Novagen®

Fractogel® Metal Affinity Chromatography (MAC) Resins and Cartridges

Tools for His•Tag® Fusion Protein Purification

Featured Products

Ni-MAC™ Purification Kit

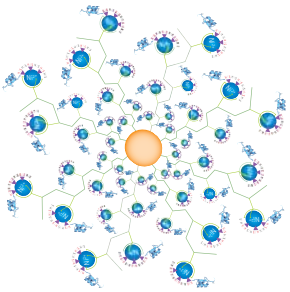
Co-MAC™ Purification Kit

u-MAC™ Cartridges

MERCK

Ni-MAC™, Co-MAC™ and u-MAC™
Metal Affinity Chromatography (MAC) Resins and Cartridges

Unique chromatography matrix with accessible tentacles



The structure of the Fractogel® matrix is unique compared to other chromatographic resins, such as dextran, agarose, or cellulose. The Fractogel matrix is a durable synthetic methacrylate-based polymeric resin that provides excellent mechanical and chemical stability, particle size of 40-90 µm, and an inert hydrophilic surface. These properties result in high flow rates and low non-specific binding, and allow for repeated use and regeneration. The resin has long polymer chain "tentacles" covalently bonded to hydroxyl groups on the Fractogel bead surface. The steric accessibility of the ligands attached to the tentacles allows high protein binding capacities. With low steric hindrance, biomolecules bind more readily during the separation process, leading to higher purification yields. Like the bead surface, the tentacles and functional ligands are stable in the presence of cleaning and regeneration buffers, so the resins can be reused many times without loss of purification performance.

Efficient metal affinity purification of His•Tag® proteins

For Metal Affinity Chromatography (MAC) applications, iminodiacetic acid (IDA) groups are attached to the Fractogel tentacles. IDA can be charged with different divalent metal ions, providing a convenient tool for rapid, efficient, one-step purification of His•Tag® fusion proteins. The Ni-MAC™, Co-MAC™, and u-MAC™ devices are pre-packed cartridges that contain 1 ml His•Bind® Fractogel Resin charged with nickel (Ni-MAC), cobalt (Co-MAC), or provided as uncharged (u-MAC) for charging with the metal of choice. The high capacity and high flow rates of the resins provide a powerful tool for metal affinity protein purification. The MAC cartridges can be used either manually, or with automated liquid chromatography (LC) instruments under low or high flow rates (up to 800 cm/h linear flow rate). Multiple MAC cartridges can be connected in series to increase binding capacity. The high chemical resistance and mechanical stability of Fractogel allows the resins to be easily regenerated and reused.

Connect to FPLC system with M6 fittings

MAC Cartridge Features:

- Rapid affinity purification of His•Tag fusion proteins
- Each cartridge packed with 1 ml Fractogel resin
- Cartridge dimensions: 2.1 cm × 0.8 cm (Fractogel bed height x diameter)
- Compatible with automated liquid chromatography systems
- Resin precharged with Ni^{2+} or Co^{2+}, or uncharged to use with choice of metal ion
- High binding capacity—up to 30 mg protein/ml
- Very low non-specific protein binding
- High mechanical and chemical stability
- Pressure stability—up to 20 bar
- High flow rates—up to 800 cm/h (~7 ml/min)
- Stable resin permits efficient regeneration
- Economical, can be reused at least 10 times

Properties of the Fractogel His•Bind tentacle affinity resins

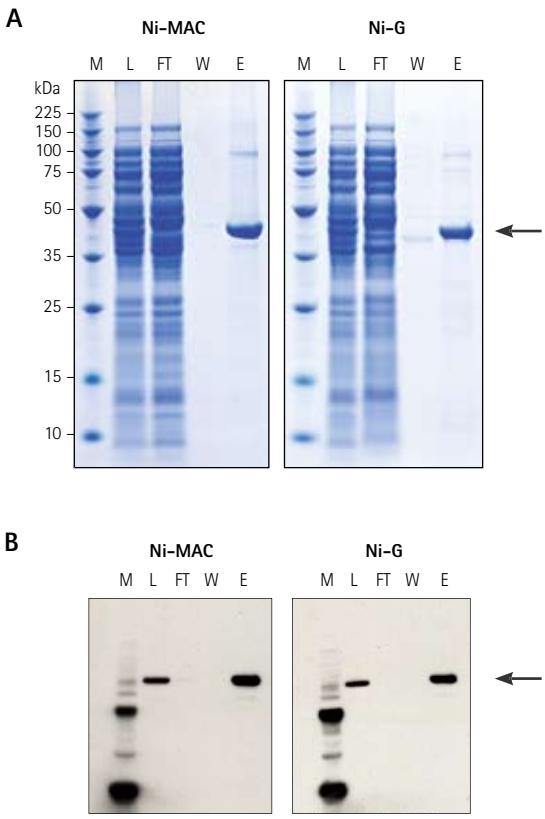
Type of Chromatography	Immobilized Metal Affinity Chromatography (IMAC)
Matrix	Fractogel crosslinked polymethacrylate
Particle size	40-90 µm
Functional group	Iminodiacetic acid (IDA)
Functional group attachment	Via long polymer chains (tentacles)
Metal ion binding capacity	80 µmol/ml of resin
Protein binding capacity	30 mg/ml of resin
Elution conditions	Increasing concentration of imidazole, free histidine, EDTA, decreasing pH
Reducing agent	Compatible with 1 mM THP
pH stability range	pH 1 to 12
Pressure limit	20 bar
Linear flow rate	up to 800 cm/h (7 ml/min)
Operating temperature	4˚C to room temperature
Storage conditions	150 mM NaCl, 20% EtOH

www. novagen.com/mac

Metal Affinity Chromatography with Ni-MAC™ Purification Kit

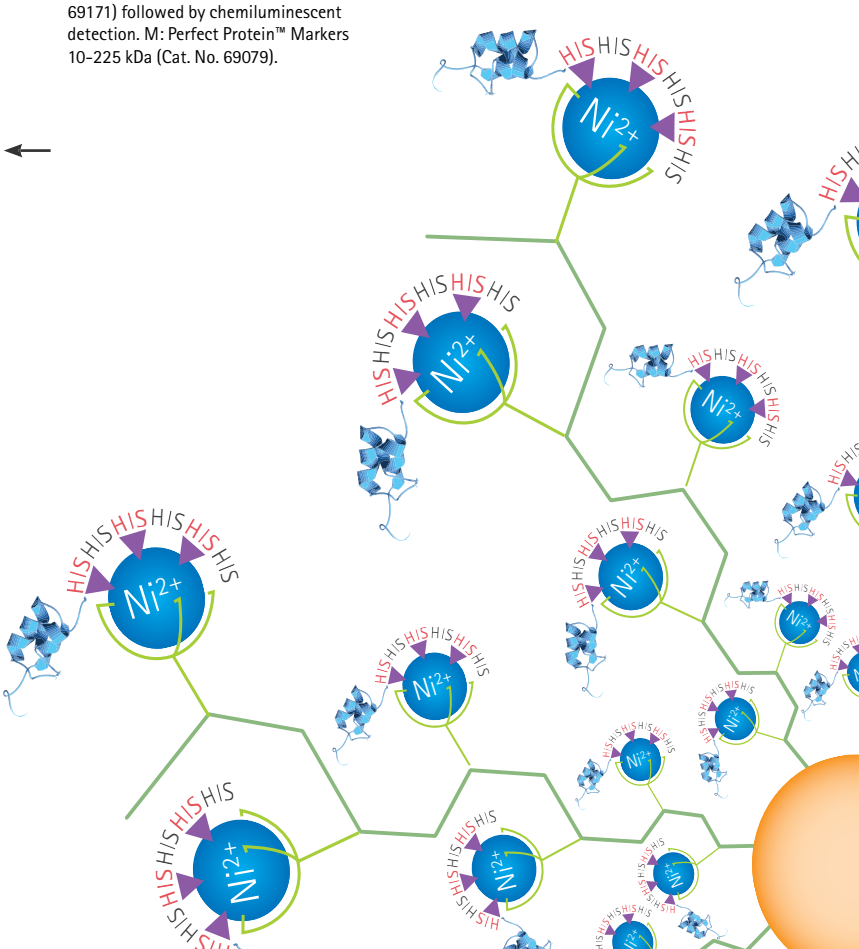
High yield specific, and efficient purification of His•Tag® proteins under denaturing and non-denaturing conditions

The Ni-MAC™ Purification Kit is designed for rapid affinity purification of His•Tag® fusion proteins by metal affinity chromatography on a Ni^{2+}-charged resin. The kit contains a set of concentrated phosphate-based buffers and 5 ready-to-use cartridges. Each Ni-MAC Cartridge is packed with 1 ml His•Bind® Fractogel® Resin, precharged with Ni^{2+}. The cartridges can be used manually with a syringe or with automated liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. Each cartridge binds up to 30 mg protein and can be reused at least 10 times.



Comparison of metal affinity purification of an ERK2–His•Tag fusion protein with Ni-MAC and competitor cartridges

An ERK2–His•Tag fusion protein (arrow) was expressed in BL21(DE3) and purified by metal affinity chromatography. The protein was purified according to manufacturers' protocols for Ni-MAC, and nickel-affinity sepharose (Competitor G, Ni-G) cartridges. Target proteins were eluted with linear gradient of 0-100% Elution buffer (10 column volumes). Crude load (L), flow-through (FT), wash (W), and elute (E) fractions (20 µl each) were collected and analyzed by 10% BIS TRIS gels. Panels show gels after (A) staining with Coomassie blue and (B) Western blot was performed using a His•Tag Monoclonal Antibody (Cat. No. 69171) followed by chemiluminescent detection. M: Perfect Protein™ Markers 10-225 kDa (Cat. No. 69079).



Metal Affinity Chromatography with Ni-MAC™ Purification Kit *continued*

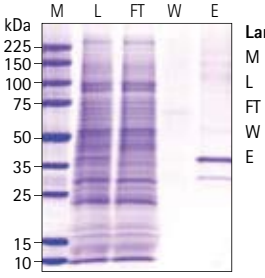
Direct Purification of His•Tag® Fusion Proteins from Insect Cell Culture

- No need to separate cells from culture media
- Lyse cells and proceed directly with the IMAC purification
- Direct affinity absorption of target proteins to Ni-MAC™ affinity resins from total culture extract

Use Insect PopCulture® Reagent and Ni-MAC™ resin

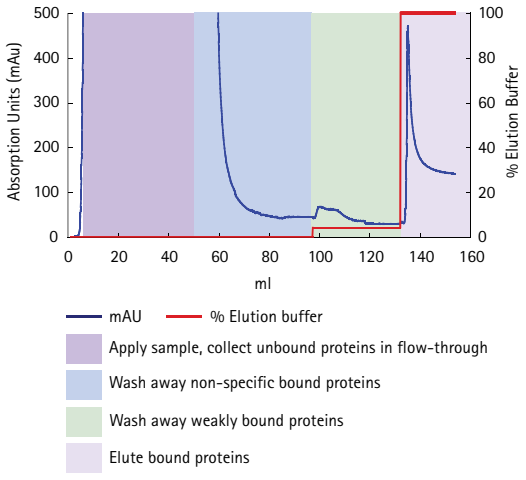
Insect PopCulture® Reagent is a detergent based lysis reagent that is specifically formulated for total insect cell culture extraction without the requirement for centrifugation. Insect PopCulture Reagent can be used for protein extraction from insect cells grown in suspension and adherent cells grown on tissue culture plates. The improved method recovers both protein released into the medium and intracellular protein, increasing processing efficiency. The method is amenable for automated expression-level screening and is compatible with the Ni-MAC affinity purification method.

A Recombinant protein kinase affinity purification

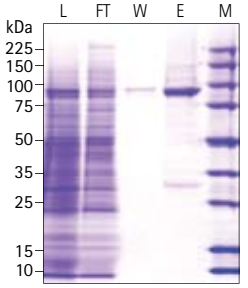


Lane	Sample
M	Perfect Protein™ Markers 10-225 kDa
L	Total cellular protein load
FT	Flow-through
W	Column wash (30 µl loaded)
E	Eluate (2 µg total protein loaded)

Kinase Ni-MAC Elution Profile

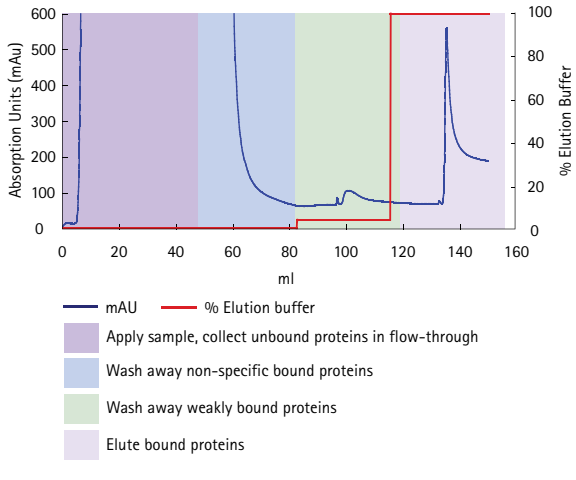


B Hsp90β affinity purification



Lane	Sample
L	Total cellular protein load
FT	Flow-through
W	Column wash (30 µl loaded)
E	Eluate (2 µg total protein loaded)
M	Perfect Protein Markers 10-225 kDa

Hsp90β Ni-MAC Elution Profile



Automated Purification of His•Tag Sf9 kinase (A) or Hsp90β (B) with Ni-MAC affinity resin

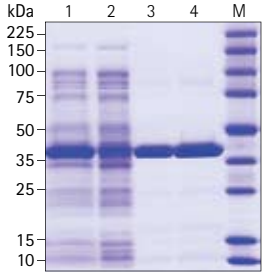
For protein expression, 50 ml TriEx™ Sf9 insect suspension cells (1.5×10^6 cells/ml) were infected with recombinant protein kinase or Hsp90β (Heat Shock Protein 90 beta) encoding baculovirus at MOI of five. 72 hours after infection, the cells were lysed using Insect PopCulture Reagent (Cat. No. 71092) and Benzonase® Nuclease (Cat. No. 70746), according to the recommended protocols. The lysate was clarified by centrifugation at 20,000 × g for 10 minutes at 4˚C. The clarified lysates were applied to two Ni-MAC columns, joined together to give a bed volume of 2.0 ml by automated LC. Contaminants were washed from the column with 1X MAC bind and wash buffers, each supplemented with 10% (v/v) glycerol. Each target protein was eluted from the columns with 1X MAC elute buffer, phosphate, supplemented with 10% (v/v) glycerol. The crude extract, flow–through, and eluate fractions were analyzed by SDS-PAGE and Coomassie blue staining. All pooled eluates were precipitated with the ProteoExtract® Protein Precipitation Kit (Cat. No. 539182), and the protein pellets resuspended in 1% SDS prior to quantification. Protein concentration of the pooled eluates was determined using the BCA Protein Assay Kit (Cat. No. 71285).

Purification of His•Tag® Fusion Proteins from Crude Bacterial Cell Lysates

Use Lysonase™ Bioprocessing Reagent and Ni-MAC™ resin

For maximum recovery of intact fusion proteins from bacterial cells the first step is to disrupt the cells and extract the relevant protein fraction. This step is critical because harsh mechanical cell disruption can result in thermal or oxidative inactivation of labile proteins. Lysonase™ Bioprocessing Reagent is an optimized, ready-to-use blend of rLysozyme™ Solution and Benzonase® Nuclease that significantly increases protein extraction efficiency and facilitates downstream processing of protein extracts. rLysozyme Solution contains a highly purified and stabilized recombinant lysozyme with specific activity 250 times greater than that of chicken egg white lysozyme. Benzonase Nuclease is a genetically engineered nonspecific endonuclease that degrades all forms of nucleic acids reducing extract viscosity, and increasing protein yield. Lysonase can be used with BugBuster® Protein Extraction Reagent or to enhance the effectiveness of non-detergent based cell lysis procedures. These extraction methods combined with the Ni-MAC™ affinity purification kits enables high-quality and quantity of purified His•Tag® fusion proteins.

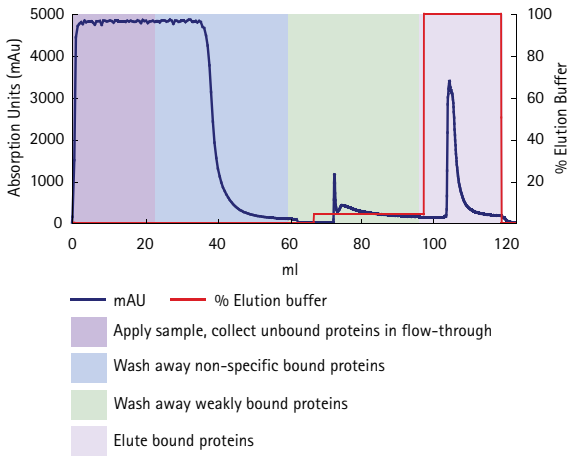
Lipocortin 1 affinity purification



Lane	Sample
1	Soluble fraction
2	Column flow-through
3	Column elution (3 µg total protein loaded)
4	Column elution (5 µg total protein loaded)*
M	Perfect Protein™ Markers 10–225 kDa

* No contaminating bands are present in the pooled fraction at the high protein load.

Lipocortin 1 Elution Profile



Lipocortin 1 purification on a single Ni-MAC column

E. coli strain BL21(DE3) was transformed with a pET vector carrying a cDNA coding for Lipocortin 1. Lipocortin 1 was expressed by using Overnight Express™ Instant TB Medium (Cat No. 71491), according to the supplied protocol. Following harvest, 20 g of wet cell paste was resuspended in 200 ml of 1X MAC bind buffer, phosphate, containing 5% (v/v) glycerol and 0.03% (v/v) BRIJ®-35 detergent. Cells were lysed by sonication and the addition of Lysonase Bioprocessing Reagent (Cat. No. 71230). The lysate was clarified by centrifugation at 10,000 × g for 10 minutes at 4°C. 25 ml of the clarified lysate was applied to a single Ni-MAC column. Lipocortin 1 was eluted from the Ni-MAC columns using 1X MAC bind, wash, and elute phosphate buffers, each supplemented with 5% (v/v) glycerol and 0.03% (v/v) BRIJ-35. Protein concentration of the pooled eluates was determined using the BCA Protein Assay Kit (Cat. No. 71285).

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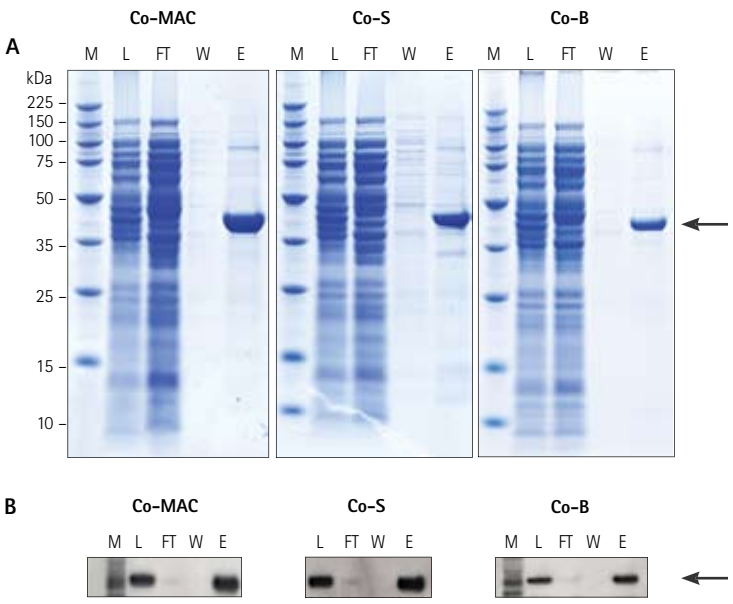
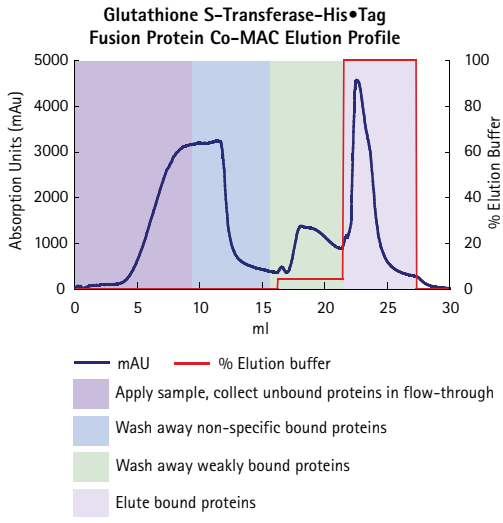
Metal Affinity Chromatography with Co-MAC™ Purification Kit

The Co-MAC™ Purification Kit is designed for rapid affinity purification of His•Tag® fusion proteins by metal affinity chromatography on a Co^{2+}-charged resin. The kit contains a set of concentrated Tris-based buffers and 5 ready-to-use cartridges. Each Co-MAC Cartridge is packed with 1 ml of His•Bind® Fractogel® Resin, precharged with Co^{2+}. The Co-MAC Cartridges can be used manually with a syringe or with automated liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. Each cartridge binds up to 30 mg protein and can be reused at least 10 times.

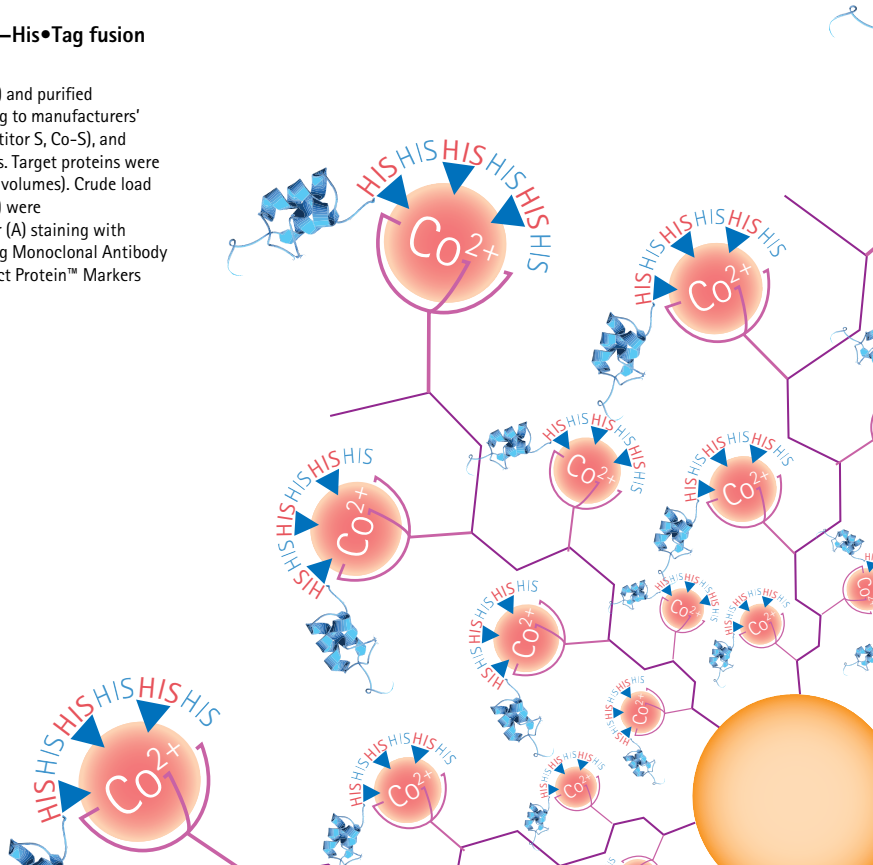


Comparison of metal affinity purification of an ERK2—His•Tag fusion protein with Co-MAC and competitor cartridges

An ERK2–His•Tag fusion protein (arrow) was expressed in BL21(DE3) and purified by metal affinity chromatography. The protein was purified according to manufacturers' protocols for Co-MAC, cobalt-affinity cross-linked agarose (Competitor S, Co-S), and cobalt-affinity cross-linked agarose (Competitor B, Co-B) cartridges. Target proteins were eluted with linear gradient with 0-100% Elution buffer (10 column volumes). Crude load (L), flow-through (FT), wash (W), and elute (E) fractions (20 µl each) were collected and analyzed by 10% BIS TRIS gels. Panels show gels after (A) staining with Coomassie blue and (B) Western blot was performed using a His•Tag Monoclonal Antibody (Cat. No. 69171) followed by chemiluminescent detection. M: Perfect Protein™ Markers 10-225 kDa.



Automated liquid chromatography (LC) of purified glutathione S-transferase—His•Tag fusion protein on Co-MAC column.



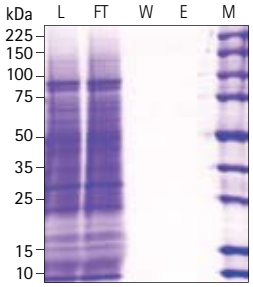
For more information or to place an order, contact your local office (see back cover).

u-MAC™ Cartridges with uncharged MAC resin

Low
Non-Specific
Binding

The u-MAC™ Cartridges are designed for rapid affinity purification of His•Tag® fusion proteins by immobilized metal affinity chromatography. Each u-MAC Cartridge is packed with 1 ml of uncharged His•Bind® Fractogel® Resin. The u-MAC Cartridges can be custom charged with different metal ions (e.g., Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}) depending on the protein characteristics and desired binding efficiency. The u-MAC cartridges can be used manually with a syringe or with automated liquid chromatography instruments at flow rates up to 7 ml/min and pressures up to 20 bar. After charging, for example with Ni^{2+} or Co^{2+}, each cartridge binds up to 30 mg His•Tag fusion protein and can be regenerated and reused at least 10 times.

Low non-specific binding on u-MAC cartridges



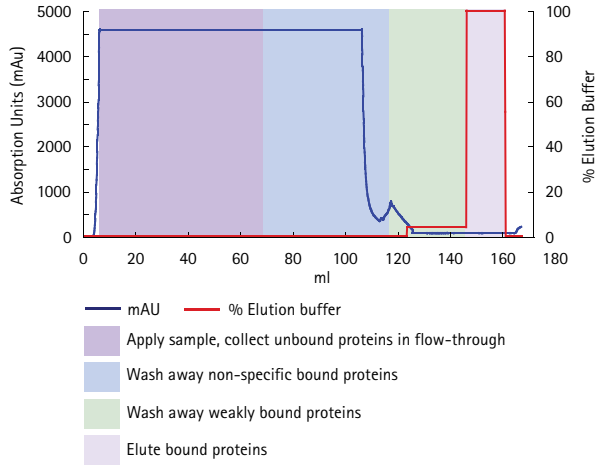
Lane	Sample
L	Total cellular protein load
FT	Flow-through
W	Column wash (maximum volume loaded)*
E	Eluate (maximum volume loaded)*
M	Perfect Protein™ Markers 10-225 kDa

*Indicates **no** non-specific protein binding

Hsp90β purification on two u-MAC columns: no detected proteins were eluted even at the high sample volume loaded onto the columns

For protein expression, 50 ml TriEx™ Sf9 insect suspension cells (1.5×10^6 cells/ml) were infected with recombinant Hsp90β (Heat Shock Protein 90 beta) encoding baculovirus at MOI of five. 72 hours after infection, the cells were lysed using Insect PopCulture® Reagent and Benzonase® Nuclease, according to the recommended protocols. The lysate was clarified by centrifugation at 20,000 × g for 10 minutes at 4°C. The clarified lysates were applied to two u-MAC columns, joined together to give a bed volume of 2.0 ml, by automated LC. Contaminants were washed from the column with 1X MAC Bind and Wash buffers, each supplemented with 10% (v/v) glycerol. Bound protein was eluted from the columns with 1X MAC elute buffer, phosphate, supplemented with 10% (v/v) glycerol. The crude extract, flow-through, and eluate fractions were analyzed by SDS-PAGE and Coomassie blue staining.

Hsp90β u-MAC Elution Profile



Product	Size	Cat. No.	Price
NEW Co-MAC™ Purification Kit	1 kit	71659-3	
NEW Ni-MAC™ Purification Kit	1 kit	71658-3	
NEW u-MAC™ Cartridges	5 cartridges	71651-3	
His•Bind® Fractogel® Resin	25 ml	70693-3	

Components

Cat. No. 71659

• 5	Co-MAC Cartridges (with Luer Lock Adaptors)
• 2 × 80 ml	8X Bind Buffer
• 3 × 25 ml	8X Wash Buffer
• 3 × 25 ml	4X Elute Buffer

Cat. No. 71658

• 5	Ni-MAC Cartridges (with Luer Lock Adaptors)
• 2 × 75 ml	4X MAC Wash Buffer, Phosphate
• 2 × 100 ml	4X MAC Bind Buffer, Phosphate
• 75 ml	4X MAC Elute Buffer, Phosphate

His•Bind® Fractogel® Resin

Rapid affinity purification of His•Tag® fusion proteins

His•Bind Fractogel Resin is a 40–90 µm methacrylate bead matrix ideal for low to medium pressure chromatography, such as FPLC with pressure up to 20 bar. The bulk resin is supplied uncharged and can be charged with different metal ions (e.g., Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}) depending on the protein characteristics and desired binding efficiency. The resin can be regenerated and reused at least 10 times for protein purification under either gentle, non-denaturing conditions, or in the presence of up to 6 M guanidine or 8 M urea.

BULK
Uncharged
Resin
(25 ml)

Features

- High mechanical and chemical stabilities
- Compatible with a syringe or liquid chromatography instruments, pressures up to 20 bar
- Charged resin binds >30 mg protein per milliliter resin
- Reuse at least 10 times

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