User Manual

HisTALON™ Superflow™ 1 ml & 5 ml Cartridges User Manual

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I. Introduction

Clontech's **HisTALON™ Superflow™ 1 ml & 5 ml Cartridges** are ready-to-use columns for the efficient purification of his-tagged proteins, using automated or syringe-based protocols, from bacterial, mammalian, and baculovirus-infected cells. The cartridges are prepacked with our TALON® Superflow Resin, which combines Superflow 6, a rigid, highly porous agarose derivative, with TALON, a highly selective immobilized metal ion affinity chromatography (IMAC) ligand. TALON is a tetradentate chelator charged with cobalt, and is specific for his-tagged proteins (Chaga *et al.*, 1994; Froelich *et al.*, 1996; Hochuli *et al.*, 1987 & 1988; Porath *et al.*, 1975; Stephens *et al.*, 1997). The stable chelation of the Co^{2+} ion, combined with the specificity of the TALON reactive core, deliver unmatched purity (*Clontechniques*, January 2009). Up to 20 mg of his-tagged AcGFP1 can be adsorbed on one 1 ml HisTALON Cartridge, and up to 100 mg on one 5 ml HisTALON Cartridge.

HisTALON Cartridges are compatible with most leading automated liquid-chromatography systems or with manual syringe processing. The cartridges can be used directly on automated ÄKTA™ FPLC™ Systems and other medium -pressure automated systems. With appropriate couplings, the cartridges may also be adapted for use with Luer Lock Syringe Fittings (GE Healthcare, Cat. No. 18-1112-51) and the original M6 FPLC fittings (GE Healthcare, Cat. Nos. 18-1112-58 & 18-1112-57).

These cartridges enable fast, easy and reproducible chromatographic separation—and can be regenerated for multiple uses. However, we recommend that you reuse a cartridge only to purify different batches of the same protein. If you plan to purify multiple proteins using the same cartridge, you must utilize the "Complete Regeneration" method described in the TALON Metal Affinity Resins User Manual (PT1320-1) at **www.clontech.com**

Figure 1. HisTALON Superflow Cartridges provide highly reproducible and rapid his-tagged protein purification.

II. List of Components

Store all components at 4°C.

HisTALON Superflow Cartridges (5 x 1 ml) (Cat. No. 635650)

- 5 HisTALON Cartridges (1 ml each)
- 5 Top Caps
- 5 Snap-Off End Caps

HisTALON Superflow Cartridge (1 x 5 ml) (Cat. No. 635683)

- 1 HisTALON Cartridge (5 ml each)
- 1 Top Cap
- 1 Snap-Off End Cap

HisTALON Superflow Cartridges (5 x 5 ml) (Cat. No. 635682)

- 5 HisTALON Cartridges (5 ml each)
- 5 Top Caps
- 5 Snap-Off End Caps

II. List of Components continued

HisTALON Buffer Set (Cat. No. 635651)

- 2 x 250 ml HisTALON Equilibration Buffer
- 200 ml HisTALON Elution Buffer
- 100 ml HisTALON xTractor Buffer

NOTE: HisTALON xTractor Buffer is equivalent to the xTractor Buffer supplied in Cat. Nos. 635623, 635625, 635656 & 635671.

HisTALON Superflow Cartridge Purification Kit (5 x 1 ml) (Cat. No. 635649)

- HisTALON Superflow Cartridges (5 x 1 ml) (Cat. No. 635650)
- HisTALON Buffer Set (Cat. No. 635651)

HisTALON Superflow Cartridge Purification Kit (5 x 5 ml) (Cat. No. 635681)

- HisTALON Superflow Cartridges (5 x 5 ml) (Cat. No. 635650)
- HisTALON Buffer Set (Cat. No. 635651)

III. Additional Materials Needed

A. Buffers

Extraction, equilibration, and elution buffers, sufficient for up to 20 purification runs (using 1 ml cartridges) or up to 5 purification runs (using 5 ml cartridges) under normal operating conditions on an automated medium-pressure chromatography system, are supplied in the HisTALON Superflow Cartridge Purification Kit (5 x 1 ml) (Cat. No. 635649), the HisTALON Superflow Cartridge Purification Kit (5 x 5 ml) (Cat. No. 635681), and the HisTALON Buffer Set (Cat. No. 635651)—see Section II.

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

- **Equilibration Buffer**: 50 mM sodium phosphate, 300 mM sodium chloride; pH 7.4
- **Wash Buffer:** 50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole; pH 7.4
  - Wash Buffer is easily made on a binary pump LC system by mixing 6.6 parts of Elution Buffer and 93.4 parts of Equilibration Buffer. This buffer ratio can be achieved by running the LC system at 6.6% Pump B.
  - Alternatively, prepare manually by mixing 660 µl of Elution Buffer with 9.34 ml of Equilibration Buffer.
- **Elution Buffer:** 50 mM sodium phosphate, 300 mM sodium chloride, 150 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, Cat. No. BP 305-50).

B. Enzymes

- **Benzonase®** (Sigma, Cat. No. E8263-5KU)
- **DNase I** (Promega, Cat. No. M6101)

C. 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.D)

III. Additional Materials Needed continued

D. 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

E. Optional

- **PD-10 desalting columns** (GE Healthcare, Cat. No. 17-0851-01) to remove incompatible reagents (Section IV) from starting samples prior to loading onto the cartridge, or to remove excess imidazole from the final sample when required for downstream applications.

IV. General Considerations

A. Use of HisTALON Cartridges

Please note the following recommendations when using HisTALON Cartridges:

- For elution in LC applications, use the Elution Buffer supplied in the HisTALON Cartridge Purification Kit (Cat. No. 635649) and the HisTALON Buffer Set (Cat. No. 635651)], or a highly pure, low-absorbance imidazole (Section III.A).
- Do not use buffers containing EDTA, chelator-containing protease inhibitors or other additives, or strong reducing agents such as DTT with the cartridges. If any of these reagents are present in the sample, it must be desalted on a PD-10 column (Section III.E) before loading it onto the cartridge.
- For best results, always filter buffers through a 0.45 µm filter and degas before use in LC applications.
- Using 2–3 HisTALON Cartridges in tandem will increase capacity, but will result in increased backpressure.
- For HisTALON Cartridge Specifications, see Table I.

Table I: HisTALON 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]	20 mg of AcGFPuv	100 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.

IV. General Considerations continued

B. Compatible Reagents

Table II shows the maximum concentrations of each reagent tested at Clontech. Higher levels may be acceptable, but they should be tested before use. Note that some of these reagents may partially or completely denature your protein.

Table II: Reagent Compatibility with TALON Superflow Resin

Reagent	Acceptable Concentration
β-Mercaptoethanol[1]	10 mM (with caution)
CHAPS[2]	1% (with caution)
Ethanol[3]	30%
Ethylene glycol	30%
HEPES	50 mM
Glycerol	20%
Guanidinium[1]	6 M
Imidazole[4]	200 mM at pH 7.0–8.0, for elution
KCl	500 mM
MES	20 mM
MOPS	50 mM
NaCl	1.0 M
NP-40	1%
SDS[2]	1% with caution
TRIS[5]	50 mM
Triton-X 100	<1%
Urea	8 M

[1] Use resin immediately after equilibrating with buffers containing these reagents. Otherwise, the resin will change color. Do not store resin in buffers containing these reagents.
[2] Ionic detergents like CHAPS (3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane-sulfonate), SDS (sodium dodecyl sulfate), and sarkosyl are compatible up to 1%. However, due to their charged nature, you should anticipate interference with binding.
[3] Ethanol may precipitate proteins, causing low yields and column clogging.
[4] Imidazole cannot be used at concentrations higher than 5–10 mM for loading his-tagged proteins, because it competes with the histidine side chains (imidazole groups) for binding to the immobilized metal ions.
[5] TRIS coordinates weakly with metal ions, causing a decrease in capacity.

C. Incompatible Reagents

These reagents are incompatible at any concentration:

- DTT (dithiothreitol) and DTE (dithioerythritol)

  NOTE: Use of strong reducing agents will interfere with the binding of the cobalt metal ions to the resin.

- EDTA (ethylenediaminetetraacetic acid) and EGTA (ethylene glycol-bis([β-amino-ethyl ether]

  NOTE: Although you can use EDTA at indicated points, it must be removed from the sample by gel filtration prior to applying it to TALON Superflow Resin.

V. Sample Preparation & Purification

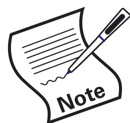
PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING
Use this procedure to (A) prepare your his-tagged protein sample for (B) automated or (C) manual purification using HisTALON Cartridges

Protocol
15 min

A. PROTOCOL: Sample Preparation

Use this method to prepare your protein sample for automated or syringe-based purification:

1. Add 2 ml of xTractor Buffer per 100 mg of cell pellet. Gently pipet up and down until the cell pellet is fully resuspended.



NOTE: xTractor Buffer is supplied in the HisTALON Cartridge Purification Kit (Cat. No. 635649) and the HisTALON Buffer Set (Cat. No. 635651), or sold separately (Cat. Nos. 635623, 635625, 635656 & 635671).

2. To the resuspended pellet, add 1 µl of Benzonase® or DNAse I (Section III.B) for every 2 ml of xTractor Buffer used (i.e., every 100 mg of cell pellet), and mix gently.
3. Incubate on ice, with intermittent mixing, for 15 min. (If desired, this step can be carried out at room temperature for 10 min.) Centrifuge for 20 min at 10,000 x g at 4°C.
4. Carefully collect the clear supernatant—this is your starting sample.

Protocol
20 min

B. PROTOCOL: Automated Purification on a Liquid Chromatography System

1. Equilibrate the cartridge and all buffers to the working temperature. Perform purifications at room temperature or at 4°C.
2. Degas all solutions.
3. Set up the LC System as follows:
   a. Prepare the LC system by filling the tubing with buffer. On a binary pump LC system, fill Pump A and B with Equilibration Buffer and Elution Buffer, respectively.
   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.
4. Equilibrate the cartridge with 5–10 column volumes of the Equilibration Buffer at a flow rate of 1 ml/min for a 1 ml cartridge or 5 ml/min for a 5 ml cartridge.
5. For maximum extraction and binding, prepare the sample using our xTractor Buffer (Section V.A). If you used incompatible reagents (Section IV) during the extraction, desalt the sample on a PD-10 column (Section III.E) before proceeding to Step 6.
6. Load the clarified sample onto the cartridge at a flow rate of 0.5–1 ml/min and collect fractions.
7. 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 10 mM imidazole). The Wash Buffer is prepared on the LC system by running pump B at 6.6%.

V. Sample Preparation & Purification continued

8. 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 150 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.
9. If necessary for downstream applications, remove excess imidazole by gel filtration on a PD-10 column (Section III.E).
10. The HisTALON cartridge can be regenerated quickly by washing with 20 column volumes of Equilibration Buffer or by washing with 10 column volumes of 20 mM MES, 0.3 M NaCl pH 5.0 buffer. 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.
11. For extended storage (over 1 week), wash the cartridge with five column volumes of water after each use and store in 20% ethanol. Attach supplied bottom cap, followed by the top plug. Store the cartridge at 4°C.

Protocol 25 min

C. PROTOCOL: Manual Purification Using a Syringe

1. Equilibrate the cartridge and all buffers to the working temperature. Perform purifications at room temperature or at 4°C.
2. Fill a Luer Lock syringe with 5–10 column volumes of Equilibration Buffer.
3. Set up the cartridge and the syringe as follows:
   a. Attach the syringe to a Luer Lock Adapter (not supplied; see Section III.D).
   b. Remove the top plug from the cartridge, add 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 the cartridge with 5–10 column volumes of Equilibration Buffer (at a flow rate of ~1 ml/min for 1 ml cartridges or ~5 ml/min for 5 ml cartridges). Remove the syringe from the Luer Lock Adapter.
5. For maximum extraction and binding, prepare the sample using our xTractor Buffer (Section V.A). If you used incompatible reagents (Section IV) during the extraction, desalt the sample on a PD-10 column (Section III.E) before proceeding to Step 6.
6. 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. Collect fractions.
7. 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 HisTALON Buffer Set (Cat. No. 635650), the HisTALON Superflow Cartridge Purification Kit (5 x 1 ml), (Cat. No. 635649), or the HisTALON Superflow Cartridge Purification Kit (5 x 5 ml) (Cat. No. 635681), prepare the Wash Buffer by mixing 6.6 parts of Elution Buffer with 93.4 parts of Equilibration Buffer.

8. 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.
9. If necessary for downstream applications, remove excess imidazole by gel filtration on a PD-10 column (Section III.E) .

V. Sample Preparation & Purification continued

10. The cartridge can be regenerated quickly by washing with 20 column volumes of Equilibration Buffer or by washing with 10 column volumes of 20 mM MES, 0.3 M NaCl pH 5.0 buffer. 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.
11. For extended storage (over 1 week), wash the cartridge with five column volumes of water after each use and store in 20% ethanol. Attach the supplied bottom cap, followed by the top plug. Store the cartridge at 4°C.

VI. Troubleshooting Guide

Table III. Troubleshooting Guide for HisTALON 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 presence of 8 M urea or 6 M guanidinium HCl.
	Inefficient target extraction	Use our xTractor Buffer.
	Inaccessible his-tag	Purify in presence of 8 M urea or 6 M guanidinium HCl.
Impurities in eluate	Insufficient washing	Increase wash volume or add intermediate wash at 20 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.

VII. References

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