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I. DESCRIPTION

GenScript's Nickel-Charged MagBeads (Cat. No. L00295) are magnetic beads with an average diameter of 40- μ m pre-charged with Ni²⁺. They are ideal for rapid purification of 6xHis-tagged target proteins and require minimal handling. Ni-charged MagBeads have a binding capacity of 5 - 20 mg of 6xHis-tagged target protein per 1 ml of beads (CV). The beads are compatible to magnetic-separation-based high-throughput applications. Proteins can be purified under non-denaturing conditions or in the presence of 8 M urea.

II. KEY FEATURES

- Quick and convenient separation accomplished by magnetic force
- High binding capacity: 5 - 20 mg of 6xHis-tagged protein/ml (CV)
- Compatible with various reagents needed in the purification process, see table 1
- Resin can be regenerated for multiple uses
- Low nonspecific binding

Table 1 Reagents Compatible with Ni-Charged Magnetic Beads

Denaturants	Detergents	Reducing Agents	Salts	Other
6 M Gu-HCl	2% Triton X-100	20 mM β -ME	4 M MgCl ₂	50% glycerol
8 M Urea	2% Tween 20	1 mM DTT	5 mM CaCl ₂	20% ethanol
	1% CHAPS		2 M NaCl	1 mM EDTA

III. SIZE

The beads are supplied in 8 ml of 25% slurry with a drained volume (column volume (CV)) of 2 ml.

IV. HIS-TAGGED FUSION PROTEIN PURIFICATION PROCEDURE

Additional Materials Required:

1.5 ml or 2.0 ml microcentrifuge tubes.

Magnetic Separation Racks, (GenScript M00112 or M00113)



1. Purification of polyhistidine-tagged proteins under native conditions

Before use, prepare the following three buffers:

Lysis-Equilibration Buffer (LE buffer, 1 liter):

- 50 mM NaH₂PO₄
- 300 mM NaCl
- Adjust pH to 8.0 using NaOH

Wash Buffer

- 50 mM NaH₂PO₄
- 300 mM NaCl
- 10 mM imidazole
- Adjust pH to 8.0 using NaOH

Elution Buffer (1 liter):

- 50 mM NaH₂PO₄
- 300 mM NaCl
- 250 mM imidazole
- Adjust pH to 8.0 using NaOH

- (1). Prepare the sample. Remove large particles and highly concentrated reagents, such as EDTA, amino acids, and reducing agents, which can reduce the binding capacity of the MagBeads.
 - A. For proteins expressed in *E. coli* or yeast cytoplasm, perform the following:
 - a) Harvest cells from a 50 ml culture by centrifugation (5,000 rpm for five minutes in a Sorvall SS-34 rotor). Resuspend the cells in 8 ml of LE buffer with an appropriate amount of PMSF or other protease inhibitors. The inhibitors must have no effect on the ability of the Ni resin.
 - b) Sonicate the solution on ice using 180 one-second bursts at high intensity with a three-second cooling period.
 - c) **Optional:** If the lysate is too viscous, add RNase A (10 µg/ml) and DNase I (5 µg/ml) and incubate on ice for 10-15 minutes.
 - c) Centrifuge the lysate at 10,000 × *g* for 15 minutes to pellet the cellular debris. Save the supernatant.
 - B. For proteins secreted into culture medium by yeast, insect, or mammalian expression systems, perform the following:
 - a) If the culture supernatant does not contain EDTA, histidine, or any other reducing agents that might affect Ni-charged MagBeads, it can be applied directly to the MagBeads. Otherwise, see step b.
 - b) Dialyze the sample against 1× PBS before applying it to the MagBeads.
 - d) For large volumes of supernatant, concentrate the proteins by ammonium sulphate precipitation, dialyze the dissolved protein solution against 1× PBS, and then apply the solution to the MagBeads.
- (2). Magnetic purification:
 - a) Shake or vortex the MagBeads gently before use. Place 100 µl of the MagBeads into a 1.5 ml microcentrifuge tube.



- b) Add 1 ml of Binding Buffer to the tube and invert it several times to mix. Use the magnet to separate the beads from the buffer. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
- c) Resuspend the beads in 100 μ l of Binding Buffer.
- d) Add 50 - 1000 μ l of the sample prepared above to the tube and gently invert it to mix. Incubate the tube at room temperature while mixing (such as on a shaker) for one hour.
- e) Magnetically separate the beads. Once the supernatant becomes clear, remove it.
- f) Add 1 ml of Wash Buffer to the tube and mix well. Magnetically separate the beads and remove the supernatant. Repeat the wash step three times.
- g) Add 100 μ l of Elution Buffer to the tube and mix well. Incubate for five minutes at room temperature while mixing occasionally. Magnetically separate the beads. Once the supernatant becomes clear, remove and save the supernatant containing the eluted protein. Repeat this elution three times to recover the target protein as completely as possible.

(3) Examples:

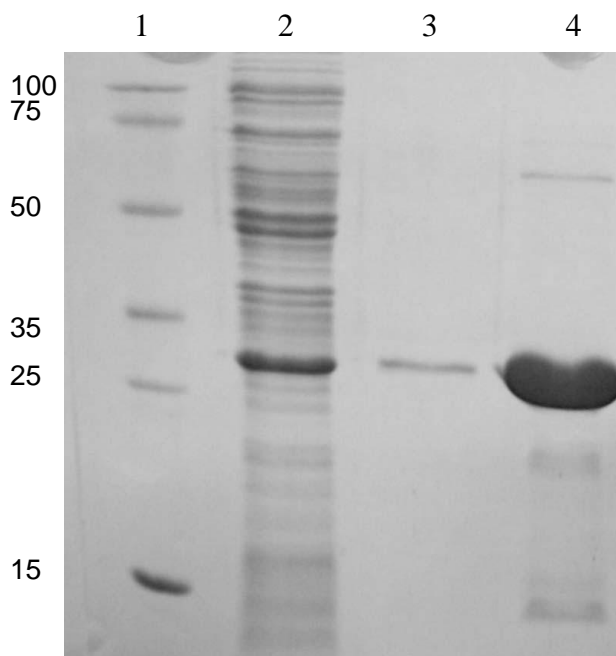


Fig. 1. Shown above is the purification of a His-tagged protein using GenScript Ni-Charged MagBeads (Cat. No. L00295).

A soluble 30 kD recombinant His-tagged protein is here purified from *E. coli* cell lysate using Ni-Charged MagBeads (Cat. No. L00295)

1. Marker
2. Flow-through
3. Wash
4. Elute



2. Purification of polyhistidine-tagged proteins from *E. coli* under denaturing conditions

This protocol is for target proteins that are expressed mainly in inclusion bodies. Before use, prepare the following three solutions:

Buffer B

- 100 mM NaH₂PO₄
- 10 mM Tris•Cl
- 8 M urea
- Adjust pH to **8.0** using 1 M NaOH

Wash Buffer C

- 100 mM NaH₂PO₄
- 10 mM Tris•Cl
- 10 mM Imidazole
- 8 M urea
- Adjust pH to **8.0** using 1 M NaOH

Elution Buffer E

- 100 mM NaH₂PO₄
- 10 mM Tris•Cl
- 250 mM Imidazole
- 8 M urea
- Adjust pH to **8.0** using 1 M NaOH

- 1) Resuspend the cell pellet in 1× PBS (about 10 - 20 ml per g of pellet), and disrupt cells by sonication as described above.
- 2) Collect inclusion bodies by centrifuging the lysate at 12,000 rpm for 10 minutes. Wash inclusion bodies with 1× PBS several times if necessary.
- 3) Solubilize the inclusion bodies in Buffer B (about 5 -10 ml per 100 mg of inclusion body), and incubate for 30 - 60 minutes at room temperature. Homogenization or sonication may be necessary to fully solubilize the pellet.
- 4) Centrifuge at 12,000 rpm for 30 minutes to remove any remaining insoluble material. Carefully transfer supernatant to a clean tube without disturbing the pellet.
- 5) Place 100 µl of the MagBeads into a 1.5 ml microcentrifuge tube.
- 6) Add 1 ml of Buffer B to the tube and invert tube several times to mix. Use the magnet to separate the beads from the buffer. Once the supernatant becomes clear, remove and discard it. Repeat this step three more times.
- 7) Resuspend the beads in 100 µl of Buffer B.
- 8) Add 50 - 1000 µl of protein sample prepared above to the tube and gently invert it to mix. Incubate the mixture at room temperature while mixing (such as on a shaker) for one hour.
- 9) Magnetically separate the beads. Once the supernatant becomes clear, remove it. Add 1 ml of Buffer C to the tube and mix well. Magnetically separate the beads and remove the supernatant. Repeat the wash step three times.
- 10) Add 100 µl of Buffer E to the tube and mix well. Incubate for five minutes at room temperature while mixing occasionally. Magnetically separate the beads. Once the supernatant becomes clear, remove and save the supernatant that contains the eluted protein. Repeat this elution three times to recover target protein as completely as possible.

Note: The process recommended here is for the purification of protein from inclusion bodies. The protein eluted from this process may need to be refolded to regain its activity and solubility.



V. REGENERATION OF THE RESIN

For complete regeneration, wash the MagBeads with the following solutions in order:

1. 2 bed volumes of 6 M GuHCl, 0.2 M acetic acid
2. 5 bed volumes of deionized water
3. 3 bed volumes of 2% SDS
4. 5 bed volumes of deionized water
5. 5 bed volumes of 100% EtOH
6. 5 bed volumes of deionized water
7. 5 bed volumes of 100 mM EDTA (pH 8)
8. 5 bed volumes of deionized water
9. 5 bed volumes of 100 mM NiSO₄
10. 5 bed volumes of deionized water

VI. TROUBLESHOOTING

Problem	Possible Cause	Solution
No recombinant protein is recovered following elution.	The His-tag is not exposed because of protein folding.	Try denaturing conditions.
	The expression level is too low.	Optimize the expression conditions.
	Not enough sample is loaded.	Load more sample.
	The protein is eluted by too much or too stringent washing.	Do not use Wash Buffer or Buffer C.
	The recombinant protein has a very high affinity for the resin.	Increase the stringency of the elution by decreasing the pH or increasing the imidazole concentration.
		Use EDTA or EGTA (10 - 100 mM) to strip the resin of nickel ions and elute the protein.
	The protein is degraded.	Perform all purification steps at 4°C and use protease inhibitors.



<p>The recombinant protein recovered is not pure.</p>	<p>The MagBeads are not washed well.</p> <p>There are other His-rich proteins in sample.</p>	<p>Wash with more bed volumes of Wash Buffer or Buffer C.</p> <p>Try an additional wash with a high-stringency buffer of lower pH (between pH 4 and pH 6) before the elution step.</p> <p>Try a pH gradient elution or an imidazole gradient elution.</p> <p>Perform a second purification over another type of column.</p>
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VII. ORDERING INFORMATION

Ni-Charged MagBeads, Cat. No. L00295

For Research Use Only.

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