3. Set the spin column upright in a 1.5 ml microcentrifuge tube.
4. Centrifuge the spin column at ~80 x g (1,000 rpm)* for 30 seconds to remove the buffer.
5. Add 500 µl of DI water to the column. Centrifuge the column to remove water. Repeat two more times. Replace bottom cap on the column.
6. Add 200 µl of 100 mM $NiSO_4$ to the column, then replace the top cap and incubate with mixing for 20 minutes at room temperature (RT).
7. Remove the top and bottom caps; set the spin column upright in a new 1.5 ml microcentrifuge tube. Centrifuge the column for 30 seconds to remove the charging solution. Repeat steps 6 and 7 once.
8. Add 200 µl of DI water to the spin column. Centrifuge the column to remove the water. Repeat two more times. Replace the bottom cap.
9. Add 200 µl of BB1 to the column; replace top cap. Mix and centrifuge the column to remove the buffer. Replace the bottom cap. Repeat two more times.
10. Resuspend the support with 90 µl of BB1.
11. Dilute 100–150 µl of sample 1:3 in BB1.

* Recommended speed for an Eppendorf 5417R centrifuge.

12. Add dilute sample to the spin column, then replace the top cap. Mix for 20–40 minutes at 4°C.
13. Remove top and bottom caps; set column upright in a new 1.5 ml microcentrifuge tube. Centrifuge column for 30 seconds and collect the flowthrough (unbound fraction). Replace the bottom cap.
14. Add 100 µl BB2 to the spin column, then replace the top cap and incubate with mixing for 5 minutes at RT. Remove the top and bottom caps; set the column upright in a new 1.5 ml microcentrifuge tube. Centrifuge for 30 seconds and collect the fraction. Replace the bottom cap.
15. Repeat step 14 and pool the fractions. Label the tube with the buffer used.
16. Repeat steps 14–15 using WB1; repeat steps 14–15 using WB2; and repeat steps 13–14 using EB.
17. The fractions are ready to be profiled on ProteinChip arrays.

Ordering Information

Catalog #	Description
C54-00027	**ProteinChip IMAC Spin Columns,** 20
C57-30078	**ProteinChip IMAC30 Arrays,** A-H format, 12

BIO-RAD

Bio-Rad Laboratories, Inc.

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ProteinChip® IMAC Spin Columns

Instruction Manual

Catalog #C54-00027

For technical support, call your local Bio-Rad office, or in the US, call **1-800-4BIORAD (1-800-424-6723)**.

BIO-RAD

Introduction

Immobilized metal affinity chromatography (IMAC) is a frequently used technique for the separation of proteins and peptides. IMAC exploits the affinity of proteins or other molecules for chelated metal ions. This affinity is due to coordination bonds formed between metal ions and certain exposed side chains of protein amino acids. IMAC is orthogonal to other classical separation methods such as ion exchange or hydrophobic interaction.

ProteinChip IMAC spin columns contain IMAC support. Prior to column use, metal ions should be immobilized on the IMAC support, which uses polymer-bound iminodiacetic acid (IDA) to chelate the metal ions. The adsorbent binds to nitrogen-containing functional groups (imidazole in histidine (His) groups) on proteins, His-tagged fusion proteins (e.g., 6x His), as well as phosphorylated proteins and peptides.

Applications

Crude biological samples contain thousands of proteins and peptides, presenting significant analytical challenges for researchers evaluating in vitro and in vivo experiments from cell lysates and plasma samples. Reducing sample complexity is essential to better understanding of cellular mechanisms and disease states. IMAC is a powerful method to enrich certain classes of proteins in samples.

Common applications include:

- Purification of 6x His-tagged recombinant proteins (when loaded with Ni^{2+})
- Purification of phosphorylated proteins and peptides (when loaded with Ga^{3+} or Fe^{3+})
- IMAC support mimics binding and elution properties of ProteinChip IMAC30 arrays. ProteinChip IMAC spin columns can be used for small-scale purification of proteins discovered on array surfaces

Storage

Store ProteinChip IMAC spin columns at 4°C.

Technical Considerations

- IMAC support is supplied in a slurry/suspension in 1 M NaCl, 20% ethanol (v/v). Before immobilizing a metal ion, wash the support extensively with deionized (DI) water to eliminate ethanol and sodium chloride preservatives in the storage solution
- Recommended sample binding time is 20–40 minutes

Recommended Buffers

Charging Solutions

Copper (Cu^{2+}) — 100 mM copper sulfate
Nickel (Ni^{2+}) — 100 mM nickel sulfate
Gallium (Ga^{3+}) — 50 mM gallium nitrate
Iron (Fe^{3+}) — 100 mM iron (III) sulfate

Binding and Equilibration Buffers

The choice of starting buffer depends on the properties of the chelated metal ion and the binding properties of the sample molecules. Sodium phosphate buffers are recommended.

Note: EDTA or EGTA should be avoided in the buffers.

Elution Buffers

Elution should be optimized on a case-by-case basis. Typically, elution can be achieved by increasing concentrations of imidazole, by reducing the pH, or a combination of both.

Imidazole-Based Elution

The concentration of imidazole needed depends on the protein, but also on the type of metal ion immobilized on the support. Typically, concentrations of 5–100 mM imidazole will be used, in the presence of 0.15–0.5 M NaCl. The presence of NaCl is not essential, but may prevent nonspecific adsorptions.

pH Dependent Elution

Sodium acetate, phosphate, or citrate buffers can be used (e.g., 50 mM sodium phosphate, pH 3.5 containing 0.14 M NaCl). Depending on the type of immobilized ion, acidic elution is not always complete and can be exploited for fractionation, e.g., with Cu^{2+},

pH dependent elution may allow you to fractionate IgG from contaminating proteins, such as transferrin in albumin-depleted human serum.

Example Protocol: Purification of 6x His-Tagged Proteins

- Binding buffer 1 (BB1) — 50 mM NaH_2PO_4, 0.5 M NaCl, 10 mM imidazole, 12 mM CHAPS, adjusted to pH 8.0 with NaOH
- Binding buffer 2 (BB2) — 1:1 dilution of BB1 with DI water (25 mM NaH_2PO_4, 250 mM NaCl, 5 mM imidazole, 6 mM CHAPS, adjusted to pH 8.0 with NaOH)
- Wash buffer 1 (WB1) — 25 mM NaH_2PO_4, 250 mM NaCl, 10 mM imidazole, 6 mM CHAPS, adjusted to pH 8.0 with NaOH
- Wash buffer 2 (WB2) — 25 mM NaH_2PO_4, 250 mM NaCl, 100 mM imidazole, 6 mM CHAPS, adjusted to pH 8.0 with NaOH
- Elution buffer (EB) — 25 mM NaH_2PO_4, 250 mM NaCl, 500 mM imidazole

1. Tap the ProteinChip IMAC spin column lightly to settle the support to the bottom (near the tapered end) of the column.
2. Remove top and bottom caps on the spin column.