Clontech Laboratories, Inc.

His60 Ni Superflow Cartridges User Manual

NOTE FOR FIRST TIME USERS:

For **optimal results**, please **follow the written protocol** when performing the first purification. Each resin (e.g., Ni-IDA, Ni-NTA, Ni-TED, TALON®) has a different chemistry.

Optimal conditions for one resin are not optimal for another resin.
If you need to modify the protocol, please refer to Table II for compatible reagents and possible effects on the resin.

PT5143-1
Cat. No(s). 635674, 635675, 635678, 635679, 635680
(031716)

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I. Introduction

Clontech's **His60 Ni Superflow Cartridges** are prefilled with 1 ml or 5 ml of His60 Ni Superflow resin and are ready to use for purification of his-tagged proteins using a syringe, peristaltic pump, or liquid chromatography systems such as ÄKTA or other FPLC systems.

A. His60 Ni Superflow 1 ml & 5 ml Cartridge Specifications

Table 1. His60 Ni Superflow Cartridge Specifications

	1 ml Cartridge	5 ml Cartridge
Support	Superflow 6 (cross-linked agarose)	
Bead diameter	60–160 µm	
Column dimensions (mm i.d.)	0.7 cm x 2.5 cm	1.6 cm x 2.5 cm
Maximum pressure[1]	5 bar, 0.5 MPa	
Typical back pressure	1.0 bar, 0.1 MPa (1 ml/min)	2.0 bar, 0.2 MPa (5 ml/min)
Recommended flow rate	1 ml/min (156 cm/hr)	5 ml/min (149 cm/hr)
Maximum flow rate[2]	10 ml/min (1,559 cm/hr)	40 ml/min (1,193 cm/hr)
pH stability short term (≤2 hr)	2–14	
pH stability long term (>2 hr)	3–14	
Binding capacity[3]	60 mg of AcGFPuv	300 mg of AcGFPuv
System compatibility[4]	Automated chromatography systems (e.g., ÄKTA, FPLC, etc.), peristaltic pump or syringe	
Cartridge body material	Polypropylene	
Connectors	1/16" (inlet); 1/16" (outlet)	

[1] The maximum pressure that can be used with the Superflow matrix itself is 10 bar. However, stability of the cartridges is only guaranteed up to 5 bar.
[2] High flow rates may lead to reduced recovery of 6xHis-tagged protein.
[3] Binding capacity may vary from protein to protein.
[4] Adaptors may be necessary.

B. His60 Ni Superflow Resin

Clontech's **His60 Ni Superflow Resin** is a high-capacity Ni-IDA resin that has been optimized for the efficient one-step purification of expressed his-tagged proteins from bacterial, yeast, mammalian, and baculovirus-infected cells. The combination of the high density of nickel (II) ion and the high flow rates of the Superflow 6 agarose beads allow the efficient capture of target his-tagged proteins. Up to 60 mg of his-tagged protein can be adsorbed onto 1 ml of His60 Ni resin (data based on purification of AcGFP1).

The **His60 Ni Superflow Cartridge Purification Kit (5 x 1 ml)** provides 5 prepacked **His60 Ni Superflow Cartridges** (each containing 1 ml of resin), as well as all the buffers needed for protein extraction and purification (also available separately as the **His60 Ni Buffer Set**)—see Section II.

The **His60 Ni Cartridge Purification Kit (5 x 5 ml)** provides 5 prepacked **His60 Ni Superflow Cartridges** (each containing 5 ml of resin), as well as all the buffers needed for protein extraction and purification (also available separately as the **His60 Ni Buffer Set**)—see Section II.

Limitations of His60 Ni Superflow Resin

Please note the following recommendations when using His60 Ni Superflow Cartridges:

- Do not use chelator-containing protease inhibitors or other additives, EDTA, or strong reducing agents (see Table 2 and the note below regarding the use of reducing agents).
- For automated liquid-chromatography (LC) applications, use highly pure, low-absorbance imidazole (Fisher, Product No. BP 305-50). Always filter buffers through a 0.45 µm filter and degas before use.
- His60 Ni allows protein purification under either native or denaturing conditions. The resin is compatible with multiple denaturants and detergents (Table 2).

NOTE: Using βME as a reducing agent with His60 Ni Superflow Resin sharply reduces protein yield. If high levels of βME are required for purification, we strongly recommend using TALON® resin, which provides high yields of the target protein in up to 30 mM βME.

Table 2. Reagent Compatibility with His60 Ni Superflow Resin (Based on Literature References)

Reagent	Notes	Acceptable Concentrations
Amino Acids		
Arginine, Glycine, Glutamine		Not recommended
Histidine	Binds to His60 Ni and competes with histidine residues in the his-tag.	Can be used at low concentrations (20 mM) to inhibit nonspecific binding; and, at a higher concentration (up to 100 mM), to elute his-tagged proteins
Buffers		
HEPES, MOPS	Amine groups that are present in these buffers can interact with Ni^{2+} ions, diminishing the resin's binding capacity.	Up to 100 mM (with caution)
Sodium acetate		Up to 100 mM (with caution)
Sodium phosphate		Up to 50 mM can be used
Tris	Coordinates weakly with metal ions, causing a decrease in binding capacity.	Up to 50 mM (with caution). Loss in binding capacity can be seen.
Chelating Agents		
EDTA, EGTA	Will strip metal ions from the resin, resulting in protein elution and a resin color change.	Not recommended
Denaturing Agents		
Gu-HCl	With high concentrations, protein unfolding generally takes place. Protein refolding on-column (or after elution) is protein-dependent.	6 M
Urea	With high concentrations, protein unfolding generally takes place. Protein refolding on-column (or after elution) is protein-dependent	8M
Detergents[1]		
CHAPS	Ionic detergents such as CHAPS, SDS, and sarkosyl are compatible at concentrations up to 1%. Even at low concentrations you should expect interference with binding.	1% (with caution)
NP-40	Has high absorbance at 280 nm.	2%
SDS	Ionic detergents such as CHAPS, SDS, and sarkosyl are compatible at concentrations up to 1%. Even at low concentrations you should expect interference with binding.	1% (with caution)
Triton X-100	Has high absorbance at 280 nm.	1%
Tween 20		2%
Reducing Agents		
ßME	Use the resin immediately after equilibrating with buffers containing βME. Otherwise the resin will change color. Do not store the resin in buffers containing βME. **A slight change in color (yellowing of the resin) will occur.**	20 mM (with caution)
DTT	Since DTT is a reducing agent, low concentrations will reduce the metal ions in His60 Ni Superflow resin. Although enough of these ions may remain unaffected to allow protein purification, please use it with caution. Do at least 20 column volumes of washes, preferably with low concentrations of imidazole (40 mM) to wash out any reduced metal ions.	1 mM (with caution)
DTE		Not recommended
Others		
$MgCl_2$		4 M
$CaCl_2$		5 mM
Ethanol	May precipitate proteins, causing low yields & column clogging.	20%
Glycerol		20%

[1]Detergents cannot be easily removed by buffer exchange.

II. List of Components

Store all components at 4°C.

His60 Ni Superflow Cartridges (5 x 1 ml) (Cat. No. 635675)

- 5 His60 Ni Superflow Cartridges (1 ml each)
- 5 Top Caps
- 5 Snap-Off End Caps

His60 Ni Buffer Set (Cat. No. 635665)

- 2 x 250 ml His60 Ni Equilibration Buffer
- 1 x 200 ml His60 Ni Elution Buffer
- 1 x 100 ml His60 Ni xTractor™ Buffer

NOTE: His60 Ni xTractor Buffer is equivalent to the xTractor Buffer supplied in Cat. Nos. 635623, 635625, 635656 & 635671 (see Section IV).

His60 Ni Superflow Cartridge Purification Kit (5 x 1 ml) (Cat. No. 635674)

- 1 His60 Ni Superflow Cartridges (5 x 1 ml) (Cat. No. 635675)
- 1 His60 Ni Buffer Set (Cat. No. 635665)

His60 Ni Superflow Cartridge (1 x 5 ml) (Cat. No. 635680)

- 1 His60 Ni Superflow Cartridge (5 ml each)
- 1 Top Cap
- 1 Snap-Off End Cap

His60 Ni Superflow Cartridges (5 x 5 ml) (Cat. No. 635679)

- 5 His60 Ni Superflow Cartridges (5 ml each)
- 5 Top Caps
- 5 Snap-Off End Caps

His60 Ni Superflow Cartridge Purification Kit (5 x 5 ml) (Cat. No. 635678)

- 1 His60 Ni Superflow Cartridges (5 x 5 ml) (Cat. No. 635679)
- 1 His60 Ni Buffer Set (Cat. No. 635665)

III. Additional Materials Required

A. Equipment

For His60 Ni Superflow Cartridges, you will need the following equipment:

- A suitable liquid chromatography system (LC procedure only) such as ÄKTA or other systems
- A syringe or manual pump

NOTE: For best results, process all buffers through a 0.45 µm filter and degas before use in LC applications.

B. Buffers – Native Conditions

For your convenience, we provide a separate kit containing a set of His60 Ni Extraction, Equilibration, and Elution Buffers, the **His60 Ni** Buffer Set (Cat. No.635665), which is sufficient for approximately 20 purifications on 1 ml **His60 Ni Superflow Cartridges (5 x 1 ml)** (Cat. No.635675), or 5 purifications on 5 ml **His60 Ni Superflow Cartridges (5 x 5 ml)** (Cat. No. 635679).

The His60 Ni Buffer Set is also available together with the 1 ml cartridges in the **His60 Ni Superflow Cartridge Purification Kit (5 x 1 ml)** (Cat. No. 635674), and together with the 5 ml cartridges in the **His60 Ni Superflow Cartridge Purification Kit (5 x 5 ml)** (Cat. No. 635678)—see Section II.

The following information is provided if you wish to prepare your own buffers for use with other applications. Please note that for FPLC and other automated applications, you need to filter the buffers through a 0.45 µm filter and degas them before use.

- **Equilibration Buffer**: 50 mM sodium phosphate, 300 mM sodium chloride, 20 mM imidazole; pH 7.4
- **Wash Buffer:** 50 mM sodium phosphate, 300 mM sodium chloride, 40 mM imidazole; pH 7.4
  - — Wash Buffer is easily made on a binary pump LC system by mixing 7.1 parts of His60 Ni Elution Buffer and 92.9 parts of His60 Ni Equilibration Buffer. This buffer ratio can be achieved by running the LC system at 7.1% Pump B.
  - — Alternatively, prepare manually by mixing 710 µl of His60 Ni Elution Buffer with 9.29 ml of His60 Ni Equilibration Buffer.
- **Elution Buffer:** 50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole; pH 7.4
- **Regeneration Buffer:** 20 mM MES (2-(N-morpholine)-ethanesulfonic acid), 0.3 M sodium chloride; pH 5.0
- **Imidazole:** Use a highly pure, low-absorbance imidazole ideal for LC applications (Fisher, Product No. BP 305-50).

C. Buffers – Denaturing Conditions – Guanidine-HCl or Urea

Denaturants, such as 5 M guanidine-HCl or 8 M urea, enhance protein solubility. Because overexpressed proteins in prokaryotic systems are sometimes insoluble, you may need to purify proteins under denaturing conditions. When using high concentrations of guanidine-HCl or urea, protein unfolding takes place. On-column refolding or post-elution refolding is protein-dependent. When purifying proteins under denaturing conditions, we recommend preparing buffers as indicated below.

Buffers with 6M Guanidine-HCl

- **Equilibration Buffer**: 50 mM sodium phosphate, 6 M guanidine-HCl, 300 mM NaCl, 20 mM imidazole; pH 7.4
- **Wash Buffer:** 50 mM sodium phosphate, 6 M guanidine-HCl, 300 mM NaCl, 40 mM imidazole; pH 7.4
- **Elution Buffer:** 50 mM sodium phosphate, 6 M guanidine-HCl, 300 mM NaCl, 300 mM imidazole; pH 7.4

Buffers with 8 M Urea

- **Equilibration Buffer**: 50 mM sodium phosphate, 8 M urea, 300 mM NaCl, 20 mM imidazole; pH 7.4
- **Wash Buffer:** 50 mM sodium phosphate, 8 M urea, 300 mM NaCl, 40 mM imidazole; pH 7.4
- **Elution Buffer:** 50 mM sodium phosphate, 8 M urea, 300 mM NaCl, 300 mM imidazole; pH 7.4

NOTE: Samples containing guanidine-HCl cannot be analyzed by SDS-PAGE. A buffer exchange to a buffer containing urea must be performed before SDS-PAGE analysis. Samples containing urea can be analyzed directly by SDS-PAGE.

D. Enzymes

- **Benzonase** (Sigma, Cat. No. E8263-5KU)
- **Recombinant DNase I** (TaKaRa, Cat. No. 2270A)

E. Accessories for Automated Purification

- A suitable liquid chromatography system (LC procedure only) with 1/16 inch tubing—or a binary pump system (a quicker and more convenient alternative)
- Additional connectors and fittings required to attach the cartridges to a Bio-Rad BioLogic or a classic FPLC System (Section III.F)

F. Accessories for Syringe-Based Purification

- **Luer Lock Syringe Fittings** (GE Healthcare, Cat. No. 18-1112-51) for syringe-based purification only
- **M6 FPLC Fittings** (GE Healthcare, Cat. Nos. 18-1112-58 & 18-1112-57) for syringe-based or automated purification

G. Optional

- **PD-10 desalting columns** (GE Healthcare, Cat. No. 17-0851-01) to remove excess imidazole from the final sample when required for downstream applications

IV. Related Products: Extraction Buffers, Protease Inhibitors, and His-Tag Antibodies

xTractor Buffer Kit (Cat. No. 635623)

Applications: extraction of insoluble protein from inclusion bodies, efficient extraction of high molecular weight proteins, and complete disruption of bacterial cell walls and membranes

- 200 ml xTractor Buffer
- 400 µl DNase I
- 2.5 ml Lysozyme

xTractor Buffer (Cat. Nos. 635656, 635671 & 635625)

Applications: bacterial lysis, extraction of proteins from yeast cells without the use of glass beads, mammalian cell pellet extraction, and purification of affinity-tagged proteins

- 100 ml (Cat. No. 635656)
- 250 ml (Cat. No. 635671)
- 500 ml (Cat. No. 635625)

ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail (Cat. No. 635673)

Complete, easy-to-use protease inhibitor cocktail that is EDTA-free (can be used on IMAC resins without interfering with protein binding).

- 10 x 100 µl (Cat. No. 635673)

Antibodies for detection of tagged proteins

- 6xHis mAb-HRP conjugate (albumin-free), 100 µl (Cat. No. 631210)
- 6xHis Monoclonal Antibody (albumin-free), 200 µg (Cat. No. 631212)
- 6xHN Polyclonal Antibody, 200 µl, (Cat. No. 631213)

V. Sample Preparation & Purification

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.
Use this procedure to (a) prepare your his-tagged protein sample for (b) automated or (c) manual purification using His60 Ni Superflow Cartridges.

A. PROTOCOL: Extracting Proteins from Cells

This procedure has been optimized for extraction of proteins from fresh or frozen cell pellets using xTractor Buffer. The extraction volumes can be adjusted, as long as **20 ml of xTractor Buffer** are used per **1 g of cell pellet**.

1. Resuspend the cell pellet

Add **20 ml of xTractor Buffer** to **1 g of cell pellet**. Mix gently. Pipet the mixture up and down to fully resuspend the pellet.

2. Optional step – lysozyme & DNase I/protease inhibitor

Add **40 µl of 1 unit/µl DNase I solution** and **200 µl of 100X lysozyme solution**. Add **EDTA-free protease inhibitor**. Mix gently, pipetting up and down several times.

NOTES:

- DNase I reduces the viscosity of the lysate, allowing for more efficient removal of cellular debris. DNase I can be used without lysozyme. However, if you are treating cells with lysozyme, you must also treat these cells with DNase I.
- Lysozyme helps to fully disrupt bacterial walls and is highly beneficial when extracting high molecular weight proteins (>40 kDa).
- The lysozyme solution may form a precipitate. Resuspend the contents of the bottle and apply 200 µl of suspension directly to the mix or (optionally) centrifuge 200 µl of lysozyme solution for 5 min at 14,000 rpm & use the supernatant to perform the lysis.
- We recommend that you use our ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. Nos. 635672 & 635673).

3. Incubation

Incubate with gentle shaking for 10 min at room temperature. (If desired, you may incubate the solution at 4°C.)

NOTE: At the end of the incubation period, there should be no visible particles. If cell pellet fragments are present, resuspend them by pipetting the solution up and down and incubating for an additional 1–2 min.

4. Lysate clarification

Centrifuge the crude lysate at **10,000–12,000 x g** for **20 min** at **4°C**. Carefully transfer the supernatant to a clean tube without disturbing the pellet. **This is your starting sample.**

NOTE: If the supernatant is not clear, centrifuge a second time or filter through a 0.45 µm membrane (e.g., cellulose acetate) to avoid clogging the IMAC column with insoluble material.

B. PROTOCOL: Automated Purification on a Liquid Chromatography System

1. Equilibration

Equilibrate the cartridge and all buffers to **room temperature** or **4°C**.

2. LC System Set up

a. Prepare the LC system by filling the tubing with buffer. On a binary pump LC system, fill Pump A with **Equilibration Buffer** and Pump B with **Elution Buffer**.

b. Remove the top plug from the cartridge and start pumping **Equilibration Buffer** at a flow rate of **1 ml/min** until a few drops fill in the top inlet.

c. Pause the pump, connect the cartridge to the pump outlet, and carefully snap off the bottom cap of the cartridge (do not twist).

d. Start the pump. To avoid introducing air into the system, allow a few drops to emerge from the cartridge before connecting to the LC UV monitor inlet port.

3. Equilibrate Cartridge

Equilibrate the cartridge with **5–10 column volumes** of the **Equilibration Buffer** at a flow rate of **1 ml/min** for **1 ml cartridge** or **5 ml/min** for **5 ml cartridge**.

NOTE: For maximum extraction and binding, prepare the sample using our xTractor Buffer (Section V.A). If you used incompatible reagents (Section I.B.) during the extraction, desalt the sample on a PD-10 column (Section III.G) before proceeding to Step 4.

4. Sample Loading

Load the clarified sample onto the cartridge at a flow rate of **0.5–1 ml/min** and collect fractions.

5. Wash Step

Wash the cartridge using a flow rate of **1 ml/min** for **1 ml cartridges** or **5 ml/min** for **5 ml cartridges** with **8 column volumes** of **Equilibration Buffer** followed by **7 column volumes** of **Wash Buffer** (i.e., Equilibration Buffer containing 40 mM imidazole). The Wash Buffer is prepared on the LC system by running Pump B at 7.1%.

6. Elution Step

Elute using a flow rate of **1 ml/min** for **1 ml cartridges** or **5 ml/min** for **5 ml cartridges** with approximately **5–8 column volumes** of **Elution Buffer** (containing 300 mM imidazole) and collect **1 ml fractions**.

Monitor protein elution by measuring the absorbance of the fractions at 280 nm or performing a Bradford assay (Bradford, 1976). The collected fractions can be analyzed by SDS-PAGE.

NOTE: If necessary for downstream applications, remove excess imidazole by gel filtration on a PD-10 column (Section III.G).

7. Cartridge Regeneration

Wash cartridge with **20 column volumes** of **Equilibration Buffer** or wash with **10 column volumes** of **20 mM MES, 0.3 M NaCl pH 5.0 buffer**.

NOTE: Regeneration allows the cartridge to be reused to purify the same protein, without significant loss of binding capacity, up to 5 times depending on the purification conditions and the target protein.

8. Extended Storage (over 1 week)

Wash the cartridge with **five column volumes** of **water** after each use. Store in **20% ethanol** at 4°C Attach supplied bottom cap, followed by the top plug.

C. PROTOCOL: Manual Purification Using a Syringe

1. Equilibration

Equilibrate the cartridge and all buffers to **room temperature** or at **4°C**.

2. Luer Lock Syringe Setup

Fill syringe with **5–10 column volumes** of **Equilibration Buffer**.

3. Cartridge Setup

a. Attach the syringe to a Luer Lock Adapter (not supplied; see Section III.F).

b. Remove the top plug from the cartridge, add a few drops of buffer from the syringe to the top inlet of the cartridge, and attach the syringe to the top of the cartridge via the Luer Lock adapter.

c. Carefully snap off the bottom cap of the cartridge (do not twist).

4. Equilibrate Cartridge

Equilibrate the cartridge with **5–10 column volumes** of the **Equilibration Buffer** at a flow rate of **1 ml/min** for **1 ml cartridge** or **5 ml/min** for **5 ml cartridge**.

Remove the syringe from the Luer Lock Adapter.

NOTE: For maximum extraction and binding, prepare the sample using our xTractor Buffer (Section V.A). If you used incompatible reagents (Section I.B.) during the extraction, desalt the sample on a PD-10 column (Section III.G) before proceeding to Step 5.

5. Sample Loading

Fill the syringe with the clarified sample and reconnect it to the Luer Lock Adapter. Slowly push the syringe plunger to pass the sample through the cartridge. For maximum binding and better yields, load the sample at an approximate flow rate **of 0.5–1 ml/min** and collect fractions.

6. Wash Step

Using a clean syringe, wash the resin with **10 column volumes** of **Wash Buffer** at a flow rate of **~1 ml/min** for **1 ml cartridges** or **~5 ml/min** for **5 ml cartridges**.

NOTE: If you are using the buffers supplied in the **His60 Ni Buffer Set** (Cat. No. 635665), the **His60 Ni Superflow Cartridge Purification Kit (5 x 1 ml)** (Cat. No. 635674), or the **His60 Ni Superflow Cartridge Purification Kit (5 x 5 ml)** (Cat. No. 635678), prepare the **Wash Buffer** by mixing **7.1 parts** of **Elution Buffer** with **92.9 parts** of **Equilibration Buffer**.

7. Elution Step

Using a clean syringe, elute the sample at a flow rate of **~1 ml/min** for **1 ml cartridges** or **~5 ml/min** for **5 ml cartridges** with approximately **five column volumes** of **Elution Buffer**, collecting 1 ml fractions.

Monitor protein elution by measuring the absorbance of the fractions at 280 nm or performing a Bradford assay (Bradford, 1976). The collected fractions can be analyzed by SDS-PAGE.

NOTE: If necessary for downstream applications, remove excess imidazole by gel filtration on a PD-10 column (Section III.G).

8. Cartridge Regeneration

Wash cartridge with **20 column volumes** of **Equilibration Buffer** or wash with **10 column volumes** of **20 mM MES, 0.3 M NaCl pH 5.0 buffer**.

NOTE: Regeneration allows the cartridge to be reused to purify the same protein, without significant loss of binding capacity, up to 5 times depending on the purification conditions and the target protein.

9. Extended Storage (over 1 week)

Wash the cartridge with **five column volumes** of **water** after each use. Store in **20% ethanol** at 4°C Attach supplied bottom cap, followed by the top plug.

D. PROTOCOL: Complete Regeneration of His60 Ni Superflow Cartridges

If you plan to purify multiple proteins using the same column, you must use the following resin regeneration protocol before you purify a new protein.

1. Strip Ni ions

Wash the cartridge with **ten column volumes** of **0.2 M EDTA (pH 7.0)** at room temperature.

Wash excess EDTA from the cartridges with an additional **ten column volumes** of **double distilled H_2O (ddH_2O)**.

2. Charge Resin

Add **two column volumes** of **100 mM $NiSO_4$ solution**.

Wash resin to remove excess ions with **seven column volumes** of **ddH_2O**, followed by **3 column volumes** of **300 mM NaCl** and **3 column volumes** of **ddH_2O**.

3. Equilibrate Resin

Add **ten column volumes** of **Equilibration/Wash Buffer**.

4. Cartridge is ready to use

Resin can be regenerated up to 5 times.

VI. Troubleshooting Guide

Table 3. Troubleshooting Guide for His60 Ni Superflow Cartridges

Description of Problem	Possible Explanation	Solution
Low target yield	Poor expression of target protein	Optimize bacterial expression conditions.
	Target protein forms inclusion bodies	• Decrease temperature to 25°C or lower during induction to minimize inclusion body formation. • Solubilize inclusion bodies and perform the purification in the presence of 8 M urea or 6 M guanidinium HCl.
	Inefficient target extraction	Use our xTractor Buffer.
	Inaccessible polyhistidine tag	Purify in presence of 6–8 M urea or 6 M guanidinium HCl.
Impurities in eluate	Insufficient washing	Increase wash volume or add intermediate wash at 60 mM imidazole. (This can result in partial loss of target protein.)
Low flow rate	Clogged cartridge	Apply only clarified extract, and decrease the amount of loaded sample.
	Viscous sample	Treat sample with Benzonase or DNase I, as described in Section V.A.
Can not detect target protein by UV		• Use low UV absorbance imidazole in the buffers. • Perform a Bradford protein assay on collected fractions to identify target protein in eluate.

Notice to Purchaser

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.