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# Anti-Myc VHH Magarose Beads

## Product Information

Catalog Number: KTSM1336

Volume: 500 μL (20 times, 50% anti-FLAG VHH coated magarose beads)

Storage condition: 4°C for 12 months

## Product Description

Myc-tag (EQKLISEEDL) has been extensively used as a general epitope tag, which does not generally interfere with the bioactivity or the biodistribution of the recombinant protein. This tag facilitates the detection, isolation and purification of the protein of interest. VHH exhibits high specificity and affinity and outperforms conventional antibodies in many aspects, such as high stability, great specificity and easy and feasible development process. Anti-Myc VHH Magarose Beads are magarose beads covalently coupled with VHH antibodies acquiring high specificity and affinity for Myc. These beads can efficiently capture and separate Myc tagged proteins, alongside with the associated proteins from cell extracts of mammal, plant, bacteria, yeast, insect, and other organisms.

## Advantages

· No heavy & light antibody chains

· Consistent and reproducible results

· Extraordinary binding capacity, even under harsh conditions

· Short incubation time (1-3 hours)

· Animal-free production

## Application

Immunoprecipitation (IP), co-immunoprecipitation (CoIP), chromatin immunoprecipitation (CHIP), RNA-binding protein immunoprecipitation (RIP), Mass Spectrometry (MS).

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## Beads Property

- Beads size: ~45 μm (cross-linked to 7.5% magarose beads)
- Storage buffer: 1XPBS, 0.03% sodium azide, 50% glycerol
- Binding capacity: 10 μL slurry bind about 18 μg of Myc tagged protein
- Specificity: Selectively recognizes Myc fusion protein

## Recommended Buffers

| Buffer | Composition |
|---|---|
| Lysis buffer | 50 mM Tris-HCl (pH 7.5); 150 mM NaCl; 1% Triton X-100; 1 mM EDTA |
| RIPA buffer | 10 mM Tris-HCl (pH 7.5);150 mM NaCl;0.5 mM EDTA; 0.1% SDS; 1% Triton X-100; 1% deoxycholate |
| Dilution /Wash buffer | 50 mM Tris-HCl (pH 7.5); 150 mM NaCl; 1 mM EDTA |
| SDS loading buffer | 120 mM Tris-HCl (pH 6.8); 20% glycerol; 4% SDS; 0.04% bromophenol blue; 10% β-mercaptoethanol |
| Glycine - elution buffer | 0.2 M glycine (pH 2.0) |
| Neutralization buffer | 1 M Tris (pH 10.4) |

Note: For yeast, plant, insect, or bacteria cell extracts, use an equally effective amount of cytolysis buffer.

## Working Procedures

### Harvest Cells

For co-immunoprecipitation, $10^6$ - $10^7$ mammalian cells expressing Myc fusion protein (about one 10 cm dish) are required.

a. Gently remove cell growth medium by aspiration;
b. Wash cells twice with 1 mL pre-chilled PBS;
c. Collect the adherent cells using cell scraper or tryptic digestion;
d. Transferred to centrifuge tubes, and centrifuge at 1,200 g for 3 - 5 min;
e. Discard the supernatant, resuspend cells with prechilled 1XPBS ;
f. Repeat cell washing twice.

### Cell Lysis

a. For cytoplasmic proteins, resuspend cells with 500 μL of pre-chilled lysis buffer;
Note: Make sure protease inhibitors and 1 mM PMSF are added.
For nuclear proteins: add 1 mg/mL DNase and 2.5 mM $MgCl_2$ to RIPA buffer (with protease inhibitor and 1 mM PMSF).

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b. Place centrifuge tubes on ice for 30 - 40 min, and resuspend cells every 10 min;
c. Centrifuge at 4°C and 12,000 g for 10 min, transfer the supernatant into a prechilled new centrifuge tube with 300 µL dilution buffer (1X PBS), discard precipitation (if required, keep 50 µL lysate for further analysis).
Note: the cell lysate collected at this point should be stored at -80°C.
Optional: Add 1 mM PMSF and protease inhibitor.

**Beads Equilibration**

a. Vortex Anti-Myc VHH Magarose Beads, and transfer 25 µL beads suspension into a 1.5 mL centrifuge tube;
b. Add 500 µL prechilled dilution buffer [1x PBST (0.05% Tween-20)];
c. Place the tube on a magnetic rack for 60 sec, until the supernatant turns clear, and repeat step b) and c) three times.

**Protein Binding**

a. Add equilibrated magnetic beads to the protein extracts (from **Cell Lysis** step);
b. Keep the tube turning on a rotating shaker for 1 - 3 hr. at 4°C.

**Washing**

a. Set the tube on a magnetic rack until the sample turns clear, and remove the supernatant (if required, keep 50 µL lysate for further analysis);
b. Add 500 µL dilution buffer (or 1x PBST) to resuspend the beads;
c. Separate the magnetic beads on a magnetic rack, then remove the supernatant;
d. Repeat step b) and c) at least 4 times.

**Protein Elution Procedure 1**

a. Add 100 µL SDS Loading Buffer to resuspend the beads and boil at 95°C for 10 min to denature and separate Myc-fusion proteins from the beads;
b. Boil at 95°C for 10 min to separate beads from the immunoprecipitation complex;
c. Separate the magnetic beads on a magnetic rack, use the supernatant for SDS-PAGE analysis.

**Protein Elution Procedure 2 (Alternative to Protein Elution Procedure 1)**

a. Resuspend beads with 50 µL elution buffer (0.2 M glycine pH 2.0), incubate on ice for 30 sec;
b. Separate the magnetic beads on a magnetic rack;
c. Add 5 µL neutralizing buffer (1M Tris-base pH 10.4) to elute protein binders.

**Elution with Myc peptide elution buffer (alternative to Protein Elution Procedure 1 or 2)**

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a. Add 20~30 μL 0.2 mM Myc peptide to every 10 μL beads, resuspend Anti-Myc Magarose Beads, incubate for 10 minutes in a mixed state, Separate the magnetic beads on a magnetic rack;
b. Transfer the supernatant to a new centrifuge tube and repeat step 1 above for at least 3 times to increase the elution efficiency;
c. Column regeneration: Add 5~10 times column volume of 0.2 M, pH2.5 glycine, keep mixing and incubate for 3 min.;
d. Place the tube on a magnetic rack for 60 sec, until the supernatant turns clear, discard the supernatant, and use 5~10 column volumes of PBS solution (pH = 7.4) to wash beads for 3 times.

## FAQs

**A. Question: Can I use an alternative Lysis Buffer with higher NaCl and NP40 concentration?**

Answer: Yes, the nanobodies conjugated to Anti-Myc VHH Magarose Beads are highly stable and resistant to harsh buffer conditions.

B. **Question: Can I incubate Anti-Myc VHH Magarose Beads with cell lysate at 4 ∞C overnight to increase binding?**

Answer: Yes, despite that the binding efficiency of our Beads and Myc tagged protein does not seem to increase very much over time, as our team has compared the results of 1 - 3 hr. and overnight co-incubation. Therefore, in most situations, 1 hr. incubation should be sufficient to achieve excellent results.