Ni-Charged MagBeads Cat. No. L00295

Technical Manual No. TM0258 **Version 02052020**

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1. Product Description

1.1 Intended Use

The GenScript Ni-Charged MagBeads are developed for quick and efficient small-scale purification of polyhistidine-tagged proteins under native or denaturing conditions.

1.2 Principle

Perform cell lysis under native or denaturing conditions. Add the cell lysate containing your polyhistidine-tagged proteins to the Ni-Charged MagBeads and allow the proteins bind to the MagBeads. Then the isolated proteins can be eluted from the beads. Magnetic separation eliminates the need for multiple tubes, minimizes the loss of sample and removes several steps of the centrifugation process.

1.3 Material Decription

Material Supplied

The GenScript Ni-Charged MagBeads are average 40μm in size, super paramagnetic beads with strong metal-chelating agent covalently bound to their surfaces. They are pre-charged with nickel and ready to use for quick and small-scale purification of polyhistidine-tagged proteins. The beads are supplied as 25% slurry in phosphate buffered saline (PBS), pH 7.4, containing 20% ethanol. The Ni-Charged MagBeads have a binding capacity of 40 mg 6×His-tagged protein per 1 ml settled beads (e.g. 4 ml 25% slurry).

Cat. No. L00295 Size: 8 ml

Additional Material Required

Bacterial pellet
Mixing/Rotation Device
Magnetic Separation Rack
Test tubes and pipettes
Buffers and solutions (see protocols below)

2. Protocols

2.1 Purification of polyhistidine-tagged proteins under native conditions

Additional Buffers Needed

Lysis Equilibration Buffer (LE buffer): 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0
Wash Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0
Elution Buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0

2.1.1 Cell Lysis

1. Completely resuspend the cell pellet in ice-cold LE Buffer. **Note:** Appropriate amount of PMSF or other protease inhibitors may be added to LE Buffer to prevent protein degradation.
2. Sonicate the solution on ice using 180 × one-second bursts at high intensity with a three-second cooling period. **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.
3. Centrifuge the lysate at 12,000 × rpm for 15 minutes at 4°C to precipitate the cellular debris. Collect the supernatant. Save 10 – 20 µl of the supernatant for SDS-PAGE analysis. **Optional**: If the supernatant contain EDTA, histidine or any other reagents that might decrease binding capacity of Ni-Charged MagBeads, dialyze the supernatant against LE Buffer to remove them.

2.1.2 Purification with Ni-Charged MagBeads

1. Completely resuspend the beads by shaking or vortexing the vial.
2. Transfer 100 µl beads into a clean tube.
3. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4. Add 1 ml LE Buffer into 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.
5. Add 1 ml of cell lysate containing polyhistidine-tagged protein prepared above to the tube and gently invert the tube to mix.
6. Incubate the tube at room temperature with mixing (on a shaker) for 30 – 60 minutes. **Note:** If the protein is unstable at room temperature, incubate at a lower temperature, e.g. 4°C.
7. Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
8. Add 1 ml 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 twice.
9. Add 100 µl Elution Buffer to the tube, mix well, and incubate for five minutes at room temperature (or at a lower temperature if the protein is unstable at room temperature) with occasional mixing.
10. Use the magnetic separation rack to collect the beads and transfer the supernatant that contains the eluted protein into a clean tube.
11. Repeat step 9 and 10 twice.

2.2 Purification of polyhistidine-tagged proteins under denaturing conditions

This protocol is recommended for the purification of proteins from inclusion bodies. The protein eluted using this protocol may need to be refolded to regain its native activity.

Additional Material Required

Lysis Equilibration Buffer (LE buffer): 100 mM NaH_2PO_4, 10 mM Tris•Cl, 8 M urea, pH 8.0
Wash Buffer: 100 mM NaH_2PO_4, 10 mM Tris•Cl, 10 mM Imidazole, 8 M urea, pH 8.0
Elution Buffer E: 100 mM NaH_2PO_4, 10 mM Tris•Cl, 250 mM Imidazole, 8 M urea, pH 8.0

2.2.1 Solubilization of Inclusion Body

1. Resuspend the cell pellet in 1×PBS, pH 8.0 and disrupt cells by sonication as described above.
2. Collect inclusion bodies by centrifuging the lysate at 12,000 rpm for 10 minutes.
3. Wash inclusion bodies with same amount of 1× PBS, pH8.0 for three times.
4. Resuspend the inclusion bodies in LE Buffer (about 0.5 -1 ml /10 mg of inclusion body) to obtain an even suspension.
5. Incubate the suspension with mixing for 30 - 60 minutes at room temperature. Sonication may be needed to fully solubilize the pellet.
6. Centrifuge the solution at 12,000 rpm for 30 minutes to remove any remaining insoluble material. Carefully transfer supernatant to a clean tube without disturbing the pellet.

2.2.2 Purification with Ni-Charged MagBeads

1. Thoroughly resuspend the beads by shaking or vortexing the vial.
2. Transfer 100 µl beads into a clean tube.
3. Use the magnetic separation rack to collect the beads and discard the supernatant.
4. Add 1 ml LE 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.
5. Add 1 ml protein sample containing polyhistidine-tagged protein prepared above to the tube and gently invert the tube to mix.
6. Incubate the tube at room temperature (or at a lower temperature if the protein is unstable at room temperature) with mixing (on a shaker) for 30 – 60 minutes.
7. Use the magnetic separation rack to collect the beads and discard the supernatant. If necessary, keep the supernatant for analysis.
8. Add 1 ml 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 twice.
9. Add 100 µl Elution Buffer to the tube, mix well, and incubate for five minutes at room temperature (or at a lower temperature if the protein is unstable at room temperature) with occasional mixing.
10. Use the magnetic separation rack to collect the beads and transfer the supernatant containing the eluted protein into a clean tube.
11. Repeat step 9 and 10 twice.

3. Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript Ni-Charged MagBeads.

Problem	Possible Cause	Solution
Sample is too viscous	Sample contains high concentration of host nucleic acid	Continue sonication until the viscosity is reduced, and/or add DNase I to 5 μg/ml, Mg^{2+} to 1 mM, and incubate on ice for 10 – 15 minutes.
The yield of the purified target protein is low or undetectable.	The polyhistidine-tag is not exposed because of protein folding.	Try denaturing conditions.
	The expression level is too low.	Optimize the expression conditions.
	Not enough samples are loaded.	Load more sample.
	Too much stringent washing was used.	Wash beads with LE buffer instead of Wash Buffer.
	The recombinant protein has very high affinity for the MagBeads.	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 beads of nickel ions and elute the protein.
	The protein has been degraded.	Perform all purification steps at 4°C and use protease inhibitors.
Multiple non-specific bands observed in the eluted sample.	The MagBeads are not washed well.	Increase wash times or volume of Wash Buffer.
	There are other polyhistidine-rich proteins in sample.	Try an additional wash with a high-stringency buffer of lower pH (between pH 4 and pH 6) before the elution step. Try a pH gradient elution or an imidazole gradient elution. Perform a second purification over another type of magbeads or resin.

4. General Information

4.1 Storage and Stability

This product is stable until the expiration date stated on the COA, when stored unopened at 2–8°C. **Do NOT freeze the product**. Keep the MagBeads in liquid suspension during storage and all handling steps. Drying will cause loss of binding capacity and result in reduced performance. Resuspend the beads well before use. Be careful to avoid bacterial/fungal contamination.

4.2 Technical Support

Please contact GenScript for further technical information (see contact details). Certificate of Analysis/Compliance is available upon request. The latest revision of the package insert/instructions for use is available on www.genscript.com.

4.3 Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. This product contains 20 % EtOH as a preservative. Flammable liquid and vapor. Flash point 38°C. R-10 flammable. Material Safety Data Sheet (MSDS) is available at http://www.genscript.com.

4.4 Related MagBeads Products

Cat. No.	Product Name
L00273	Protein A MagBeads
L00274	Protein G MagBeads
L00277	Protein A/G MagBeads
L00327	Glutathione MagBeads
L00275	Mouse Anti-His mAb MagBeads
L00336	Mouse Anti-GST mAb MagBeads

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