

NT-R45631

PDC (Pentadentate Chelator) Kit

Metal-Pentadentate Chelator linked to Affarose in ready to use and pre-packed columns

R45631

The kit consist of :

PDC (Pentadentate Chelator) Kit

1 ml Cu-PDC column, 1 ml Zn-PDC column, 1 ml Ni-PDC column, 1 ml Co-PDC column, Buffers
PDC - Affarose free of metal ions

This technical notice presents interesting applications showing PDC kit offers solutions for the purification of His tagged proteins. The PDC pentadentrate chelator and the choice of complexed metal allows original purifications with result overcoming conventionnal Ni- or Co-resins.

Applications : 6X His -Tagged protein and Zn-Protein purification in a single step

[Application 1: HSP 60 from Helicobacter pylori](#)

[Application 2: Urease from Helicobacter pylori](#)

Zn protein purification

[Application 3: mesophilic alkaline protease](#)

General protein purification

[Application 4: triosephosphate isomerase \(Mutated timase\)](#)

[Application 5: alpha2-Macroglobulin purification](#)

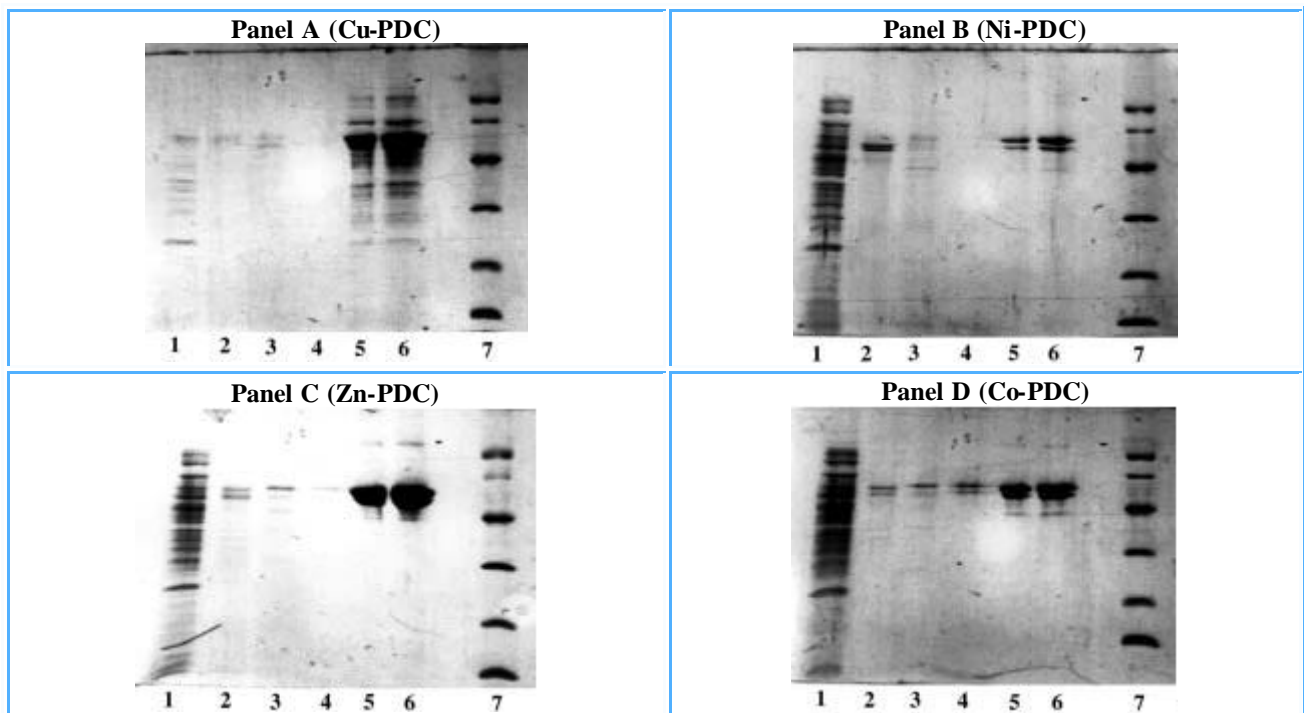
[Application 6: Mouse monoclonal IgG purification](#)

Application 1: HSP 60 from Helicobacter pylori

The crude clarified lysate of 6x His -tagged HSP 60 from Helicobacter pylori expressed in E. Coli was kindly supplied by E. Baise, Laboratoire de Biochimie B6, Sart Tilman 4000 Liège (Belgium).

Sample volume that was loaded onto each column: 500 µl (conc. in HSP 60 : approx. 5 mg/ml).

SDS-PAGE 12% in the presence of beta-mercaptoethanol
(15 µl of sample - stained with Coomassie blue)



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Panel A, B, C & D

Lane 1: flow through + buffer A; Lane 2: buffer A; Lane 3: buffer B; Lane 4: buffer C; Lane 5,6: buffer E; Lane 7: Markers (97,400; 66,200; 45,000; 31,000; 21,500 & 14,400 daltons).

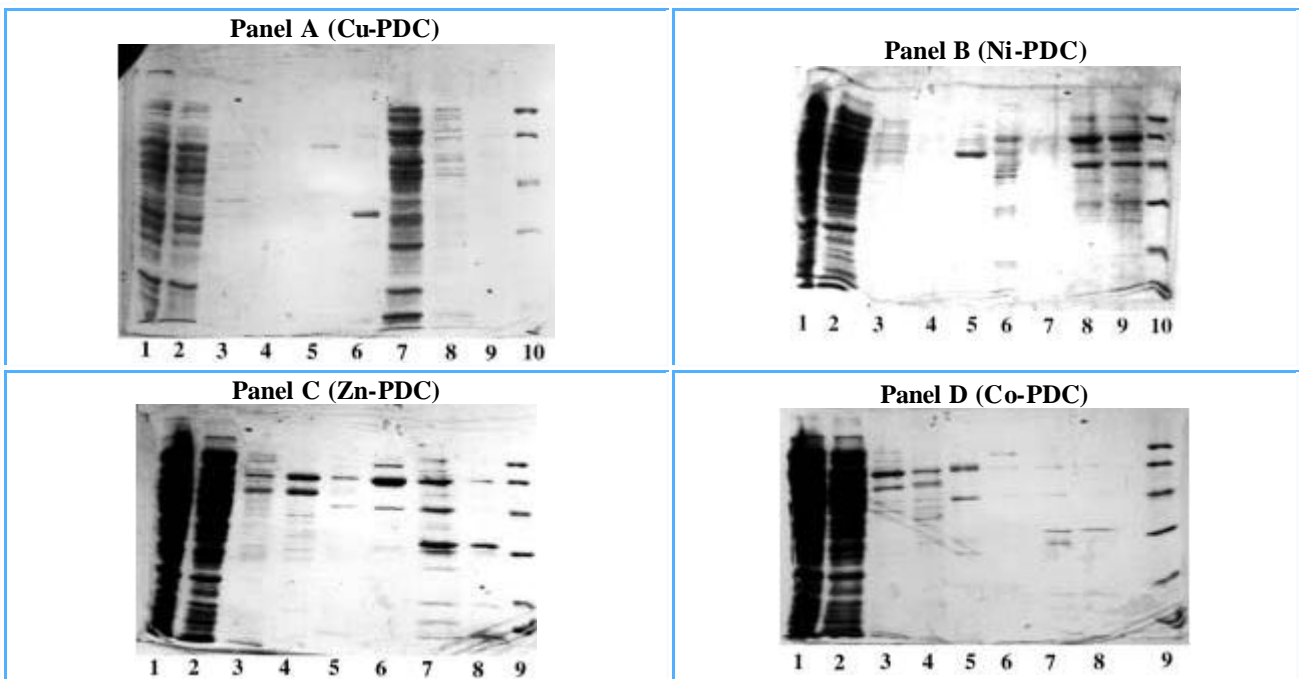
Conclusion: Zn-PDC allowed the purification of HSP 60 in a single step with a recovery of 15 mg of protein per ml of wet gel (Panel C, lane 5 & 6).

Application 2: Urease from *Helicobacter pylori*

The crude clarified lysate of 6x His-tagged Urease from *Helicobacter pylori* expressed in *E. Coli* was kindly supplied by J.M. François, Laboratoire de Biochimie B6, Sart Tilman 4000 Liège (Belgium).

Sample volume that was loaded onto each column: 2 ml (conc. in urease: approx. 1 mg/ml).

SDS-PAGE 12% in the presence of beta-mercaptoethanol (15 µl of sample - stained with Coomassie blue)



Panel A

Lane 1: flow through; Lane 2,3,4: buffer A; Lane 5: buffer B; Lane 6: buffer C; Lane 7,8: buffer E; Lane 9: buffer F and Lane 10: Markers (97,400; 66,200; 45,000; 31,000; 21,500 & 14,400 daltons).

Panel B

Lane 1: flow through; Lane 2,3,4: buffer A; Lane 5: buffer B; Lane 6: buffer C, Lane 7: buffer D; Lane 8,9: buffer E and Lane 10: Markers (97,400; 66,200; 45,000; 31,000; 21,500 & 14,400 daltons).

Panel C & D

Lane 1: flow through; Lane 2,3: buffer A; Lane 4: buffer B; Lane 5: buffer C; Lane 6: buffer D; Lane 7,8: buffer E and Lane 9: Markers (97,400; 66,200; 45,000; 31,000; 21,500 & 14,400 daltons).

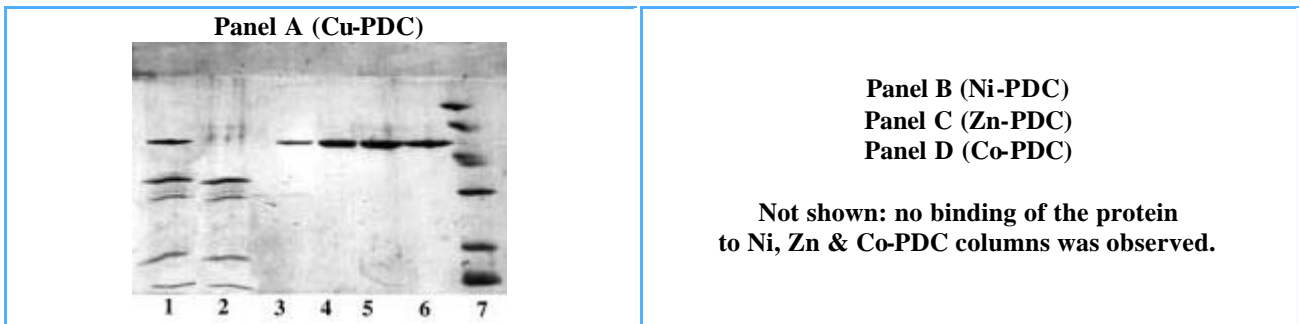
Conclusions: Ni-PDC can be used to purify the native urease (Panel B, lane 8 & 9) and Zn-PDC to obtain the alpha-chain (MW 60,000 daltons) and the beta-chain (MW 30,000 daltons) of urease (Panel C, lane 6 and 7 & 8).

Application 3: mesophilic alkaline protease

The crude extract (redissolved ammonium sulfate precipitate) containing a mesophilic alkaline protease MW=50,000 daltons (Zn protein) from *Pseudomonas aeruginosa* IFO 3455 was kindly supplied by J.P. Chessa and E. Planamente, Laboratoire de Biochimie, Institut de Biochimie B6, Sart Tilman 4000 Liège (Belgium).

Sample volume that was loaded onto each column: 1 ml (conc. in alkaline protease: approx. 1 mg/ml).

SDS-PAGE 12% in the presence of beta-mercaptoethanol (15 µl of sample - stained with Coomassie blue)



Panel A

Lane 1: redissolved ammonium sulfate precipitate

Lane 2: buffer A

Lane 3,4,5,6: buffer B

Lane 7: markers (97,400; 66,200; 45,000; 31,000; 21,500 & 14,400 daltons).

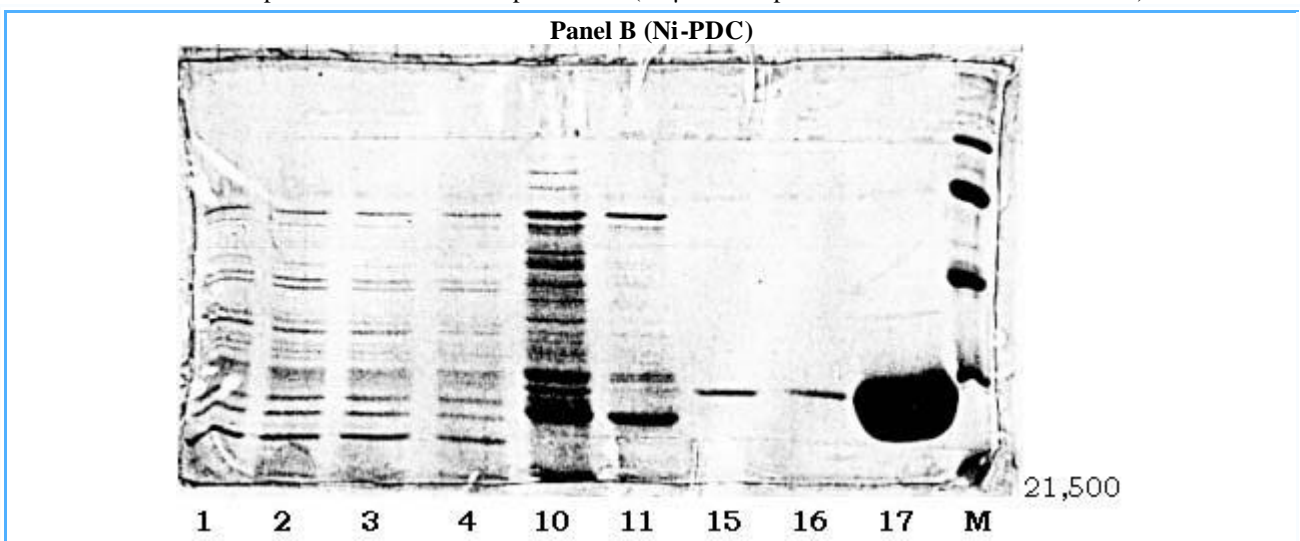
Conclusion: Cu-PDC was the only chelate gel allowing the purification of this protease in a single step (Panel A, lane 4,5,6). No binding to Ni-PDC, Zn-PDC and Co-PDC was observed.

Application 4: triosephosphate isomerase (Mutated timase)

By kind permission of the author, Dr P.A. Flamée, Laboratoire de Génie génétique et de Biologie moléculaire, Institut de Biochimie B6, Sart Tilman 4000 Liège (Belgium).

Sample volume that was loaded onto each column: 10 ml of crude clarified lysate of Mutated triosephosphate isomerase (Mutated timase) from *E. Coli*, containing 8 histidine residues (5 accessible).

SDS-PAGE 12% in the presence of beta-mercaptoethanol (15 µl of sample - stained with Coomassie blue)



Panel B

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Fractions 1,2,3 & 4: washing with K_2PO_4 20mM pH 7.5, NaCl 50mM.
 Fractions 10-11: fractions eluted at pH 5.7.
 Fractions 15-17: mutated timase (MW 27,000 daltons) eluted at pH 5.0.
 M: Markers 14,400; 21,500; 31,000; 45,000; 66,200 & 97,400 daltons.

Conclusion: Ni-PDC allows the purification of mutated timase in a single step with a recovery of 15 mg of protein per ml of wet gel (Panel B, lane 15, 16 & 17).

Panels A, C & D (Cu-PDC, Zn-PDC & Co-PDC) are not shown

Application 5: alpha2-Macroglobulin purification

The PDC KIT allows the purification of alpha2-Macroglobulin in a single step with the purity of approx. 90% (w/w) as follows

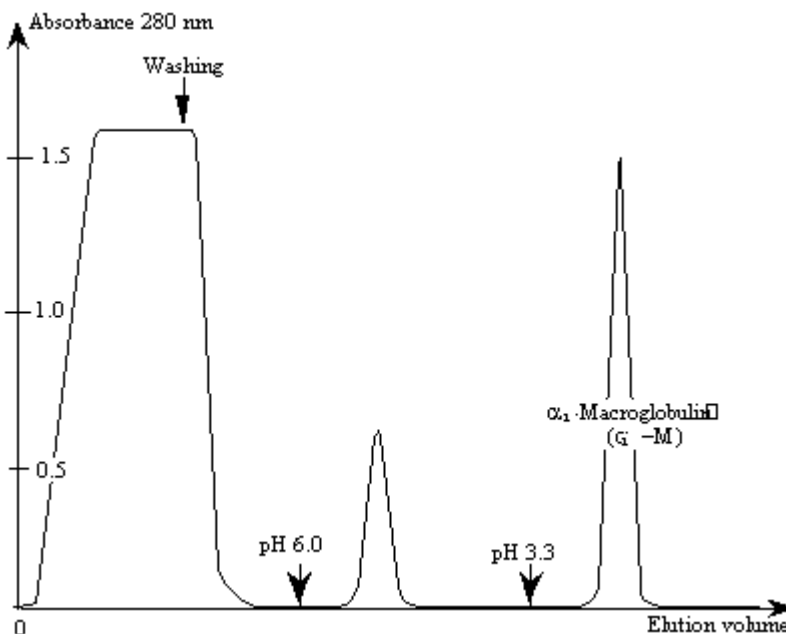
Gel volume: 25 ml Ni^{2+} PDC- Affarose.

Sample: 100 ml human serum.

Binding buffer: K_2HPO_4 50mM, NaCl 0.15M pH 8.0.

Washing buffer: 1. K_2HPO_4 50mM, NaCl 0.15M pH 8.0. / 2. K_2HPO_4 50mM, NaCl 0.15M pH 6.0.

Elution buffer: AcONa 50mM pH 3.3.



Purity of alpha2-M (containing 38 histidine residues) was determined to be approx. 90% by SDS-PAGE in the presence of beta-mercaptoethanol and its activity by immunodiffusion on Affarose gel.

Application 6: Mouse monoclonal IgG purification

Ni^{2+} -PDC-Affarose is also an excellent affinity support for the purification of Mouse monoclonal IgG1 with a recovery greater than 90% and the degree of purity greater than 90% (w/w).

Results not shown.

Sample: Clarified Ascite fluids.

Binding buffer: K_2HPO_4 50mM, NaCl 0.15M pH 8.0.

Washing buffer: K_2HPO_4 50mM, NaCl 0.15M pH 8.0.

Elution buffer: K_2HPO_4 50mM, NaCl 0.15M pH 6.0.

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Reagent for R&D *in vitro* use only

Literature references and applications:

J. Biol. Chem. 2000 275: 11147-11153

J. Biol. Chem. 2000 275: 25411-25417

J. Biol. Chem. published November 13, 2001 as 10.1074/jbc.M105043200

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