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Ni Focurose 6HP(IMAC)

Catalog No: E-CM-AF23

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Please read this manual carefully before use to ensure the performance and successful operation. If you have any questions, please contact our Technical Support.

Product introduction

Ni Focurose 6HP(IMAC) can be used for separation and purification with the interactions between $Ni^{2+}$and some amino acids (mainly include histidine, cysteine, tryptophan) on the side chain of protein, and it is suitable for separation and purification of His-tagged protein and biological molecules which interact with Ni^{2+}.

Advantages

1. Quick and easy (one-step purification).
2. Wide application, simple-operation. Suitable for gravity column and prepacked column.
3. Lower Ni^{2+} abscission probability and wider compatibility with reagents (Table 2) when compared with Ni-6FF (IDA).
4. High resolution.

Table 1: Performance index

Matrix	Highly cross-linked with 6% agarose
Particle size range	25-45 μm
Average particle size	37 μm
Binding capacity	40 mg (His-tagged protein)/mL (media)
pH stability*	3-12 (long-term) 2-14 (short term)
Chemical stability*	0.01M HCl, 0.01M NaOH (1 week) 1M NaOH, 70% Ethanol (12 hours) 2% SDS (1 hour) 30% Isopropyl alcohol (30 minutes)
Flow rate	600 cm/h
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30℃ (4~8℃ is preferred)

*: The stability of media when unchelated with metal ions.

Table 2: Compatibility with common reagents

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
Reductant*	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 Na_2SO_4 1.5M NaCl 0.001M EDTA** 0.06M Citrate

* During the operation with Ni Focurose 6HP(IMAC), it is allowed to add low concentration of reductant, but it must be avoided of being immersed or stored for a long time with solution with reductant.

** During the operation with Ni Focurose 6HP(IMAC), it is allowed to add nominal concentration of metal ions chelating agent (e.g. 0.0001M EDTA) into samples of small volume, but it must be avoided of adding or loading a large volume of samples containing chelating agent into the purified solution.

Operation (take 1 mL column and 5 mL column for example)

1. Wash (water)

Wash the media with 5~10 CV (column volume) of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This operation is used to remove the 20% ethanol in media.

2. Equilibration

Balance the media with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Make sure that the pH and component in media are in accordance with sample.

3. Sample application

Apply the sample with flow rate of 0.2 mL/min (1 mL) or 1.0 mL/min (5 mL) after centrifugation and filtration (0.45 μm). Wash with the equilibrium liquid until the baseline tends to zero.

Note: The binding capacity of proteins varies with the type of lysates, the properties of target proteins, flow rate, temperature, and pH. Low flow rates often increase the binding efficiency of samples.

4. Wash

Wash out the impurity substance with 5~10 CV of washing solution with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the washed solution.

Note: The washing solution is used to wash out some non-specific adsorbed impurity proteins.

5. Elution

Elute with 5~10 CV of eluent with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Low flow rate may increase the concentration of target protein in the eluted solution.

6. Wash with water

Wash the media with 5~10 CV of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This procedure is used to remove the eluent in media.

7. Storage

Wash the media with 5~10 CV of 20% ethanol with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and the store the media.

Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

8. Preparation of buffer

Add 8M urea or 6M guanidine hydrochloride to the following equilibrium liquid, washing buffer and eluent if the sample is inclusion body.

Equilibrium liquid: 0.02M PB, 0.5M NaCl, adjust the pH to 7.4. Store the prepared equilibrium liquid at room temperature.

Note: NaCl in equilibrium liquid is used to inhibit the ion exchange of media.

Washing buffer: 0.02M PB, 0.5M NaCl, 0.02-0.04M Iminazole, adjust the pH to 7.4. Store the prepared washing buffer at room temperature.

Note: Add 0.02-0.04M Iminazole to the washing buffer (yield preferred) or to the equilibrium liquid directly (purity preferred) according to the final application demands.

Eluent: 0.02M PB, 0.5M NaCl, 0.5M Iminazole, adjust the pH to 7.4. Store the prepared eluent at room temperature.

Note: 0.05-0.25M Iminazole in eluent is enough to elute the target protein in general condition.

Cleaning

The excellent performance of media (e.g. loading ability, mobility, column efficiency, etc.) can be recovered after cleaning the strong coupling substance (e.g. some strong coupling protein, denatured protein, lipids, etc.).

It is recommended to wash the media after used for each 5 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 5~10 CV of purified water.
   Note: This procedure is used to remove the eluent (wash after using) or 20% ethanol (wash before using).
2. Wash the media with 5~10 CV of 0.02M Tris-HCl, 0.1M EDTA (pH 8.0), then wash the media with 5~10 CV of purified water immediately.
   Note: This procedure is used to remove the Ni^{2+}.
3. Wash the media with 5~10 CV of 1.0M NaOH, Wash the media until the pH to neutral with purified water after standing for 1 hour.
   Note: This procedure is used to remove the protein precipitates or lipids accumulated in the media.
4. Wash the media with 5~10 CV of 0.1M $NiSO_4$, then wash the media with 5~10 CV of purified water after standing for 0.5 hour.
   Note: This procedure is used to couple Ni^{2+}.
5. Store the media after washed with 5~10 CV of 20% ethanol.
   Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

Trouble shootings

Problem	Possible cause	Suggestion
The target compound does not combine with the media or the combining amount is low when purifying	Overloading of sample volume.	Decrease the sample volume.
	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely and effectively.
	The expression condition is too severe that the His-tag was enwrapped and cannot combine with the media.	An empty vector is suggested to set as control of expression and purification to check whether the expression condition is suitable.
	The initial sample does not contain His-tagged protein.	Verify through the gene sequence or His-tag antibody.
	The target protein occurs in the flowthrough.	The target protein has not been expressed successfully, or the pH and components in sample and equilibrium liquid were incorrect.
No target compound was collected or only a small amount of target compound was collected	The target compound does not combine with the media or the combining amount is low	Confirm whether the target combine with media or not.
	Unsuitable elution condition.	Increase the iminazole concentration in eluent.
	Insufficient elution time.	Decrease the flow rate and prolong the retention time of eluent.
	Insufficient elution volume.	Increase the elution volume.
	Target protein was washed out during the washing.	Decrease the iminazole concentration in washing buffer.
	The target compound accumulates in the elution buffer.	Check the solubility and stability of target compound in the elution buffer (salt concentration, pH, etc.) Add some additives into the eluent, such as 0.2%Triton X-100 or 0.5% Tween 20.
Low purity of target compound	Sample has not been pretreated.	Samples must be centrifuged or filtered before loading.
	High viscosity of sample.	Dilute the sample properly with equilibrium liquid to decrease the viscosity.
	Insufficient washing.	Increase the washing volume until the

		baseline is stable and consistent with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Impurity substances have strong-affinity with Ni^{2+}.	Purify with other type of media (e.g. ion–sieve or molecular-sieve).
	The target compound degrades.	Determine the stability of target compound and add protease inhibitors.
	Bad loading effect of column resin.	Reload or re-purchase.
	Non-specificity absorption of impurity materials and media.	Add appropriate additive to reduce the non-specific absorption, such as 0.5%Triton X-100, 1.0% Tween 20 or 50% glycerol.
	The top of separation column has a large volume of sample.	Reload the column or reduce the volume of the sample.
	There is microbial grow in the media.	Correctly store the media after used.
Decrease of loading volume	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely.
	Ligand dropped off due to excessive use.	Re-couple with new media.
	The expression condition is too severe that the His-tag was enwrapped and cannot combine with the media.	An empty vector is suggested to set as control of expression and purification to check whether the expression condition is suitable.
The chromatographic peak rises slowly	The media was loaded too tight.	Reload the column.
The chromatographic peak trails	The media was loaded too loose.	Reload the column.
The column bed cracks or being dry	Leakage occurred or a large volume of bubbles was introduced.	Check whether there is leakage or bubble, reload the column.
Flow of the column is exceedingly slow	Protein or lipids accumulate in the media.	Wash the media or filter membrane timely.
	Protein precipitates in the media.	Adjust the content of equilibrium liquid and wash buffer to maintain the stability of target compound and binding efficiency of media.

	There is microbial grow in the media.	Filter and degas all the reagents. Samples must be centrifuged or filtered before loading.