng/μl final concentration).
5.2.Add 5 beads volumes of 3×Flag elution solution to each sample and control beads.
5.3.Incubate the samples and controls with gently shaking or on a rotator for 30 minutes at 2~8℃.
5.4.Place tube in the appropriate magnetic separator to collect the beads. Transfer the supernatants to fresh tubes. Be careful not to transfer any beads.
5.5.Repeat steps 5.1-5.4, pooling elutes in the same tube.
5.6.For cleaning and storage of used beads, see steps 3.3 and 3.4.

### Elution with 0.1 M Glycine HCl, pH 3.5

The procedure should be performed at room temperature. Do not leave the beads in this buffer more than 20 minutes.
6.1.Add 5 beads volumes of 0.1 M glycine HCl buffer, pH 3.5, to each sample and control beads.
6.2.Incubate the samples and controls with gentle shaking or on a rotator for 5 minutes at room temperature.
6.3.Place tube in the appropriate magnetic separator to collect the beads. Transfer the supernatants to fresh tubes containing 10 μl of 0.5 M Tris HCl, pH 7.4, with 1.5 M NaCl. Be careful not to transfer any beads.
6.4.Repeat steps 6.1-6.3, pooling eluates in same tube.
6.5.For immediate use, store the combined eluates at 2~8℃. Store at -20℃ for long term storage.
6.6. For cleaning and storage of used beads, see steps 3.3 and 3.4.

### Elution with SDS-PAGE Sample Buffer

The procedure should be performed at room temperature. Sample buffer should be at room temperature before use.In order to minimize the denaturation and elution of the antibody, no reducing agents, e.g., 2-mercaptoethanol or DTT, should be included in the sample buffer. The addition of reducing agents will result in the dissociation of the heavy and light chains of the immobilized antibody (25 and 50 kDa bands). If reducing conditions are absolutely necessary, a reducing agent may be added. The final concentration of 2-mercaptoethanol or DTT in the 1× sample buffer (62.5 mM Tris HCl, pH 6.8, 2% SDS, 10% (v/v) glycerol, and 0.002% bromphenol blue) should be 5% or 50 mM, respectively.Note: Elution of the bound Flag fusion protein as a SDS-PAGE sample results in damage to the Anti-Flag Magnetic Beads and they can not be used again. The SDS in the sample buffer will denature the antibody and boiling will damage the bead structure.
7.1.Add 2 beads volume of 2×sample buffer (125 mM Tris HCl, pH 6.8, 4% SDS, 20% (v/v) glycerol, and 0.004% bromphenol blue) to each sample and control.
7.2.Boil the sample and control tubes for 3 minutes.
7.3.Place tubes in the appropriate magnetic separator to collect the beads. Transfer the supernatants to fresh tubes. The samples and controls are ready for loading on SDS-PAGE and immunoblotting using Anti-Flag or specific antibodies against the fusion protein.




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For *in vitro* research use only and not intended for use in humans or animals.