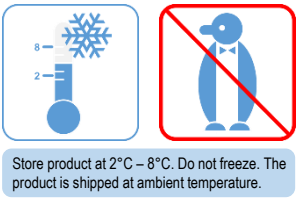

Store product at 2°C – 8°C. Do not freeze. The product is shipped at ambient temperature.

ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose

Cat. No.	Package Size
11-0227-010	ProteIndex Chemical-Tolerant Ni-Penta Agarose, 10 mL settled resin
11-0227-050	ProteIndex Chemical-Tolerant Ni-Penta Agarose, 50 mL settled resin
11-0227-100	ProteIndex Chemical-Tolerant Ni-Penta Agarose, 100 mL settled resin

Intended Use

This product is intended for research use only. Not intended for any animal or human therapeutic or diagnostic use.

Table of Contents

1. Product Description

ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose is a new immobilized metal ion affinity chromatography (IMAC) medium precharged with nickel ion via a proprietary, chemical-stable pentadentate ligand. It is designed mainly for capturing and purification of histidine-tagged proteins secreted into eukaryotic cell (i.e. mammalian, and insect) culture supernatants. The strong nickel ion binding provides excellent resistance to EDTA and reducing agents such as DTT and β-ME. Ni-Penta Agarose enables direct loading of a large amount of culture sample, unlike conventional IMAC medium that requires a preceding step to remove nickel-chelating agents.

Table 1. Characteristics of Chemical-Tolerant Ni-Penta™ Agarose resin.

Matrix	4% agarose supplied as 50% slurry
Binding capacity	>10 mg 6XHis-tagged protein/mL medium
Particle size	45 – 165 µm
Maxi pressure	0.1MPa, 1 bar
Storage solution	1x PBS containing 20% ethanol
Storage	2°C – 8°C

Table 2. Chemical compatibilities of Ni-Penta Agarose.

Solution	Tested Duration
0.01 M HCl, 0.01 M NaOH	One week
10 mM EDTA, 1 M NaOH, 5 mM DTT, 5 mM TCEP, 20 mM β-ME, 6 M guanidine-HCl	24 hours
500 mM imidazole, 100 mM EDTA	2 hours
30% isopropanol	20 minutes

2. Operation

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers by passing through a 0.22 µm or 0.45 µm filter before use.

Equilibration Buffer: 20mM sodium phosphate, 0.5 M NaCl, pH7.4

Wash Buffer: 20mM sodium phosphate, 0.5 M NaCl, 0 – 5 mM imidazole, pH7.4

Elution Buffer: 20mM sodium phosphate, 0.5 M NaCl, 250 mM imidazole, pH7.4

Note:

1. It is not recommended to include imidazole in the sample and equilibration buffers. To minimize host cellular proteins in the eluate, low concentration of imidazole can be added to the Wash Buffer. However, the concentration of imidazole should be determined empirically. For some target proteins, even a small increase of the imidazole concentration in the wash buffer may lead to partial elution. To elute bound His-tagged proteins, we suggest titrate the amount of imidazole needed for sufficient elution, starting from lower concentrations, and use up to 250 mM in concentration.
2. Adding salt (e.g. 0.5 to 1.0 M NaCl) to buffers may help minimize ion-exchange effects.
3. Other methods can also be employed to elute the target protein, for example lowering pH to the range 2.5 to 5.0. Combining different approaches may also be helpful for elution of difficult proteins.

2.2 Sample Preparation

1. Before sample loading, remove cells by centrifugation at 7,000 rpm for 10 – 15 min at 4°C. To avoid clogging of the column, it is also recommended to remove cell clumps and debris by filtering the sample solution through a 0.45 µm filter.
2. For optimal binding, it is **NOT** recommended to include imidazole in the sample and Equilibration Buffer.

3. When the concentration of target protein is low in the sample, pre-treatment is unnecessary and a batch purification procedure is usually recommended.

Cleared culture medium or lysate is now ready to be purified on Ni-Penta Agarose resins using either batch or column protocol.

2.3 Batch Purification

2.3.1 Preparation of resins

1. Determine the volume of resins required for your purification. For example, use 1 ml of 50% resin slurry for every 4 ml medium or lysate.
2. Gently shake the bottle to resuspend the slurry and transfer a desired amount to a tube.
3. Settle the resins by centrifugation at 500 x g for 5 min. Carefully discard the supernatant.
4. Add 5 volumes of Equilibrate Buffer to the tube to equilibrate the resins.
5. Sediment the resins by centrifugation at 500 x g for 5 min. Carefully remove the supernatant.
6. Repeat Steps 4 and 5 once.

2.3.2 Batch sample purification

1. Add the cleared culture medium or lysate to the tube containing the equilibrated resins. Mix gently by shaking (e.g., 200 rpm on a rotary shaker) for 15–60 min at room temperature.
2. Load lysate–resin mixture carefully into an empty gravity column with the bottom cap still attached.
3. Remove the bottom cap and drain the flow-through. Collect a sample of flow-through for SDS-PAGE analysis.
4. Wash the column with 10 column volumes of Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5. Elute the target protein with Elution Buffer and collect the eluate fractions.

6. Equilibrate the column with 5 column volumes of Equilibrate Buffer, distilled water, and then 1xPBS containing 20% ethanol. Finally store the beads with 1xPBS containing 20% ethanol at 4°C.

2.4 Column Purification

2.4.1 Packing Columns

1. Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
2. Close the column outlet leaving the net covered with packing buffer.
3. Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

   If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
4. Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, Chemical-Tolerant Nickel-Penta Agarose 6 should be packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
5. When the bed has stabilized, close the bottom outlet and stop the pump.

   If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6. With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
7. Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
8. Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4.2 Sample Purification

1. Mix the slurry by gently inverting the bottle several times to completely suspend the Ni-Penta Agarose**.** Close the column outlet leaving the net covered with packing buffer. Transfer the slurry to the column.
2. Allow the resin to settle down and the buffer to drain from the column. Add 5 column volumes Equilibrate Buffer to the column to equilibrate the beads.
3. Load the sample to the column. Collect the flow-through for measuring the binding efficiency to the beads, i.e. by SDS-PAGE.
4. Wash the column with 10 column volumes Wash Buffer or until the absorbance of the effluent at 280 nm is stable.
5. Elute the target protein with Elution Buffer and collect the eluate.
6. Equilibrate the column with 5 column volumes of Equilibrate Buffer, distilled water and 1x PBS containing 20% ethanol. Finally store the beads with 1x PBS containing 20% ethanol at 4°C.

2.5 Analysis

Identify the fractions that contain His-tagged protein, by using UV absorbance, SDS-PAGE, or Western blot.

3. Cleaning-in-Place

A column used to purify protein from cell extract usually contains some soluble substances and cell debris that can be non-specifically absorbed onto the matrix support. Cleaning-in-Place

eliminates materials that are not removed by regeneration and prevents progressive buildup of contaminants. If the column is to be reused, these contaminants should be cleaned from the column, as they were not completely removed during the sample clarification steps.

To remove strongly bound proteins via hydrophobic interaction, lipoproteins, and lipids:

Option I: Wash with 1 M NaOH

1) Wash the column with 1M NaOH. The contact time is usually 1 to 2 hours;
2) Finally wash the column with 10 CV of Equilibration Buffer.

Option II: Wash with 30% isopropanol

1) Wash the column using 5 – 10 column volumes of 30% isopropanol, and contact for 15 – 20 min.
2) Wash the column with 10 CV distilled water.

Option III: Wash with non-ionic detergent in acidic or alkaline solution

1) Wash the column with 2 CV of 0.1 – 0.5% non-ionic detergent in 0.1 M acetic acid. Contact for 1 – 2 hours.
2) Wash the column with 10 CV distilled water.

To remove proteins that are bound via ionic interactions:

1) Wash the column with 1.5 M NaCl solution contacting for 10 – 15 min.
2) Finally wash the column with 10 CV distilled water.

4. Storage

For long-term storage, Ni-Penta™ Agarose should be stored in 20% ethanol at 2 – 8°C.

5. Trouble Shooting Guide

Problem	**Probable cause**	**Solution**
Back pressure exceeds 1 bar	Column is clogged	Cleaning in place (Part 3).
		Increase the centrifugation speed or filtering the sample.
No protein is eluted	Expression of target protein in extract is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply large sample volume.
	Target protein is found in the flow through	Reduce imidazole concentration in Lysis Buffer and Wash Buffer. Increase buffer pH.
	Elution conditions are too mild.	Increase imidazole concentration in Elution buffer. Or decrease buffer pH.
	Target protein is degraded or the his-tag is missing	Operate at 4°C. Add protease inhibitors.
		Make a new construct with his-tag attached to other terminus.
His-tagged protein is not pure	Wash is not enough	Increase the volume of Wash Buffer.
	Association between the His-tagged protein and protein contaminants.	Optimize the wash condition by adjusting the pH, salt concentration, and imidazole concentration.
		Add an additional chromatography step, which can be of either ion exchange, hydrophobic interaction, or size exclusion.

Protein precipitates during purification	Temperature is too low	Perform the purification at room temperature.
	Aggregate formation	Add solubilizing agents to the samples and buffers, such as 0.1% Triton X-100, Tween-20 and ≤20% glycerol to maintain protein solubility.

6. Related Products

Product Name	Package Size	Cat. No.
ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose 6 FF		
	10 mL settled resin	11-0228-010
	50 mL settled resin	11-0228-050
	100 mL settled resin	11-0228-100
ProteIndex™ Co-NTA Agarose 6 Fast Flow		
	10 mL settled resin	11-0231-010
	50 mL settled resin	11-0231-050
	100 mL settled resin	11-0231-100
ProteIndex™ Ni-NTA Agarose 6 Fast Flow		
	10 mL settled resin	11-0225-010
	50 mL settled resin	11-0225-050
	100 mL settled resin	11-0225-100
ProteIndex™ Ni-IDA Agarose 6 Fast Flow		
	25 mL settled resin	11-0222-025
	250 mL settled resin	11-0222-250
ProteIndex™ Ni-IDA Agarose		
	25 mL settled resin	11-0221-025
	250 mL settled resin	11-0221-250
ProteIndex™ Ni-NTA Agarose		
	10 mL settled resin	11-0224-010
	50 mL settled resin	11-0224-050
	100 mL settled resin	11-0224-100
ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Coarse		
	100 g	11-0248-100
	500 g	11-0248-500
ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Medium		
	100 g	11-0249-100
	500 g	11-0249-500
ProteIndex™ SEC G-25 Cross-linked Dextran Resin, Fine		
	100 g	11-0250-100
	500 g	11-0250-500
Empty Gravity Flow Columns		
	12 mL, pack of 50	11-0257-050
	3 mL, pack of 50	11-0258-050
	6 mL, pack of 50	11-0259-050
	30 mL, pack of 25	11-0260-025
	300 mL, pack of 10	11-0261-010

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