

# IPAC™ Resin Protein Purification Protocol

**Product No: IPAC40-M-10, IPAC40-M-25, IPAC40-M-50, IPAC40-M-100**

The following protocol has been optimized for use with **copper or nickel ions** on the IPAC support. Suggestions on buffers and experimental conditions for different classes of proteins are found on the last page of this protocol.

## Immobilization of Metal Ions on the IPAC resin support

1. Fully resuspend the IPAC resin slurry in the container provided. Using a pipette fitted with a dropper bulb, slowly add the slurry to the inside wall of the column and allow it to run to the bottom. Allow the column to flow until the settled resin bed reaches the desired height. Allow the remainder of the storage solution to run through the support until its level reaches the top of the gel bed (do not allow the gel bed to run dry).
2. Wash the column with 5-10 bed volumes of deionized water to remove all preservatives.
3. Prepare a 50 mM metal sulfate solution to immobilize on the IPAC support. Pass three bed volumes of this solution through the column.
4. Wash the column with 5 bed volumes of water to remove any unbound metal ions.
5. If storing the column for later use, wash the column with 5 bed volumes of deionized water containing 0.02% sodium azide, or a 20% methanol or ethanol solution in water and store at room temperature. If the resin is going to be used immediately, proceed with "Purification of a Protein Using IPAC."

## Purification of a Protein using IPAC

1. Equilibrate the column with 5 bed volumes of a 20 mM sodium phosphate, 0.5 M sodium chloride solution, pH 7.0-7.5.
2. Pass the sample containing protein through the column. The sample should be in the same buffer as described in Step 1 (20 mM sodium phosphate, 0.5 M sodium chloride solution, pH 7.0-7.5).
3. Wash the column with 5-10 bed volumes of a 20 mM sodium phosphate, 0.5 M sodium chloride solution, pH 7.0-7.5, to remove any proteins in the sample nonspecifically bound to the support.
4. Elute the column-bound protein using one of the methods described below:
  - a. Wash the column with 5-10 bed volumes of a solution containing 2 mM Imidazole, 20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0. Next, wash the column with 5-10 bed volumes of a solution containing 20 mM imidazole, 20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0. Collect 0.5-1.0 mL fractions as they elute from the column.

**Note:** For a high resolution elution scheme, apply a linear elution gradient from 2 mM to 20 mM imidazole in a volume that is equal to 20 times the column bed volume.

**Note:** 6xHis fusion proteins often bind strongly to immobilized  $\text{Cu}^{2+}$  columns. If you wish to elute a 6xHis fusion protein from such a column, perform the following elution scheme: wash the column with 5 bed volumes of a solution containing 5 mM imidazole, 20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0. Next, wash the column with 5 bed volumes of a solution containing 20mM imidazole, 20 mM sodium phosphate, 0.5 M sodium chloride, pH 7.0. For the final step, wash the column with 5 bed volumes of a solution containing 200 mM imidazole, 20 mM sodium phosphate and 0.5 M sodium chloride.

- b. Wash the column with 5 bed volumes of a solution containing 20 mM sodium phosphate and 0.5 M sodium chloride, pH 5.8. Next, wash the column with 5-10 bed volumes of a 20 mM sodium phosphate, 0.5 M sodium chloride solution, pH 3.8. Collect 0.5-1.0 mL fractions as they elute from the column.

**Note:** For a high resolution elution scheme, apply a linear elution gradient from pH 5.8 to pH 3.8 in a volume that is equal to 20 times the column bed volume.

## Regeneration of the IPAC resin

1. Wash the column with 5-10 bed volumes of 0.1 M EDTA to strip away the metal ions.
2. When using  $\text{Fe}^{3+}$  ion as an immobilized metal, use a solution containing 0.5 M sodium phosphate, 1 M HEDPA (1- hydroxyethylidenediphosphonic acid), pH 1.2.
3. Wash the resin with 5-10 bed volumes of 0.5 M NaOH.
4. Wash with 5-10 bed volumes of distilled water.
5. If you would like to reload a metal ion onto the IPAC resin, please repeat the steps found under "Immobilization of Metal Ions on the IPAC resin support".
6. If storing the column for later use, wash the column with 5 bed volumes of deionized water containing 0.02% sodium azide, or a 20% methanol or ethanol solution in water and store at room temperature.

Please refer to the table on the following page and the IPAC resin brochure for examples in which IPAC has been tested in-house with different types of proteins and buffer solutions, in order to further assist you in any type of developmental research.

<b>Protein</b>	<b>Metal Ion(s)</b>	<b>Starting Buffer (SB) and Wash Buffer (WB)</b>	<b>Elution Buffer(s)</b>
Ovalbumin	Fe <sup>3+</sup>	SB & WB1: 0.1 M Acetic Acid, pH 5.0  WB2: 0.05 M MES, pH 5.69	EB1: 50/50 mix of 0.5 M MES and 0.05 M PIPES, pH 6.3  EB2: 0.05 M PIPES, pH 7.2
Transferrin	Fe <sup>3+</sup>	SB & WB: 50 mM Sodium Acetate, pH 5.5	0.2 M Potassium Phosphate, pH 5.2
Calmodulin	Ca <sup>2+</sup> , Eu <sup>3+</sup>	SB & WB1: 2.0 M NaCl, 0.1 M Tris-HCl, 0.2 M Calcium Chloride, pH 7.4  WB2: 2.0 M NaCl, 0.1 M Tris-HCl, 0.6 M Sodium Sulfate, pH 7.5	0.2 M Citrate, 3.0 M Sodium Chloride, 0.2 M Potassium Phosphate, pH 6.9
Horse Myoglobin Skeletal Muscle	Cu <sup>2+</sup>	WB: 20 mM Sodium Phosphate, 0.5 M NaCl, pH 7.3	200 mM Imidazole, 20 mM Sodium Phosphate, 0.5 M NaCl, pH 7.2
Bovine serum Albumin	Cu <sup>2+</sup> , Ni <sup>2+</sup> , Zn <sup>2+</sup> , Co <sup>2+</sup>	SB & WB: Distilled Water, pH 7.26	20 mM Imidazole, pH 7.4
Human Serum Albumin	Cu <sup>2+</sup>	SB & WB: Distilled Water, pH 7.26	20 mM Imidazole, pH 7.4