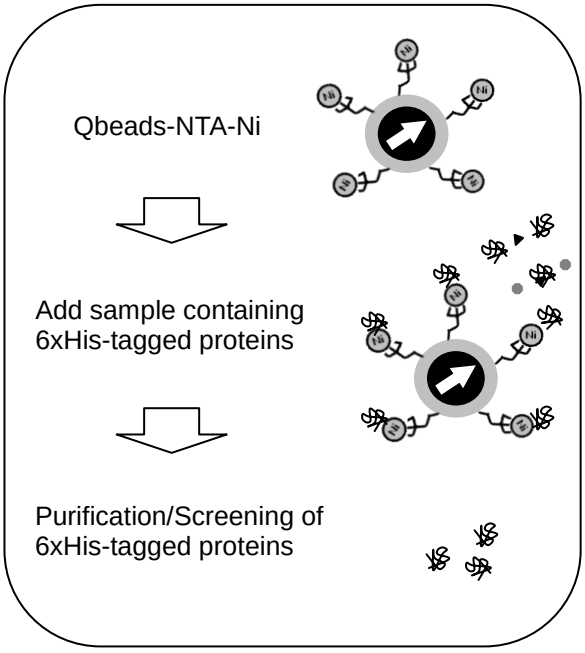
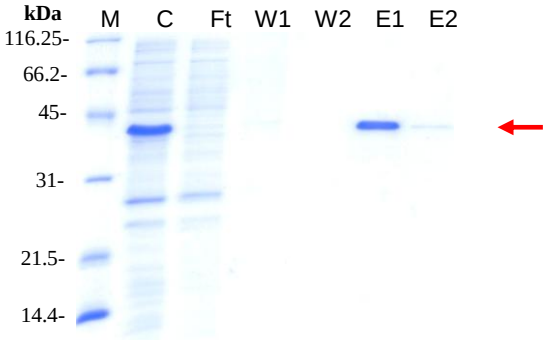
Cat. No. MF-HIS-3000

Qbeads-NTA-Ni

Product description

Qbeads-NTA-Ni is designed for rapid purification of 6xHis-tagged proteins. Qbeads-NTA-Ni has nitrilo- triacetic acid (NTA) groups with charged nickel covalently bind to surface dextran of Qbeads. Due to the high affinity, Qbeads-NTA-Ni can be used for capturing 6xHis-tagged proteins. Bound 6xHis-tagged proteins can be temporarily immobilized under magnetic attraction, so the other parts in supernatant can be removed easily and efficiently. Bound proteins can be directly used in downstream applications or be eluted off the beads. The capacity of purified 6xHis-tagged proteins (~35kDa) captured by Qbeads-NTA-Ni is approximately 5 mg/mL (Fig. 1).





M: Marker
C: Crude *E.coli* lysate
Ft: Flow through
W1 & W2: wash before elution
E1 & E2: 1st and 2nd eluate

Figure 1. Purification of 6xHis-tagged protein (indicated by arrow) from *E.coli* lysate with Qbeads-NTA-Ni. (SDS-PAGE stained with Coomassie blue)

Material supplied

Qbeads-NTA-Ni contains coated magnetic beads of an average ~1 μm in diameter. The beads are suspended in 20% ethanol reagent.

Additional material required

- Qbeads-NTA-Ni Binding/Wash Buffer
  50 mM sodium phosphate , pH 7.4
  300 mM NaCl
  0.02 % Tween 20
- Magnetic stand: **Magdorf** (MDF-08) for the best performance
- Tilt rotation device
- Qbeads-NTA-Ni Elution Buffer
  50 mM sodium phosphate, pH 7.0
  300 mM NaCl
  500 mM Imidazole
  0.1 % Tween 20
- Eppendorf tubes & pipettes

Protocol

Preparation of Qbeads-NTA-Ni for use

1. Resuspend the Qbeads-NTA-Ni thoroughly by pipetting or vortexing the vial.
2. Transfer 100 µL Qbeads-NTA-Ni suspension into a clean Eppendorf tube.
3. Add 900 µL Binding/Wash buffer and resuspend the beads by pipetting.
4. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
5. Discard the supernatant by aspiration with a pipette.
6. Remove the tube from magnetic stand.
7. Add 1 mL Binding/Wash buffer and resuspend the beads by pipetting.
8. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
9. Discard the supernatant, and then remove the tube from the magnetic stand.
10. Repeat steps 7-9 twice.
11. Ready for purification of 6xHis-tagged proteins.

    NOTE: Wash the Qbeads-NTA-Ni at least three times before use.

Purification of 6xHis-tagged proteins

12. Mix 100 µL clear lysate sample and 900 µL Binding/Wash Buffer with beads thoroughly by pipetting.
13. Incubate with tilt rotation for 30 minutes at room temperature.
14. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
15. Collect (or discard) the supernatant as unbound substances by aspiration with a pipette, and then remove the tube from the magnetic stand.
16. Add 1 mL Binding/Wash buffer and resuspend the beads by pipetting.
17. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall.
18. Collect (or discard) the supernatant as unbound substances, and then remove the tube from the magnetic stand.
19. Repeat steps 16-18 two more times.
20. Proceed to elution of 6xHis-tagged proteins.

Elution of 6xHis-tagged proteins

21. Add 500 µl Elution Buffer and gently resuspend the Qbeads-NTA-Ni- (6xHis-tagged proteins) complex by vortex AND pipetting.
22. Incubate with tilt rotation for 15 minutes at room temperature.
23. Place the tube on the magnetic stand for 30-60 seconds and **collect the supernatant** to a clean tube.
24. If required, repeat steps 21-23.

    NOTE: The first eluate (from step 22) contains the majority of the purified 6xHis-tagged proteins. If required, both eluates (from steps 22 & 23) can be combined.

Trouble Shooting

Troubles	Solutions
The yield of protein is low.	1. Check collecting supernatant from some steps (step 14,17 and 22) by SDS-PAGE. 2. Incubation time and temperature can be optimized depending on each sample. 3. We strongly recommend that use 500 mM imidazole in the elution buffer. 4. Check the pH and composition of all buffers and solutions. 5. Make sure the beads are suspended thoroughly during both the binding and elution steps.
Proteins degrade during purification.	1. Carry out the purification procedures at low temperature (e.g. 2 ~8˚C). 2. Use proper protease inhibitors in lysis, Bindin/Wash & Elution Buffer.
The beads adhere on the tip or tube.	1. Decrease the salt concentration (e.g. 50 mM NaCl) in the Binding/Wash Buffer. 2. Increase Tween 20 concentration (e.g. 0.1 %) in the Binding/Wash Buffer.
Qbeads are hard to immobilize using the magnet stand.	1. Make sure the tube is directly contact with the magnet stand. 2. Use **Magdorf** magnetic stand for the best performance.
Purified His-tagged protein can't be quantified using the standard methods such as Bradford or BCA.	1. The imidazole in the elution buffer may interfere with these assays. Either dialyze the sample or dilute to the optimal imidazole concentration for the protein quantification reagent used. 2. Check the beads are not in suspension. The remaining beads may interfere.

Storage

Please keep the reagent at 2 – 8 ℃. The validity is warranted for 12 months.

Contact Information

Please contact us when you have any questions or comments via e-mail: info@maggu.com, or phone: +886-2-8667-1897.

Remarkable Notes

1. Please keep Qbeads away from magnets during storage.
2. Do not freeze.
3. Qbeads-NTA-Ni is for research use only.

Product Information

Magnetic Qbeads Series

Products	Cat. No.
Qbeads-Protein A	MF-PRA-3000
Qbeads-Protein G	MF-PRG-3000
Qbeads-NTA-Ni	MF-HIS-3000
Qbeads-Streptavidin	MF-STA-3000
Qbeads-Silica	MF-SIL-5010
	MF-SIL-5024
Qbeads-Hydroxyl	MF-DEX-3000
Qbeads-Carboxyl	MF-COO-3000
Qbeads-Amine	MF-NHH-3000
Qbeads-Carboxyl Labeling Kit	KT-COO-3000-5SE

Accessory

Products	Description	Cat. No.
Magdorf	for 1.5 ml eppendorf tube	MDF-08
Magstand	for magnetic separating column	MSD-01
	for 15 ml falcon tube	MSD-15
	for 50 ml falcon tube	MSD-50
Magtractor	for 96-well culture plates	MTR-96
	for 24-well culture plates	MTR-24
	for 6-well culture plates	MTR-06

Magnetic NanoParticle Series

Products	Particle size	Cat. No.
Magnetic Fluid- Hydroxyl	30 nm	MF-DEX-0030
	60 nm	MF-DEX-0060
	90 nm	MF-DEX-0090
Magnetic Fluid- Carboxyl	30 nm	MF-COO-0030
	60 nm	MF-COO-0060
	90 nm	MF-COO-0090
Magnetic Fluid- Amine	30 nm	MF-NHH-0030
	60 nm	MF-NHH-0060
	90 nm	MF-NHH-0090
NanoQ-Carboxyl Labeling Kit	60 nm	KT-COO-0060-1SE

Fluorescent Magnetic Nanoparticles

Products	Particle size	Cat. No.
Blue FluoroNanoQ	60 nm	MF-FBL-0060
Green FluoroNanoQ	60 nm	MF-FGR-0060
Red FluoroNanoQ	60 nm	MF-FRE-0060

Customized Conjugation Service

Products	Particle size	Cat. No.
Customized conjugated magnetic beads Antibody or peptide provided by customers (100 ug)	3 μm	MF-CCS-3000
	30 nm	MF-CCS-0030
	60 nm	MF-CCS-0060
	90 nm	MF-CCS-0090

www.magqu.com

磁量生技股份有限公司

新北市 231 新店區中正路 538 巷 12 號 3 樓
電　　話　+886-2-8667 1897
傳　　真　+886-2-8667 1809
統一編號　28953128
電子郵件　info@magqu.com

MAGQU CO. LTD.

3F, No.12, Lane 538, Zhongzheng Rd., Xindian Dist.,
New Taipei City 231, Taiwan
TEL　+886-2-8667 1897
FAX　+886-2-8667 1809
Email　info@magqu.com