
NW Rose Ni FF

Immobilized Metal Affinity Chromatography (IMAC) Resin

Product Instruction

File code: NM-A-DF-0202

Version: A0

NW Rose Ni FF

Immobilized metal affinity chromatography resin

NW Rose Ni FF is immobilized metal affinity chromatography resin, it can be used for His-Tag protein purification, and also some protein has exploited side-chains of amino acid like histidine, cysteine and tryptophan which can complexes with the transition metal ions. NW Rose Ni FF is used highly cross-linked agarose beads (NW Rose6FF beads) coupled with chelating group NTA, the chelating group has then been charged with nickel (Ni^{2+}) ions. It easy to used, and easy to scale-up to pilot, process scale. IMAC is a very versatile purification technique. The NTA group has four sites for chelating metal ions. So, it can withstand a higher reduction agent, physical and chemical stability.

NW Rose Ni FF is an established affinity resin for capture of proteins with His-Tag.

- Pre-charge nickel ions, it easy to used.
- High dynamic binding capacity.
- Fast, reliable scale-up of His-tag purification.
- Compatible for reducing agents, detergents and other additives.
- Available as prepacked column NmTRAP 1 mL, NmTRAP 5 mL for lab scale purification, NmSCREEN 4.7 mL column for Process development.

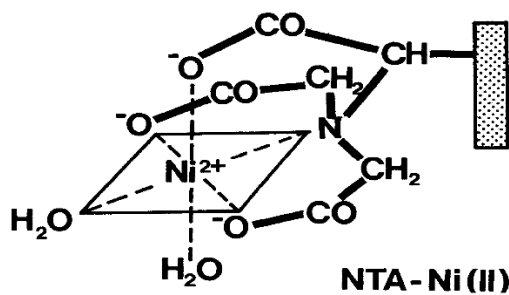


Fig 1. The ligand of NW Rose Ni FF.

Table 1. The characteristics of NW Rose Ni FF

Product name	NW Rose Ni FF
Chromatography technique	Histidine-tagged protein purification
Matrix	Highly cross-linked agarose with fast flow rate
Particle Size	45-165 μm
Ligand	NTA
Dynamic Binding Capacity	$\sim 40 \text{ mg} \cdot \text{mL}^{-1}$
Metal Ion Capacity	$15 \text{ } \mu\text{mol} \cdot \text{mL}^{-1}(\text{Ni}^{2+})$
Maximum Pressure	0.3 MPa
Clean in Place	0.5 M NaOH (Ni^{2+} must be removed)
Maximum Flow Velocity	150 cm/h (50/30 Column, bed height 10 cm)
Recommended flow velocity	600 cm/h, 16/20 Column, bed height 10 cm
pH stability	3-12 (operational) 2-14 (CIP, Ni^{2+} must be removed)
Chemical stability	Stable in commonly used aqueous buffers, 2% SDS, 30% isopropyl alcohol, 6 M guanidine hydrochloride, 8 M urea, 70% ethanol (if Ni^{2+} is removed)
Operational temperature	4-30 $^{\circ}\text{C}$
Storage	20% ethanol, 4-30 $^{\circ}\text{C}$

Lab scale Purification His-tag protein

Use NmTRAP 1 mL, NW Rose Ni FF purify GST-His protein.

Column: NmTRAP Ni FF 1 mL 0.7×25 mm

Sample: GST-his protein in E.coli lysate buffer

Buffer A: 20 mM PB + 500 mM NaCl pH 7.4

Buffer B: 20 mM PB + 500 mM imidazole + 500 mM NaCl pH 7.4

Flow rate: 1ml/min

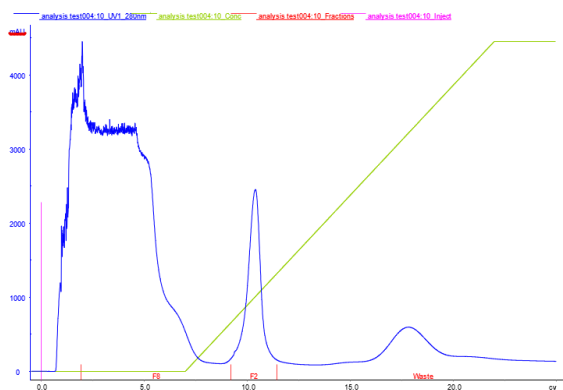


Fig 3. NmTRAP 1ml column for purify His-tag protein.

Process development

Use NmSCREEN 4.7 mL, NW Rose Ni FF for process development.

For Process development, 100mm bed high column is normally used.

NmSCREEN column is 0.77 mm×100 mm PP column, only 4.7 ml column volume. The benefit is fast, lower sample. If want to test 200 mm bed high, it easy to connect two columns for 200mm bed high. Fig 4. Show 10× scale-up.

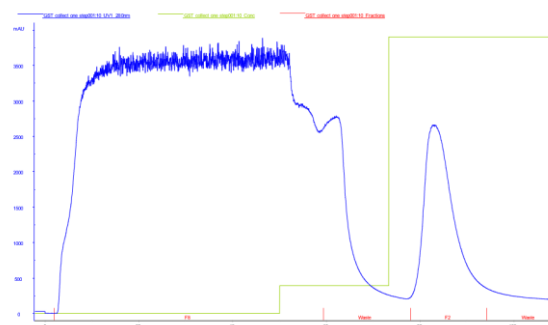


Fig 4. NmSCREEN 4.7 mL column for Process development.

Repeated Purification without Ni recharging

Reproducibility in terms of purity, selectivity, and recovery over a number of repeated runs without stripping, cleaning, or Ni^{2+} recharging was investigated. Five repeated purifications of GST-His and five repeated purifications of his-tag from E. coli extract, were run on NmSCREEN 4.7 mL, NW Rose Ni FF column. The number of reproducible runs without Ni^{2+} recharging and cleaning will depend on the sample and protein used. When the column pressure growth, or DBC decrease, or recovery decrease, the column needs to be cleaning.

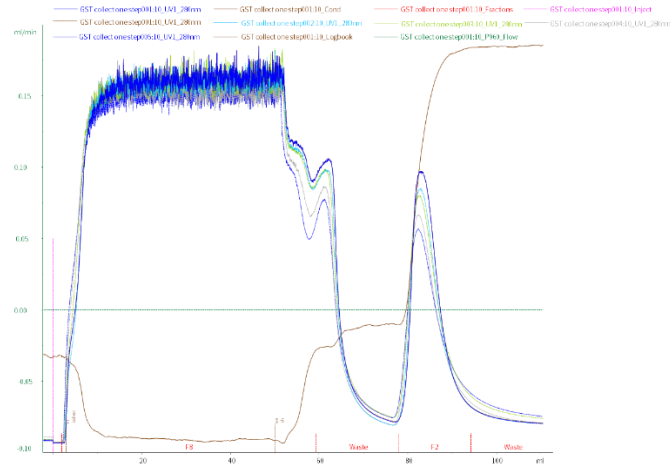


Fig 5. Repeated loading without Ni recharging.

Note: the sample is the centrifugal supernatant of Ecoli. lysate.

Stability of Ni capacity after purification

After 40 cycles purification, Ni capacity is investigated.

Use the same sample, after five times repeat purification of GST-His protein, then strip Ni^{2+} , use 1M NaOH to do CIP. Recharge Ni^{2+} , and analysis Ni^{2+} capacity, Fig 6. shows the Ni capacity stable after 40 cycles purification.

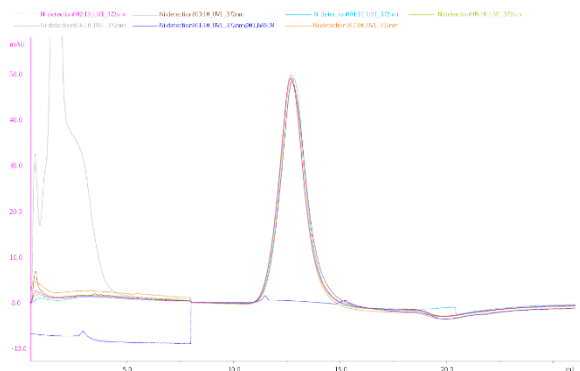


Fig 6. Show the Ni capacity stable after 40 cycles purification.

Stability with NaOH

Sodium hydroxide is widely accepted for cleaning, sanitizing. The Benefits of NaOH for GMP production is high efficacy and lower cost ease of detection, removal, and disposal, and also sodium hydroxide saponifies fats and dissolves proteins and nucleic acids. It is good reagent for sanitizing, sodium hydroxide can inactive viruses, bacteria, yeast, fungi and endotoxins.

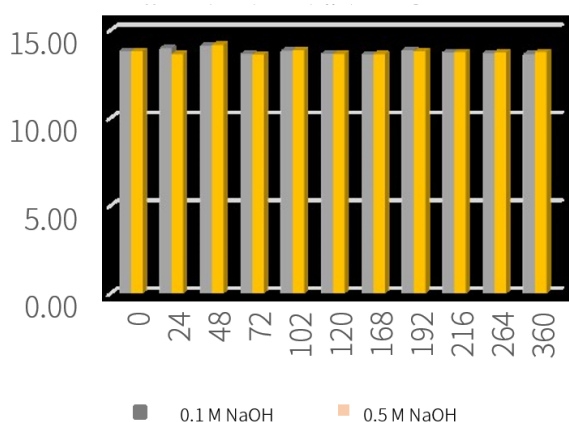


Fig7. Show the stable of the Ni²⁺ capacity.

Stability with NaOH of NW Rose Ni FF was investigated, after strip the Ni²⁺, the resin was treated with NaOH at room temperature. The result show after 360 hours 0.1, 0.5 M NaOH treatment NW Rose Ni FF Ni²⁺ capacity is stable.

Optimization

Use purification system will get good resolution, At the first run, the liner gradient elution is the best choice, liner gradient has good resolution, it can separation the impurity.

If the sample too viscous, please dilution the sample; if the sample is from E.coli lysate, please use ultrasonic processor for 3-5 min on ice. And use centrifuge for removed cell debris.

Adding 20-40 mM imidazole in starting buffer will get good purify, if you find the his-protein in flowthrough, it can decrease the imidazole concentration in starting buffer.

Lower pH can elution the his-tag protein, but the Ni²⁺ will leakage increase.

Nonspecific hydrophobic or other interaction impact, add nonionic detergent to the elution buffer (like 0.2% Triton X-100) or increase the NaCl concentration.

Order information

Product name	Package size	Code number
NW Rose Ni FF	25 mL	60042-531800-2025
	100 mL	60042-531800-2100
	500 mL	60042-531800-2500
	1 L	60042-531800-1001
	5 L	60042-531800-1005
	10 L	60042-531800-1010

We also offer 7.7 mm × 22 mm 、 16 mm × 25 mm 、 7.7 mm × 100 mm preppacked columns. More requirements about products or customized needs, please contact us.

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