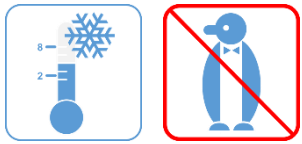

Store product at 2°C – 8°C. Do not freeze. The product is shipped at ambient temperature.

ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose 6 FF Prepacked Cartridge

Cat. No.	Package Size
11-0229-5x1ML	ProteIndex Chemical-Tolerant Ni-Penta Agarose 6 FF Prepacked Cartridge, 5 x 1 mL settled resin
11-0229-1x5ML	ProteIndex Chemical-Tolerant Ni-Penta Agarose 6 FF Prepacked Cartridge, 1 x 5 mL settled resin
11-0229-5x5ML	ProteIndex Chemical-Tolerant Ni-Penta Agarose 6 FF Prepacked Cartridge, 5 x 5 mL settled resin

Intended Use

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

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1. Product Description

ProteIndex™ Chemical-Tolerant Ni-Penta™ Agarose 6 FF Prepacked Cartridge is a prepacked, ready-to-use column for capturing and purification of histidine-tagged proteins that are secreted from eukaryotic cells into the culture medium. The special design of the column, together with the matrix, provides a fast, simple and easy separation tool.

Chemical-Tolerant Ni-Penta™ Agarose 6 FF Prepacked Cartridge are packed with 1 ml and 5 ml of Ni-Penta Agarose 6 Fast Flow. The columns have standard interface that can be adapted to most commonly used chromatography systems, such as ÄKTA.

Table 1. Characteristics of the cartridges.

Cartridge Volume	1 mL	5 mL
Column inner diameter	7 mm	16 mm
Cartridge dimensions	7 × 25 mm	16 x 25 mm
Cartridge body material	Polypropylene	Polypropylene
End plug material	Polypropylene	Polypropylene
Compatible chromatography systems	AKTA, syringe, other peristatic pumps	AKTA, syringe, other peristatic pumps

Table 2. Characteristics of Ni-Penta™ Agarose 6 FF.

Matrix	Highly cross-linked 6% agarose
Binding capacity	>10 mg 6xHis-tagged protein/ml medium
Particle size	45 – 165 µm
Maxi pressure	0.3 MPa, 3 bar
Storage solution	1x PBS containing 20% ethanol
Storage	2°C – 8°C

The base matrix of Chemical-Tolerant Ni-Penta Agarose 6 Fast Flow is highly cross-linked 6% agarose, the chelating group has then been charged with nickel ions (Ni^{2+}). The strong nickel ion binding provides very high resistance to EDTA and reducing agents like DTT. Chemical-Tolerant Nickel-chelating Agarose 6 Fast Flow enables direct loading of large sample volumes without having to remove agents that cause stripping of nickel ions from conventional IMAC medium. It is stable in all buffers commonly used in IMAC.

Table 3. Chemical compatibilities of Ni-Penta™ Agarose 6 FF.

Solution	Test Time
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-mercaptoethanol, 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, up to 30 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, it is recommended to add imidazole at low concentrations in the wash buffer. However, the amount 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.

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 loading the sample, 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 filter the sample solution through a 0.45 µm filter to remove cell clumps and debris.

2 For optimal binding, it is not recommended to include imidazole in the sample and Equilibration Buffer.

3 Sample pre-treatment may not be necessary if the concentration of target protein is low in the sample.

2.3 Sample Purification

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.
2. Wash the column with 10 column volumes of binding buffer.
3. Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.
4. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
5. Elute with 5 column volumes of Elution Buffer. Other volumes may be required if the protein is difficult to elute.

2.4 Analysis

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

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 6 FF Prepacked Cartridge should be stored in 20% ethanol at 2 - 8°C. Tightly seal the cartridge outlet with an end cap to avoid dry out.

5. Trouble Shooting Guide

Problem	Probable cause	Solution
Back pressure exceeds 1 bar.	Column is clogged	Cleaning in place (Section 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 the concentration of imidazole in Lysis and Wash Buffers. 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 a 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™ Ni-NTA Agarose 6 FF Prepacked Cartridge		
	5x 1 mL settled resin	11-0230-5x1ML
	1x 5 mL settled resin	11-0230-1x5ML
	5x 5 mL settled resin	11-0230-5x5ML
ProteIndex™ Co-NTA Agarose 6 FF, Prepacked Cartridge		
	5x 1 mL settled resin	11-0253-5x1ML
	1x 5 mL settled resin	11-0253-1x5ML
	5x 5 mL settled resin	11-0253-5x5ML
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

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