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A Geno Technology, Inc. (USA) brand name

Ni-NTA Magnetic Beads

(Cat. # 786-910, 786-911)

INTRODUCTION

G-Biosciences' Ni-NTA Magnetic Beads are 3µm beads designed for the rapid purification of 6x His-tagged proteins. Ni-NTA Magnetic Beads have nitrilotriacetic acid (NTA) groups with charged nickel covalently bound to the surface dextran of the beads. Due to the high affinity, Ni-NTA Magnetic Beads can be used for capturing 6xHis-tagged proteins.

Bound 6xHis-tagged proteins can be temporarily immobilized under magnetic attraction, so non-tagged proteins in the 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 G-Biosciences' Ni-NTA Magnetic Beads is ≈5 mg/ml.

ITEMS SUPPLIED

Cat. #	Description	Size
786-910	Ni-NTA Magnetic Beads	1ml
786-911	Ni-NTA Magnetic Beads	5ml

STORAGE CONDITIONS

The beads are shipped at ambient temperature. Upon arrival, store the beads at 4°C. If stored and handled correctly the beads have a 1 year shelf life.

SPECIFICATIONS

Fe_3O_4 beads coated with dextran of an average 3µm in diameter. Nitrilotriacetic acid (NTA) groups with charged nickel covalently bound to the surface dextran. Supplied in 20% ethanol.

PRECAUTIONS

- Do not freeze the magnetic beads
- Do not store near magnetic sources

IMPORTANT INFORMATION

- The purity and yield of the recombinant fusion protein is dependent of the protein's confirmation, solubility and expression levels. We recommend optimizing and performing small scale preparations to estimate expression and solubility levels.
- Avoid EDTA containing protease inhibitor cocktails, we recommend our Recom ProteaseArrest™ (Cat. # 786-376, 786-436) for inhibiting proteases during the purification of recombinant proteins.
- For recombinant proteins that are sequestered to inclusion bodies we recommend out IBS™ Buffer (Cat. # 786-183)

ADDITONAL ITEMS REQUIRED

- Binding Buffer and Elution Buffer, see protocol for details.
- Magnetic stand

PREPARATION BEFORE USE

Sample preparation: Refer to manufacturer's protocols for optimal conditions for growth, induction and lysis of recombinant His-tagged clones. The preferred buffers that improve binding affinity are 50mM acetate or 10-150mM phosphate buffers with pH 7-8, although this can fluctuate between pH 5.5-8.5. Avoid buffers with primary amines (Tris, Glycine) as these weaken binding affinity and can even strip metal ions. The buffer should be supplemented with 0.15-0.5M NaCl to suppress secondary ionic interactions and proteins/protein interactions.

PROTOCOL

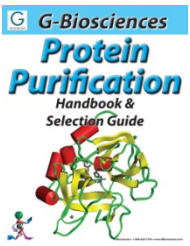
1. Resuspend the beads thoroughly by pipetting or vortexing the vial.
2. Transfer 100µl bead suspension into a clean tube.
3. Add 900µl Binding/Wash buffer (i.e. i.e. 20mM Na_2HPO_4, 0.5M NaCl pH 7.4, 0.05% Tween-20) and resuspend the beads by pipetting.
   NOTE: *The buffer can be supplemented with 5-10mM imidazole to reduce non-specific interactions.*
4. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall. Discard the supernatant by aspiration with a pipette and remove the tube from magnetic stand.
5. Add 1ml Binding/Wash buffer and resuspend the beads by pipetting.
6. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall. Discard the supernatant, and then remove the tube from the magnetic stand. Repeat steps 5-6 twice.
7. Mix 100µl clear lysate sample and 900µl Binding/Wash Buffer with beads thoroughly by pipetting, then incubate with tilt rotation for 30 minutes at room temperature.
8. Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall. Collect (or discard) the supernatant as unbound substances by aspiration with a pipette, and then remove the tube from the magnetic stand.
9. Add 1ml Binding/Wash buffer and resuspend the beads by pipetting.
   Place the tube on the magnetic stand for 30-60 seconds to immobilize the beads at tube wall. Collect (or discard) the supernatant as unbound substances, and then remove the tube from the magnetic stand. Repeat step 9 two more times.
10. Add 500µl Elution Buffer (20mM Na_2HPO_4, 0.5M NaCl, 0.5M Imidazole, pH 7.4) and gently resuspend the beads by vortexing and pipetting.
11. Incubate with tilt rotation for 15 minutes at room temperature.
12. Place the tube on the magnetic stand for 30-60 seconds and remove the supernatant to a clean tube. If required, repeat steps 10-12.
    NOTE: *The first eluate contains the majority of the purified 6xHis-tagged proteins. If required, both eluates can be combined.*

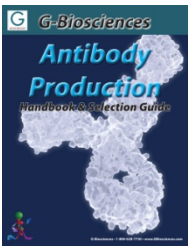
TROUBLESHOOTING

Issue	Possible Reason	Solution
Low protein yield	Poor protein expression	Optimize expression
	Proteins formed inclusion bodies	For recombinant proteins that are sequestered to inclusion bodies we recommend out IBS™ Buffer (Cat. # 786-183)
	Poor cell lysis and extraction	Optimize cell lysis protocol
	Fusion protein failed to bind to beads	Check the sequence. Check expression with ELISA or Western blotting
	Protein degraded during purification	Perform purifications at 4°C Avoid EDTA containing Use an EDTA free protease inhibitor cocktails (Recom ProteaseArrest™, Cat. # 786-376, 786-436
Poor purity of protein	Poor washing	Increase number of washes at least 2 fold Adjust imidazole concentration in wash buffer
Beads aggregate/ adhere to tip or tubes	Lack of detergent	Increase the binding/wash buffer detergent concentration to 0.1%

RELATED PRODUCTS

Download our Protein Purification and Antibody Production Handbooks.





http://info.gbiosciences.com/complete-protein-purification-handbook/

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