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Polar MC-IMAC Excel Resins

(Immobilized Metal Affinity Chromatography Resins)

Product Description

Polar MC-IMAC Excel resins are metal affinity chromatography resins for the purification of recombinant proteins with a histidine (His) tag, which are widely used in the biotech industry. The resins are composed of hydrophilic polymethacrylate beads with high physical and chemical stability. The resins have a particle size of 30 μm or 60 μm with a pore size of 800 Å. The resin surface is highly hydrophilic, which minimizes non-specific interaction with biological analytes. On the hydrophilic surface, carboxylic acid groups which can chelate multivalent metal ions are chemically attached via a proprietary linker optimized for bio-separations.

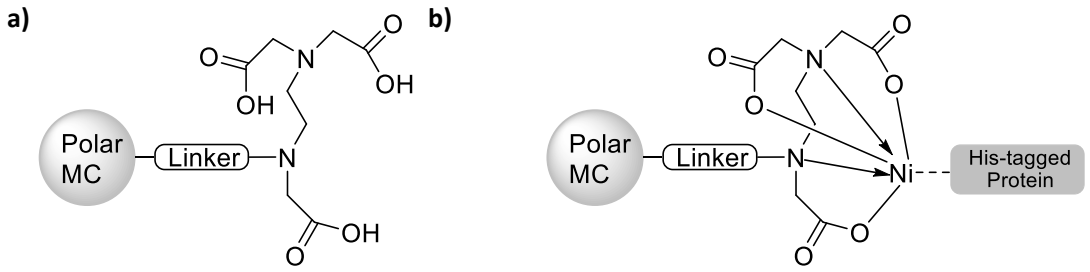

Figure 1. Chemical Structures of a) Polar MC30/60-CA Excel and b) Polar MC30/60-Ni Excel

Polar MC-IMAC Excel resins are offered in two forms: a free acid form (Polar MC30-CA Excel or Polar MC60-CA Excel, carboxylic acid) and a chelated Ni^{2+} form (Polar MC30-Ni Excel or Polar MC60-Ni

Excel). The free acid form can be used for metal ion removal and can be customized with a metal ion of the customer's choice. The structures of these two forms IMAC Excel resins are shown in **Figure 1**. The resins are highly stable and can tolerate 0.5 M NaOH close to 100 cleaning cycles. The metal ion chelated resin can be washed with 0.1 M EDTA without loss of metal ions. Therefore, no recharge of metal ion is necessary after each use. Both resins can be offered as loose resin, in FPLC cartridges, or in regular HPLC column format.

Technical Specifications

Resin Type	Polar MC30-CA Excel, Polar MC30-Ni Excel Polar MC60-CA Excel, Polar MC60-Ni Excel	
Packing	50% (v/v) slurry in 20% ethanol	
Matrix	Hydrophilic polymethacrylate	
Particle Size	20 ~ 45 µm (mean 30 µm) 45 ~ 90 µm (mean 60 µm)	
Pore Structure	800 Å	
pH Stability	1-13 for base resin, Polar MC-CA Excel	
	1-13 for metal ion complex, Polar MC-Ni Excel	
Loading Capacity	Polar MC30-CA Excel	>120 µeq. carboxylic group /mL
	Polar MC30-Ni Excel	40-60 µeq. Ni^{2+} /mL
	Polar MC60-CA Excel	>100 µeq. carboxylic group /mL
	Polar MC60-Ni Excel	40-60 µeq. Ni^{2+} /mL
HIS-Protein Loading	Polar MC30-Ni Excel, ~20 mg/mL Polar MC60-Ni Excel, ~15 mg/mL	
Operating Temperature	Up to 40 °C	
Operating Pressure	Up to 60 bar	
Mobile Phase Compatibility	aqueous solutions, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, Tris, and acetate, also compatible with Guanidine HCl 6 M, Urea 8 M, EDTA 0.1 M, DTT 10 mM	
Linear Flow Rate	Up to 1800 cm/hour	

The resins can be used as batch mode or column mode as shown in **Figure 2**. A crude His-tagged protein 300 mL was loaded to 15 x 220 mm Sepax Generik FPLC column (PN# 202000-1525-AF) packed with Polar MC60-Ni Excel resin. After washing with loading buffer, the targeted protein was eluted at a

flow rate of 7.5 mL/min with 500 mM imidazole in 25 mM sodium phosphate pH 8 buffer. The collected fractions were confirmed with 10% tris-glycine SDS-Pages, as shown in **Figure 3**.

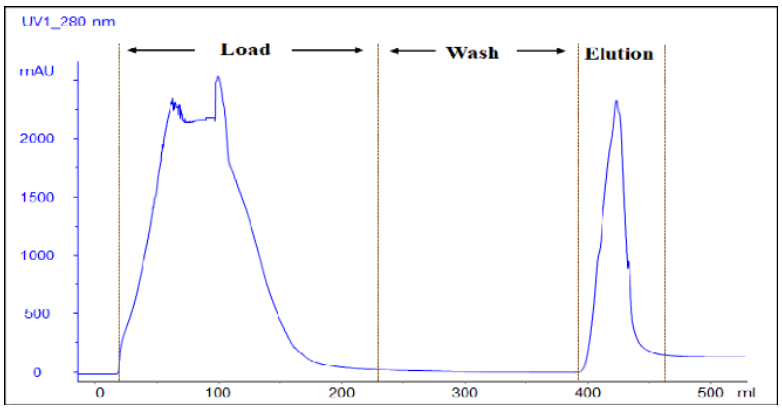

Figure 2. Purification of an exemplary His-tagged protein on Polar MC60-Ni Excel. Crude His-tagged protein in 25 mM sodium phosphate pH 8, 0.5 M NaCl was loaded onto Polar MC60-Ni Excel resin at a flow rate of 5.0 mL/min. The column was washed with 25 mM sodium phosphate pH 8, 0.5 M NaCl. The target His-tagged protein was eluted with 25 mM sodium phosphate pH 8, 0.5 M NaCl, 0.5 M Imidazole. Fractions were collected. Load was collected as Flowthrough.

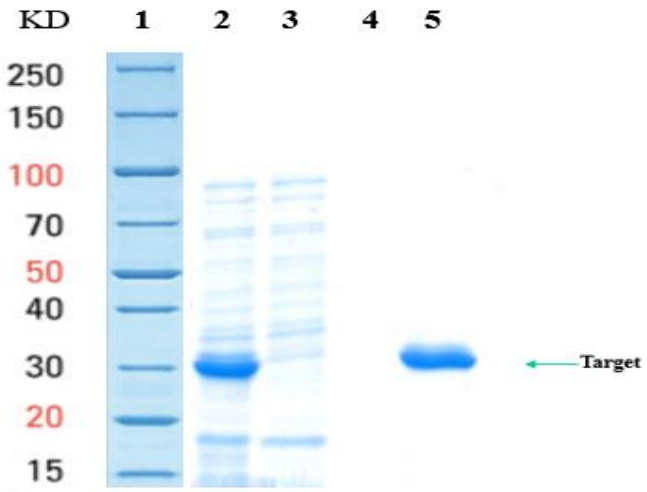

Figure 3. 10% Tris-Glycine SDS-Page analysis of fractions collected from the above purification as shown in **Figure 2**.

Lane 1: Molecular weight ladder

Lane 2: Crude His-tagged protein sample

Lane 3: Flowthrough collected as the sample was loaded onto the column

Lane 4: washing fraction after the sample loaded

Lane 5: Elution of the target with 500 mM imidazole

Caustic stability and reusability of the IMAC Excel resin are demonstrated as the resin can tolerate 90 column volume wash with 0.5 M NaOH (**Figure 4**). After each 30 cycle wash with 0.5 M NaOH at flow rate of 0.25 column volume/min with a total contact time of 2 hrs, the resin was repeated used for the purification of the same His-tagged protein. The result showed the resin binding character remained the same.

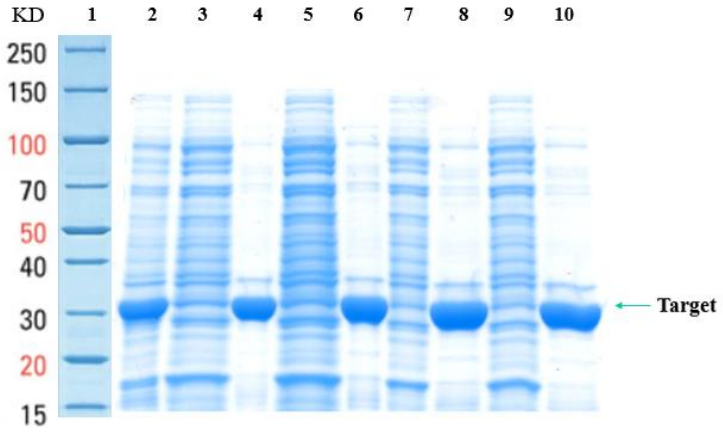

Figure 4. 10% Tris-Glycine SDS-Page analysis of fractions collected from the above purification as shown in **Figure 2**.

Lane 1: Molecular weight ladder

Lane 2: Crude His-tagged protein sample

Lane 3: Flowthrough collected as sample the was loaded on the fresh column

Lane 4: Elution of the target with from fresh Ni column

Lane 5: Flowthrough collected after column was cleaned with 30 column volumes of 0.5 M NaOH at a flow rate of 0.25 column volume/min with a total 2 hrs contact time

Lane 6: Elution of the target from Ni column cleaned with 30 column volumes of 0.5 M NaOH

Lane 7: Flowthrough collected after column was cleaned with another 30 column volumes of 0.5 M NaOH at a flow rate of 0.25 column volume/min with a total 2 hrs contact time

Lane 8: Elution of the target from Ni column cleaned with another 30 column volumes of 0.5 M NaOH

Lane 9: Flowthrough collected after column was cleaned with a third 30 column volumes of 0.5 M NaOH at a flow rate of 0.25 column volume/min with a total 2 hrs contact time

Lane 10: Elution of the target from Ni column cleaned with a third 30 column volumes of 0.5 M NaOH

Guideline for Resin Use

- Resins are shipped and stored in 50% suspension of 20% ethanol. Prior to first usage, wash the IMAC Excel resin with metal-free water (> 10 volumes of resin amount).
- Settle the resin three times in 5 volumes of water to remove the small broken particles or debris. For 60 µm resin, it takes about 1.5 hrs to settle, 30 µm resin will take about 3 hrs to settle.
- If sanitization is necessary, soak the resin in 5 volumes of 0.5 M NaOH for more than 1 hour. Filter and wash the resin with water to pH 7 before packing.

- Equilibrate column with selected binding buffer.
- It is recommended to carry out the binding of recombinant His-tagged proteins in sodium or potassium phosphate. Typical binding buffer: 20-50 mM phosphate, pH 7-8, with 0-1.0 M NaCl without imidazole.
- After binding, wash the chelated His-tagged protein resin with the binding buffer without imidazole.
- It is important to note that elution of the His-tagged proteins from the IMAC Excel resins can be affected by the following: Concentrated imidazole solution (0.1-0.5 M) in phosphate buffers with or without NaCl (0-1.0 M).
- Various proteins may require different methods and should be tested experimentally to determine the most suitable method.
- Inclusion bodies may require denaturants to allow for solubilization (6 M Guanidine HCl or 8 M Urea).
- If suitable, refolding of proteins can be performed on column.

Storage of the resin and column

For the extended storage, it is recommended that the column and resin be stored in 0.02% azide or 20 % ethanol at 2-8 °C.

CIP and Regeneration of IMAC Excel resin

- If some proteins are deposited onto the resins, denaturing chemicals such as urea and organic solvents can be used to clean the resins.
- To remove the endotoxin and HCP, the resins can be washed with 0.5 M or 1.0 M NaOH for extended period of time, then equilibrated with appropriate binding buffer for 20 column volumes.
- IMAC Excel resin can be repeatedly used without recharging of Ni2+ ion. The same resin can be used up to 100 times of the same protein. It is recommended to use new IMAC Excel resin for each different protein.
- Wash the column with 20-50 mM sodium phosphate pH 7-8 for 10-20 column volumes, before next use.

Product Order Information

Description	Particle size	P/N
Polar MC30-CA Excel	30 µm	270530800
Polar MC30-Ni Excel	30 µm	270630800
Polar MC60-CA Excel	60 µm	270560800
Polar MC60-Ni Excel	60 µm	270660800

Standard package size: 25 mL, 100 mL and 1 L.

Package sizes for bulk orders are also available.