Ni -IMAC Pre-Packed Column

Catalog # 9078

For Research Use Only - Not Human or Therapeutic Use

DESCRIPTION:	Ni-IMAC: 6% cross-linked Sepharose
APPLICATION:	Separation and purification of Histidine (His)-tagged proteins and biological molecules which interact with Ni^{2+}
QUANTITY:	5 ml (resin volume)
FORM:	Suspension in 20% ethanol (50% slurry)
BINDING CAPACITY:	40 mg (His-tagged protein)/ml media
ION CAPACITY:	16-23 µmol Ni^{2+}/ml media
pH RANGE:	pH 3 - 12
STORAGE:	4 - 30 degrees C (DO NOT FREEZE)
STABILITY:	1 year
COLUMN DIMENSIONS:	9.5 cm x 1-2 cm
NOTES:	Avoid using chelating agents (e.g. EDTA and EGTA) and strong reducing agents (e.g. DTT and DTE)
REFERENCES:	N/A

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INTRODUCTION

Immobilized Metal Ion Affinity Chromatography (IMAC) is a powerful purification technique based on the principle of specific amino acid side chains of a protein binding to metal ions immobilized on a matrix. Ni^{2+} is commonly used for Histidine (His)-tagged purification as it gives a high yield. Chondrex, Inc. offers prepacked Ni-IMAC columns (5 ml) which are applicable for the separation and purification of His-tagged proteins and biological molecules which interact with Ni^{2+}. The product is compatible with common reagents listed in Table 1.

Table 1. Compatibility with Common Reagents

Buffer Reagents	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
Denaturants	8M Urea
	6M Guanidine-HCl
Detergents	2% Triton X-100
	2% Tween 20
	2% NP-40
	2% Cholate
	1% CHAPS
Reducing Agents	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

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SAMPLE PROTOCOL (His-tagged protein purification)

Native Conditions

Buffer Preparation:

- Equilibration and Wash Buffer: 50 mM sodium phosphate (pH 7.4) with 0.3 M sodium chloride and 10 mM imidazole.
- Elution buffer: 50 mM sodium phosphate (pH 7.4) with 0.3 M sodium chloride and 250 mM imidazole.

  NOTE: imidazole concentration should be optimized for each sample.

Sample Preparation:

- The buffer of the samples should be the same as the equilibration buffer.

  NOTE: the buffer can be exchanged by dialysis or diafiltration
- Clarification of the samples by centrifugation or filtration.

Purification Protocol:

1. Ensure the resin in the column is packed at the bottom of the column.
2. Remove the cap, attach a 3-way stopcock, open the valve, and drain the storage solution in the column.
3. Keep the stopcock valve open and equilibrate the column with 50 ml of equilibration buffer.
4. When equilibrating is complete, drain the column until no buffer remains in the resin and close the stopcock valve.
5. Open the stopcock valve and load the sample onto the column.
6. Pool the flow through sample.
7. Drain the column until no sample remains in the resin and close the stopcock valve.
8. Open the stopcock valve and wash the column with equilibration buffer.
9. Collect 5 to 10 tubes of 5 ml fractions. Keep collecting until the A280 OD value stabilizes.
10. Drain the column until no buffer remains in the resin and close the stopcock valve.
11. Open the stopcock valve and elute the column with elution buffer.
12. Collect 5 to 10 tubes of 5 ml elution fractions.
13. Check the A280 OD values of each fraction.
14. Drain the column until no buffer remains in the resin and close the stopcock valve.
15. Combine all positive fractions into a single purified sample.

    NOTE: if needed, concentrate the sample by ultrafiltration and then dialyze to change the buffer.

16. Open the stopcock valve and wash the column with 25 ml of distilled water.
17. Drain the column until no water remains in the resin and close the stopcock valve.
18. Open the stopcock valve and wash with 25 ml of distilled water containing 20% ethanol until the liquid level is several millimeters above the top of the resin bed (do not allow the resin to dry out).
19. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.
20. Store at 4-30 degrees C.

Denaturing Conditions for Samples Forming Inclusion Bodies

Buffer Preparation (8M Urea is necessary for purifying inclusion bodies)

- Equilibration Buffer: 0.1 M sodium phosphate, pH 8.0, with 8 M Urea and 10 mM β-mercaptoethanol
- Wash Buffer: 0.1 M sodium phosphate, pH 6.3, with 8 M Urea and 10 mM β-mercaptoethanol
- Elution Buffer: 0.1 M sodium phosphate, pH 4.3, with 8 M Urea and 10 mM β-mercaptoethanol

  NOTE: some recombinant His-tagged proteins may elute in the pH range of 4.3-6.3 so some elution may occur while washing the column after loading.

Sample Preparation:

- The buffer of the samples should be the same as the equilibration buffer.

  NOTE: the buffer can be exchanged by dialysis or diafiltration

- Clarification of the samples by centrifugation or filtration.

Purification protocol:

1. Ensure resin in the column is packed at the bottom of the column.
2. Remove the cap, attach a 3-way stopcock, open the valve, and drain the storage solution in the column.
3. Keep the stopcock valve open and equilibrate the column with 50 ml of equilibration buffer.
4. When equilibrating is complete, drain the column until no buffer remains in the resin and close the stopcock valve.
5. Open the stopcock valve and load the sample onto the column.
6. Pool the flow through sample.
7. Drain the column until no sample remains in the resin and close the stopcock valve.
8. Open the stopcock valve and wash the column with wash buffer.
9. Collect 5 to 10 tubes of 5 ml fractions. Keep collecting until the A280 OD value stabilizes.

10. Drain the column until no buffer remains in the resin and close the stopcock valve.
11. Open the stopcock valve and elute the column with elution buffer.
12. Collect 5 to 10 tubes of 5 ml elution fractions.
13. Check the A280 OD values of each fraction.
14. Drain the column until no buffer remains in the resin and close the stopcock valve.
15. Combine all positive fractions into a single purified sample.

    NOTE: if needed, concentrate the sample by ultrafiltration and then dialyze to change the buffer.
16. Open the stopcock valve, wash the column with 25 ml of distilled water.
17. Drain the column until no water remains in the resin and close the stopcock valve.
18. Open the stopcock valve and wash with 25 ml of distilled water containing 20% ethanol until the liquid level is several millimeters above the top of the resin bed (do not allow the resin to dry out).
19. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.
20. Store at 4 - 30 degrees C.

Regeneration of Ni-IMAC Resin

When elution speed gets slow due to protein/lipid contamination or the resin changes its color from blue to brown (reduced), it is necessary to regenerate the Ni-IMAC resin.

1. Wash the column with 25 ml of distilled water.
2. Wash the column with 25 ml of 0.2 M NaOH to remove contaminant proteins/lipids.
3. Wash the column with 25 ml of distilled water.
4. Wash the column with 25 ml of 0.1M of EDTA at pH 8.0 to remove Ni.
5. Wash the column with 25 ml of distilled water.
6. Wash the column with 25 ml of 0.1 M $NiSO_4$ to recharge Ni.
7. Wash the column with 25 ml of distilled water.
8. Wash the column with 25 ml of distilled water containing 20% ethanol. Stop when the liquid level is still several millimeters above the top of the resin bed (do not allow the resin to dry out).
9. Close the stopcock, replace the cap, and ensure all connections are tight and sealed.
10. Store at 4 - 30 degrees C.

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