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AmMag™ Ni magnetic beads
Cat.No. L00776

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I PRODUCT DESCRIPTION
GenScript's AmMag™ Ni magnetic beads are designed for purification and screening of histidine-tagged (his-tagged) proteins. The beads are highly EDTA and DTT resistant (Table2), enabling them to bind proteins in the presence of chelators. The beads are also optimized for easy cleanup and regeneration, for reuse with multiple samples. Table 1 describes the basic properties of the AmMag™ Ni magnetic beads.

Table 1. Characteristics of AmMag™ Ni magnetic beads

Ligand	Nickel-charged TED (tris-carboxymethyl ethylene diamine)
Binding capacity	about 10 mg histidine-tagged protein /ml settled beads
Matrix	Highly cross-linked spherical 4% agarose including magnetite
Average particle size	~70 μm
Storage solution	20% ethanol (25% slurry)
Storage conditions	2-8 °C (**Do NOT freeze**)

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Table 2. Reagents Compatible with AmMag™ Ni magnetic beads

Reagents	Resistant-time
20 mM EDTA, 10 mM DTT,	24 h
100 mM EDTA, 20 mM DTT	1h
Other Reagents that are commonly used in IMAC-Ni resin/ magnetic beads	

II BUFFER PREPARATION

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 μm filter before use.
Binding/Wash Buffer: 20 mM Na_2HPO_4, 0.15 M NaCl, pH 7.0
Elution Buffer: 20 mM Na_2HPO_4, 0.15 M NaCl, 0.5M imidazole pH 7.0
Cleaning Buffer: 1M NaOH with 2M NaCl
Note: When purify inclusion bodies, add 8M Urea or 6M Gu•HCl into the Binding/Wash and Elution buffers to improve the solubility of proteins.

III. INSTRUCTIONS FOR USE

This protocol uses 100 μl of AmMag™ Ni magnetic beads. However, depending on the amount of beads used, the protocol can be scaled up or down accordingly.

3.1 Preparation of the magnetic beads

1. Completely re-suspend the magnetic beads by shaking or vortexing the vial.
2. Transfer 100 μl of the beads into a clean tube.
3. Place the tube on the AmMag™ MR-mini magnetic separation rack (L00722) to collect the beads. Remove and discard the supernatant.
4. Add 1 mL Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.

3.2 Separation of His-tagged protein

1. Add the sample containing the His-tagged protein to the tube containing magnetic beads from step 3.1-4, and gently invert the tube to mix.
2. Incubate the tube at room temperature with mixing for 60 minutes. For best results, use a rocker, tumbler, or a rotator platform that allows a 360º rotation of the tubes for optimal mixing.
3. Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
4. Add 1 mL Binding/Wash Buffer to the tube and mix well, use the magnetic separation rack to collect the beads and discard the supernatant. Repeat the wash step 3 times.

3.3 Elution of His-tagged protein

1. Add 500 μl of Elution Buffer to the tube and mix well. Incubate for 5 minutes at room temperature with occasional mixing.
2. Use the magnetic separation rack to collect the beads and transfer the supernatant that contains the eluted His-tag protein into a clean tube.
3. Repeat Step 1 and 2 three times.

3.4 Preparation of beads for reuse without regeneration

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To clean and reuse the AmMag™ Ni magnetic beads to purify the same protein, please use the method below. (**Note:** to thoroughly clean the beads to purify different His-tagged proteins, please follow the regeneration protocol (Section 3.5)). This protocol may be used to prepare the beads for reuse up to three times.

1. Vortex the beads with 4x volume dI (deionized) water following the elution step. Vortex for 10 seconds, and discard the water. Repeat this step three times.

2. Vortex the beads with 4x volume 20% ethyl alcohol. Vortex for 10 seconds, and discard the 20% ethyl alcohol. Repeat this step three times.

3. Store the magnetic beads in 20% ethyl alcohol until further use.

3.5 Regeneration of the AmMag™ Ni beads

The regeneration protocol below allows thorough clean-up of the AmMag™ Ni magnetic beads, enabling reuse of the magnetic beads. When used in the presence of high concentration of chelators (for e.g. 100 mM EDTA), the AmMag™ Ni magnetic beads can be reused up to 3 times. When used in absence of chelators, the AmMag™ Ni magnetic beads can be reused for up to 100 purification cycles, depending on the purity and composition of the samples purified.

1. To regenerate the beads, vortex the beads with 4x volume dI water following the elution step. Vortex for 10 seconds, and discard the water. Repeat this step twice.

2. Add 4x volume Cleaning Buffer (1M NaOH with 2M NaCl) and vortex the beads for 30 minutes.

3. Wash the beads with 4x volume dI water until the water turns into pH neutral (about 4-5 times).

4. Stored the magnetic beads in 20% ethanol solution at 4°C until further use.

IV. Troubleshooting

Review the information below to troubleshoot your experiments using the AmMag™ Ni magnetic beads.

Problem	Possible Cause	Solution
The yield of the purified target protein is low or undetectable	The polyhistidine-tag is not exposed because of protein folding.	Try protein purification under denaturing conditions.
	Low expression of His-tagged proteins	Increase sample amount
	The protein has been degraded	Perform all purification steps at 4°C and use protease inhibitors
	Elution buffer is not appropriate	Increase the Imidazole concentration of elution buffer
	Elution or incubation time is not sufficient	Increase the elution or incubation time
	Protein forms aggregates in the solution during binding or elution	Add Tween 20, Tween 80, Triton-100, or another non-ionic surfactant (0.1-0.5%)
	Target-protein is washed out when washing	Decrease the imidazole concentration of washing/binding buffer

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The purity of the target protein is low	The magnetic beads are not washed well	Increase wash time or the volume of Wash Buffer.
	There are other polyhistidine-rich proteins in sample	Try a pH step gradient elution or an imidazole step gradient elution.
The binding capacity of the magnetic beads has declined	Proteins or lipids have aggregated on the beads	Wash the beads with NaOH
	The beads have been reused too many times.	Use new beads

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.

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