Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Ni Sepharose High Performance

Product Number: HQ060212001L

Shipping and Storage

4-30℃ in 20% ethanol

Description

Ni Sepharose High Performance is capable of separation and purification by the interactionbetween Ni^{2+} and certain amino acids (mainly histidine, cysteine, and tryptophan) on theprotein side chain, and is applicable for the separation and purification of His-tag proteinsand biomolecules that interact with Ni^{2+}.

Matrix	Highly cross-linked 6% sepharose
Range of particle size	25-45μm
Mean particle size	35±5μm
Binding capacity	≥ 40 mg (His-tag protein)/mL (media)
pH stability*	3 - 12 (long-term) 2 - 14 (short-term)
Chemical stability**	All common aqueous solutions and buffers; Avoid chelating agents (such as EDTA and EGTA) and reducing agents (such as DTT and DTE)
Flow rate	≥150cm/h

*: Stability refers to the stability of the media before chelating with metal ions.

Buffer	0.05M sodium phosphate, pH 7.4 0.1M Tris-HCl, pH 7.4 0.1M Tris-acetate, pH 7.4 0.1M HEPES, pH 7.4 0.1M MOPS, pH 7.4 0.1M sodium acetate, pH 4
Denaturant	8M Urea 6M Gua-HCl
Detergent	2% Triton X-100 2% Tween 20 2% NP-40 2% Cholate 1% CHAPS
Reducing agent*	0.005M DTE 0.005M DTT 0.02M β-mercaptoethanol 0.005M TCEP 0.01M reduced glutathione
Other additives	0.5M Imidazole 20% Ethanol 50% Glycerol 0.1M Na2SO4 1.5M NaCl 0.001M EDTA** 0.06M Citrate

Specification

Product Name	Specification	Product Number
Ni Sepharose High Performance	25ml	HQ060212025M
Ni Sepharose High Performance	100ml	HQ060212100M
Ni Sepharose High Performance	500ml	HQ060212500M
Ni Sepharose High Performance	1L	HQ060212001L
Ni Sepharose High Performance	5L	HQ060212005L
Ni Sepharose High Performance	20L	HQ060212020L

Features

1. Fast and simple operation (one-step purification).
2. It has wide scope of application, with simple operation, and is applicable for gravity column and pre-packed column (peristaltic pump or chromatography system).
3. Multiple options are available: it can be chelated with various metal ions (such as Cu^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, Ca^{2+}, etc.) for use.
4. Compared with Ni Focurose FF (IDA), it has advantages of low Ni^{2+} dropout and wide reagent compatibility.
5. Small particles, high resolution.

Note: Avoid using phosphate buffers (since there may be precipitates) when chelating withCa2+.

Preparation of Solution

Equilibrium solution: 0.02M PB, 0.5M NaCl, 0.02 - 0.04M Imidazole, pH 7.4. Eluent: 0.02M PB, 0.5M NaCl, 0.5M Imidazole, pH 7.4.

Note: When purifying inclusion bodies, 8M Urea or 6M Gua-HCl should be added to the solution.

Preparation of Sample

1. The solution containing the sample should be consistent with the equilibration solution,and the buffer can be exchanged by lysis with the equilibration solution, or by dialysis/ultrafiltration/G25.
2. The samples are filtered (filter membrane selection: 0.22 µm if mean particle size is <45µm; 0.45µm if 45µm < mean particle size <165µm; 0.8 µm if mean particle size is >165µm).

Purification Process (taking XK16/10 as an example)

1. Connect the chromatographic column to the chromatography system drop-to-drop (to avoid introducing air bubbles).
2. Wash 5 CV with purified water at a flow rate of 5 mL/min.
3. Wash 5 CV with eluent at a flow rate of 5 mL/min.
4. Wash 10 CV with equilibrium solution at a flow rate of 5 mL/min.
5. Inject the samples at a flow rate of 5 mL/min.
6. Wash with equilibration solution at a flow rate of 5 mL/min until the UV absorption value is stable (10 - 15 CV).
7. Elute 5 CV or 20 CV with the eluent (5 CV by stepwise elution/20 CV by linear gradient elution).

Regeneration (taking XK16/10 as an example)

1. Wash 10 CV with purified water at a flow rate of 5 mL/min.
2. Wash 10 CV with stripping solution at a flow rate of 5 mL/min.
3. Wash 10 CV with equilibrium solution at a flow rate of 5 mL/min.
4. Wash 10 CV with purified water at a flow rate of 5 mL/min.
5. Wash 5 CV with 0.1M NiSO4 at a flow rate of 5 mL/min.
6. Wash 5 CV with purified water at a flow rate of 5 mL/min.
7. Wash 5 CV with equilibrium solution at a flow rate of 5 mL/min.

8. Wash 5 CV with 20% ethanol at a flow rate of 5 mL/min.

Note: Stripping solution, 0.02M PB, 0.5M NaCl, 0.05 EDTA, pH 7.4.

Washing (taking XK16/10 as an example)

1. Strip off Ni2+ according to operation steps in 5.1 - 5.4.
2. Wash 10 CV with 1.5M NaCl at a flow rate of 5 mL/min.
3. Wash 10 CV with purified water at a flow rate of 5 mL/min.
4. Wash 30 CV with 1.0M NaOH at a flow rate of 2.5 mL/min.
5. Wash 10 CV with equilibrium solution at a flow rate of 5 mL/min.
6. Wash 10 CV with purified water at a flow rate of 5 mL/min.
7. Wash 10 CV with 30% isopropanol at a flow rate of 2.5 mL/min.
8. Chelate with Ni2+ according to operation steps in 5.4 - 5.8.

Common Problems

Common problems and solutions

Problem	Possible causes	Solution
The target substance is not bound to the media during purification or has a lower binding capacity	1. Injection overload	Decrease the injection volume
	2. The injection flow rate is too fast	Decrease the injection flow rate
	3. Aggregation of proteins or lipids in the media affects the binding.	Clean the media in a timely and effective manner or replace with new media
	4. The expression conditions are too severe, so the His tag is wrapped and cannot be bound with the media	It is recommended to prepare an empty vector as a control for expression and purification, so as to determine whether the expression conditions are suitable
	5. No His-tag proteins in the initial sample	Verify by gene sequence or His-tag antibody
	6. The target protein appears in flow-through mode	The target protein is not successfully expressed, or the pH and components of the sample and the equilibration solution are incorrect
No target substance is collected during elution, Or only minor target substance is collected	1. The target substance is not bound to the media, or has a lower binding capacity	Confirm whether the target substance is bound to the media
	2. Inappropriate elution conditions	Increase the concentration of imidazole in the eluent
	3. Insufficient elution duration	Decrease the flow rate, and extend the retention time of the eluent
	4. The elution volume is too low	Increase the elution volume
	5. The target protein is washed off when washing the impurities.	Decrease the concentration of imidazole in the eluent
	6. The target substance aggregates and precipitates under the eluent conditions	Check the solubility and stability of the target protein under eluent conditions (pH and salt concentration). Add some additives to the eluent: such as 0.2% Triton X-100 or 0.5% Tween 20

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Low purity of the target substance	1. Lack of pretreatment for the sample	The samples must be centrifuged or filtered before being injected on the column
	2. The viscosity of sample is too high	Dilute the sample appropriately with the equilibration solution to decrease the viscosity
	3. Inadequate impurity washing	Increase the washing volume until being stable at the baseline and consistent with the equilibration solution
	4. Impurity proteins or lipids aggregate and precipitate in the media	Clean the media in a timely and effective manner
	5. The impurity has high affinity with Ni^{2+}.	Purify with other types of media (such as ionic or molecular sieves)
	6. Degradation of the target substance	Check the stability of the target protein, and add protease inhibitors
	7. Poor column media	Re-pack the media or purchase again
	8. The impurity has non-specific adsorption with the media.	Select appropriate additives to decrease the non-specific adsorption. Add some additives to the sample: such as 0.5% Triton X-100, 1.0% Tween 20 or 50% glycerol.
	9. Larger sample storage volume at the top of the separation column	Re-pack the column or decrease the sample storage volume
	10. Microbial growth in the media	Please store the media in time and correctly after use
Media capacity decreased	1. The injection flow rate is too fast	Decrease the injection flow rate
	2. Proteins or lipids aggregate in the media, resulting in decreased capacity.	Clean the media in time
	3. Repeated use for too many times	Replace with a new media
	4. The expression conditions are too severe, so the His tag is wrapped and cannot be well bound with the media	It is recommended to prepare an empty vector as a control for expression and purification, so as to determine whether the expression conditions are suitable
Chromatographic peaks rise too steeply	The media is packed at higher concentration	Re-pack the column
Chromatographic peaks rise slowly or have tailing	The media is packed at lower concentration	Re-pack the column
Cracked or dry column bed	There is any leakage, or large air bubbles are introduced	Check the tube lines for leakage or air bubbles, and re-pack the column
Slow flow rate	1. Aggregation of proteins or lipids	Clean the media or filter membrane in time
	2. Proteins precipitate in the media	Modify the components of the equilibration solution and eluent to maintain the stability of

		target substance and the binding efficiency of media
	3. Microbial growth in the separation column	The reagents used must be filtered and degassed;The samples must be centrifuged or filtered before being injected on the column