Clontech Laboratories, Inc.

TALON® Metal Affinity Resins User Manual

Cat. Nos. Many

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I. Introduction

A. Summary

TALON Metal Affinity Resin is a durable immobilized metal affinity chromatography (IMAC) resin, consisting of a tetradentate chelator charged with cobalt, which has a remarkable affinity and specificity 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. This resin is compatible with all commonly used IMAC reagents and allows protein purification under native or denaturing conditions, and in the presence of ß-mercaptoethanol. It is available as a 50% slurry in three different formats.

B. Overview of TALON Resin Formats

- **TALON Metal Affinity Resin** is useful for batch and low-pressure chromatographic applications. This resin utilizes Sepharose CL-6B (GE Healthcare), a durable substrate that performs very well under native and denaturing conditions in centrifuge-mediated purification schemes. The large pore size resin has a high-binding capacity.
- **TALON Superflow Resin** is specifically designed for quick, effective purification of his-tagged proteins at high flow rates (up to 5 ml per cm^2 per min) and medium pressure (up to 150 psi). This resin is recommended if short purification times are essential, or if purification protocols developed at bench scale will be scaled up for larger volumes. It utilizes Superflow-6 (Sterogene Bioseparations, Inc.), an agarose-based medium featuring a unique polysaccharide composition that resists biological degradation. Superflow-6 beads are also stabilized by a chemical cross-linking reaction that allows flow rates up to 10 times higher than are possible with regular cross-linked beads.
- **TALON CellThru Resin** is a novel IMAC resin for purifying his-tagged proteins from crude cell lysates, sonicates, and fermentation liquids. The larger bead size of TALON CellThru Resin (300–500 µm) permits cellular debris to flow through the column, eliminating the need for high-speed centrifugation. Destabilizing factors are removed more quickly with this resin than with other IMAC resins, because the number of steps is reduced.

Table 1. Physiochemical Properties of TALON Resins

Features	TALON Resin	TALON Superflow Resin	TALON CellThru Resin
Batch/gravity flow applications	Yes	Yes	Yes
FPLC applications	No	Yes	Yes
Scale	Analytical, preparative, production	Analytical, preparative, production	Preparative, production
Capacity* (mg protein/ ml adsorbent)	5–15	5–18	5–10
Matrix	Sepharose 6B-CL (6% crosslinked agarose)	Superflow (6% crosslinked agarose)	Uniflow (4% crosslinked agarose)
Bead size (µm)	45–165	60–160	300–500
Maximum linear flow rate (cm/hr)**	30	3,000	800
Maximum volumetric flow rate (ml/min)**	0.5	50	13
Recommended volumetric flow rate (ml/min)	0.3	1.0–5.0	1.0–5.0
Maximum pressure	2.8 psi 0.2 bar 0.02 MPa	150 psi 10 bar 0.97 MPa	9 psi 0.62 bar 0.02 MPs
pH stability (duration)	2–14 (2 hr) 3–14 (24 hr)	2–14 (2 hr) 3–14 (24 hr)	2–14 (2 hr) 3–14 (24 hr)
Protein exclusion limit (Da)	4×10^7	4×10^6	2×10^7

* The binding capacity of individual proteins may vary.

** For washing and elution only.

II. List of Components

TALON Resin, TALON Superflow Resin, and TALON CellThru Resin are supplied as 50% (w/v) slurries in nonbuffered 20% ethanol. Please note that during shipping and storage, the resin will settle; thus, we recommend that you thoroughly resuspend it before aliquotting. 2 ml of homogeneously resuspended slurry will provide 1 ml of resin with a binding capacity of at least 5 mg of his-tagged protein.

Store all of these resins, columns and buffers at 4°C unless otherwise indicated. **Do not freeze TALON Resins**.

A. Resins

TALON Metal Affinity Resin	Cat. No.	Amount
	635501	10 ml
	635502	25 ml
	635503	100 ml
	635504	250 ml
TALON Superflow Resin	**Cat. No.**	**Amount**
	635506	25 ml
	635507	100 ml
TALON CellThru Resin	**Cat. No.**	**Amount**
	635509	10 ml
	635510	100 ml

B. Columns

TALON 2 ml Disposable Gravity Columns*	Cat. No.	Amount
	635606	50 x 2 ml columns
TALON CellThru Disposable Columns*	**Cat. No.**	**Amount**
	635513	20 x 10 ml columns

*Use one of these empty disposable columns for Step 3.f of the Batch/Gravity-Flow Column Purification Protocol (Section V.A).

C. Buffers

xTractor™ Buffer*	Cat. No.	Amount
	635656	100 ml
	635671	250 ml
	635625	2 x 250 ml

xTractor Buffer Kit (Cat. No. 635623)	
200 ml	1X xTractor Buffer*
2.5 ml	100X Lysozyme
400 µl	DNase (5 units/ml)**

HisTALON™ Buffer Set (Cat. No. 635651)	
2 x 250 ml	HisTALON Equilibration Buffer
200 ml	HisTALON Elution Buffer
100 ml	HisTALON xTractor Buffer*

* xTractor Buffer is equivalent to HisTALON xTractor Buffer.

**Store DNase I at –20°C.

III. Additional Materials Needed

A. Buffer Preparation

Use the buffers supplied in the HisTALON Buffer Set (Section II.B) to extract and purify proteins under native and denaturing conditions as follows:

1. Native Conditions

- **Lysis Buffer:** use HisTALON xTractor Buffer (or TALON xTractor Buffer)
- **Equilibration Buffer:** use HisTALON Equilibration Buffer
- **Wash Buffer:** mix 660 µl of HisTALON Elution Buffer with 9.34 ml of HisTALON Equilibration Buffer
- **Elution Buffer:** use HisTALON Elution Buffer

2. Denaturing Conditions

- **Equilibration Buffer:** Add guanidine-HCl to HisTALON Equilibration Buffer to a final concentration of 6 M, and, if necessary, readjust the pH to 7.4.
- **Wash Buffer:** Add guanidine-HCl to Wash Buffer (III.A) to a final concentration of 6 M, and, if necessary, readjust the pH to 7.4.
- **Elution Buffer:** Add guanidine-HCl to HisTALON Elution Buffer to a final concentration of 5.4 M, and, if necessary, adjust the pH to 7.4.

3. Resin Regeneration

- **Regeneration Buffer (pH 5.0):** 20 mM MES (2-(N-morpholine)-ethanesulfonic acid), 0.3 M sodium chloride

4. Protease Inhibitors

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

IV. Sample Preparation Protocols

Please read each protocol completely before starting. Successful results depend on understanding and performing the following steps correctly.

IMPORTANT: We strongly recommend using **ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail** (Cat. No. 635673) when preparing your protein extract. Add 10 µl of Protease Inhibitor Cocktail per ml of lysis buffer [xTractor Buffer for native proteins (Section IV.A) or denaturing Equilibration Buffer for denatured proteins (Section IV.B)] **before** lysing cells to yield a 1X final concentration of inhibitors. For more information, refer to the ProteoGuard Protocol (type PT5140-2 in the keyword field at www.clontech.com/manuals).

A. Protocol: Sample Preparation to Isolate Native Proteins

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

1. Add 20 ml of xTractor Buffer to 1 g of bacterial cell pellet. Gently pipet up and down to fully resuspend the pellet.

   NOTE: A log-phase culture of *E. coli* (O.D.=0.6–0.8) when induced for 2–4 hours, would be expected to provide ~20–40 mg bacterial pellet from 2 ml of the culture.

2. **[Optional]**: Add 40 µl of 5 units/µl DNase I solution and 200 µl of 100X lysozyme solution.

   NOTES:

   - DNase I reduces lysate viscosity, allowing more efficient cellular debris removal. DNase can be used without lysozyme—but cells treated with lysozyme must also be treated with DNase I.
   - Lysozyme helps to fully disrupt bacterial walls, and thus to extract high molecular weight proteins (>40 KDa). However, lysozyme should be omitted for mammalian extraction procedures as well as when lysozyme interferes with your protein's functionality.
   - If the lysozyme solution forms 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, and use the supernatant for the lysis.

3. Mix gently, pipetting up and down several times.
4. Incubate with gentle shaking for 10 min at room temperature or at 4°C.

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

5. The resulting cell lysate can now be applied directly to a TALON CellThru Column, or the lysate supernatant can be applied to any other TALON Resin column after centrifuging (10,000–12,000 x g for 20 min at 4°C).

B. Protocol: Sample Preparation to Isolate Denatured Proteins

1. Harvest 20–25 ml of cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C.
2. Resuspend pellet in 2 ml of **denaturing** Equilibration Buffer (Section III.A.2) per 20–25 ml of culture.
3. Gently agitate or stir the sample until it becomes translucent.
4. Centrifuge the sample at 10,000–12,000 x g for 20 min at 4°C to pellet any insoluble material.
5. Carefully transfer the supernatant (the clarified sample) to a clean tube without disturbing the pellet.
6. Set aside a small portion of the clarified sample for SDS/PAGE analysis before starting purification.

NOTE: Samples containing 6 M guanidinium must be dialyzed overnight against buffer containing 8 M urea before loading on a gel.

V. Protein Purification Protocols

Please read each protocol completely before starting. Successful results depend on understanding and performing the following steps correctly. Use the appropriate set of buffers depending on whether you are purifying protein under native or denaturing conditions (Section III.A).

A. Protocol: Batch/Gravity-Flow Column Purification

For IMAC columns using TALON, we recommend a hybrid batch/gravity-flow procedure. This method combines the speed and convenience of a batch procedure with the higher purity of the gravity-flow column method. In this hybrid procedure, the binding and initial washing steps are performed in a batch format to save time, eliminate extraneous debris, and avoid column clogging. After the initial washes, the resin is transferred to a column for additional washing and protein elution.

1. Resin Equilibration

a. Thoroughly resuspend the TALON Resin.

b. Immediately transfer the required amount of resin suspension to a sterile tube that will accommodate 10–20 times the resin bed volume.

c. Centrifuge at 700 x g for 2 min to pellet the resin.

d. Remove and discard the supernatant.

e. Add 10 bed volumes of Equilibration Buffer and mix briefly to pre-equilibrate the resin.

f. Recentrifuge at 700 x g for 2 min to pellet the resin. Discard the supernatant.

g. Repeat Steps e and f.

2. Sample Application

a. Add crude lysate (TALON CellThru) or clarified sample (TALON & TALON Superflow) from Section IV to the resin.

b. Gently agitate at room temperature or on ice for 20 min on a platform shaker to allow the his-tagged protein to bind the resin.

NOTE: Incubation on ice will decrease proteolysis.

c. Centrifuge at 700 x g for 5 min. Carefully remove as much supernatant as possible without disturbing the resin pellet.

3. Washing

a. Wash the resin by adding 10–20 bed volumes of Equilibration Buffer. Gently agitate the suspension at room temperature or on ice for 10 min on a platform shaker for thorough washing.

b. Centrifuge at 700 x g for 5 min.

c. Remove and discard the supernatant.

d. Repeat Steps a–c with 10–20 bed volumes of Equilibration Buffer

e. Add one bed volume of Equilibration Buffer to the resin, and resuspend by vortexing.

NOTE: Steps f–h can be performed on ice or at room temperature, but incubation on ice will decrease proteolysis.

f. Transfer the resin to a 2 ml gravity-flow column with an end-cap in place, and allow the resin to settle out of suspension.

g. Remove the end-cap and allow the buffer to drain until it reaches the top of the resin bed, making sure no air bubbles are trapped in the resin bed.

h. Wash column once with 5 bed volumes of Wash Buffer.

4. Elution & Analysis

a. Elute the his-tagged protein by adding 5 bed volumes of Elution Buffer to the column. Collect the eluate in 500 µl fractions.

NOTE: Under most conditions, the majority of the his-tagged protein will be recovered in the first two bed volumes.

b. Use spectrophotometric and SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the his-tagged protein.

NOTE: Use a Bradford protein assay (Bradford, 1976) or UV absorbance at 280 nm.

B. Protocol: Large-Scale Batch Purification

This method purifies his-tagged proteins faster than gravity-flow columns; however, batch washes remove impurities less efficiently than gravity-flow columns. Therefore, they require larger wash buffer volumes to obtain pure his-tagged proteins.

1. Resin Equilibration

a. Thoroughly resuspend the TALON Resin.

b. Transfer the required amount of resin to a glass filter with a pore size of 10–20 µm.

c. Apply a vacuum to the filter to remove excess ethanol.

d. Add 5 bed volumes of deionized water to the resin and apply vacuum.

e. Add 5 bed volumes of Equilibration Buffer to the resin and apply vacuum.

f. Repeat Step e two times.

2. Sample Application

a. Add crude lysate (TALON CellThru) or clarified sample (TALON & TALON Superflow) to the resin and mix for 3–5 min.

b. Apply vacuum and collect the filtrate.

3. Washing

a. Wash the resin by adding 10–20 bed volumes of Equilibration Buffer. Gently agitate the suspension at room temperature for 10 min on a platform shaker to promote thorough washing.

b. Apply vacuum to remove buffer.

c. Repeat the above wash (Steps a–b) 2–3 times with 10–20 bed volumes of Equilibration Buffer.

d. **[Optional]:** If necessary, repeat Step c under more stringent conditions using 5 mM imidazole in Equilibration Buffer.

4. Elution & Analysis

a. Elute the his-tagged protein by adding 5 bed volumes of Elution Buffer.

b. Gently agitate suspension at room temperature for 5 min.

c. Apply vacuum, and collect the purified his-tagged protein.

d. Repeat Steps a–c two times, collecting separate fractions.

e. Use spectrophotometric analysis (absorbance at 280 nm) or a Bradford protein assay and SDS-PAGE analysis to determine which fraction(s) contain(s) the majority of the his-tagged protein.

VI. TALON Resin Regeneration and Storage

Generally, reuse TALON Resins 3–4 times before discarding. The exact number of uses varies among preparations because of differences in redox potential, organic complexity, and debris content. To avoid possible cross-contamination, use a particular aliquot of resin to purify a single type of his-tagged protein.

A. Important Precautions

- Do not store TALON Resin in denaturants such as 6 M guanidinium.
- Do not store TALON Resin with bound imidazole; the resin should be washed with 2-(N-morpholine)-ethanesulfonic acid (MES) buffer (pH 5.0) before reuse to remove the bound imidazole.

B. Protocol: Resin Regeneration & Storage

1. Wash resin with five bed volumes of 20 mM MES buffer (pH 5.0) containing 0.3 M NaCl.
2. Rinse resin with five bed volumes of distilled H_2O.
3. Store resin at 4°C in 20% nonbuffered ethanol containing 0.1% sodium azide.

VII. Troubleshooting

A. Loading/Washing

Description of Problem	Possible Explanation	Solution
Protein elutes in the wash buffer	Problems with vector construction	Ensure that the protein and tag are in-frame.
	Buffer compositions are not optimal	Check the pH and composition of all buffers. Use a lower-stringency wash buffer for all washing steps. *For example, slightly increase the pH of the wash buffer.*
	Protein is degraded during extraction	• Perform initial purification step more quickly. • Use mild extraction conditions in the presence of ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. No. 635673). • For expressed proteins, make a C-terminal construct.
	Reagent interferes with binding	• Check Appendix A: Reagent Compatibilities • Dilute an aliquot of lysate (1:10), or sonicate, and check binding on a small scale. • Try using a different his-tagged protein as a control.
	Tag is not accessible under native conditions	• If the “under native conditions” protein fails to bind under native conditions, treat a small aliquot (<1 ml) with 6 M guanidinium and bind to 50 µl of TALON and then proceed with protein purification. • If the target protein is now bound to the resin, then try to move the tag to the other terminus of the protein where it may be more exposed under native conditions. • Add small amounts of nonionic detergent(s) to improve tag accessibility.

B. Elution

Description of Problem	Possible Explanation	Solution
High amount of coeluted impurities	Insufficient wash	Use larger volumes of Wash Buffer
	Buffer compositions are not optimal	• Check buffers used for sample preparation and wash steps. • Check pH. The Wash Buffer should be pH 7.4. Contaminants will coelute in buffers with pH <7.0. • Increase the volume of wash buffer and continue to wash resin bed until the A_{280} drops to zero. • Increase counterion concentration up to 0.5 M NaCl or KCl to inhibit nonspecific ionic interactions. • Add small amounts of nonionic detergent(s); this is particularly important when isolating proteins from a eukaryotic expression system. • Add ethylene glycol or glycerol to inhibit nonspecific hydrophobic interactions.
	Resin was used in large excess	Estimate the fusion protein content of your lysate and use only as much resin as required. (See Table III for binding capacities of TALON resins.)
	Proteolytic product	Use mild extraction conditions in presence of ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. No. 635673).
	Covalent attachment (Cys-Cys, disulfide bonds) of impurities to the protein	Use 5–10 mM of β-ME in the Wash Buffer
	Copurifying histidine-rich proteins	• For HAT- or his-tagged proteins, use enterokinase to remove the HAT tag and rerun IMAC with the mixture. Target protein will pass through the column, while impurities and tag will be adsorbed. (Remove chelating ligands by gel filtration before loading the proteolytic mixture onto TALON Resin.) • Buffer pH is not optimal. Adjust pH as needed. • Use second purification scheme, such as size exclusion, ion exchange, hydrophobic chromatography, etc.
	Protein sample is too concentrated and/or viscous	• Dilute sample 1:5 or 1:10 with additional buffer and centrifuge again before proceeding. • Digest sample for 20–30 min at room temperature with 2.5 µg/ml of DNase I, taking into account that proteolytic activity is much higher at room temperature. • Alternatively, dilute the sample fivefold with Equilibration Buffer before applying it to the resin. This procedure should not significantly affect recovery.

B. Elution (continued)

Description of Problem	Possible Explanation	Solution
Column ceases to flow	Frit or filter is clogged with subcellular debris	Change column filters and centrifuge sample at 12,000 x g for 20–30 min at 4°C
	The lower resin bed support may be clogged with cellular debris	• Remove resin from clogged column and resuspend. Then wash it in a batch format and transfer to a fresh column. • Use a syringe filled with wash buffer or reverse the pump on the column to gently run the column backwards. In addition, test for tubing blockages in a similar manner. Apply gentle pressure. Do not exceed a 1 drop/sec flow rate.
His-tagged proteins do not elute	Elution Buffer is not optimal	• Elute with 150 mM imidazole or pH 4.0 buffer. • For proteins that will not elute otherwise, you can strip off the protein using 100 mM EDTA (pH 8.0). (This will remove the Co^{2+} from the resin and deposit it in your protein sample.) • Add 1–5 mM β-ME to reduce disulfide linkages. Supplement buffer with 1% nonionic detergent. • Purify his-tagged protein under denaturing conditions.

C. Changes in Resin

Description of Problem	Possible Explanation	Solution
White or pale-colored resin	Presence of chelators in the sample causing loss of Co^{2+}	Remove chelators from sample by gel filtration and use fresh resin.
Gray or brown resin	TALON Resin was overexposed to reducing agents or high concentration of β-ME	Completely remove reducing agents, such as DTE or DTT, or by gel filtration chromatography in the presence of β-ME. Reduce β-ME concentration (≤5 mM).
Resin particles aggregate or exhibit a change in consistency	DNA crosslinking	• Increase ionic strength of the buffers by using ≤ 500 mM NaCl. • Vigorously sonicate samples before loading to shear DNA. • Pretreat sample with 100 µg/ml DNase I at 30°C for 30 min. • Dilute sample 1:5–1:10 with buffer before loading on column. • Avoid long-term storage of resin in denaturants.

D. Analysis

Description of Problem	Possible Explanation	Solution
High background on silver-stained gels	Nucleic acid contaminant	• Supplement buffer with 0.5 M NaCl or KCl. Repeat purification • Shear DNA more vigorously. • Use DNAse I in the extraction procedure.
Nonfunctional protein	Protein was damaged by sonication.	• Conduct a time-course assay to determine the minimum sonication time needed to disrupt the cells while maintaining the native protein/enzyme function. *For example, sonicate samples at a medium-high setting for 0, 20, and 30 sec. Then perform protein or enzyme functional assays and measure the A_{280} of each sample.* • Perform the lysis or sonication procedure on ice.
	Protein has degraded	• Keep protein samples at 4°C during purification • Reduce purification time for initial steps. • Add ProteoGuard EDTA-Free Protease Inhibitor Cocktail (Cat. No. 635673).
	Protein may not be folded properly	Try purifying protein under denaturing conditions, then try refolding (Lin *et al.*, 2007)
	Protein may require a posttranslational modification to be active	In this case, it may be necessary to change the expression system (e.g., switch from *E. coli* to insect or mammalian cells.)

E. Resin Reuse

Description of Problem	Possible Explanation	Solution
Binding drops below original capacity	Lysate contains naturally occurring reducing agent or a nonspecific polyanion may be obscuring the metal binding sites.	• Use a larger volume of the previously-used resin. • Replace used resin with fresh resin. • Wash resin with 6 M guanidinium (pH 5.0) and 1% Triton X-100 or SDS, and re-equilibrate before use.
	Resin is dirty or has not been fully regenerated.	Resin has been damaged or has worn out. TALON Resins can be reused at least 3–4 times with proper handling.

Appendix A: Reagent Compatibilities and Incompatibilities

A. Compatible Reagents

Table 2 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 2. Reagent Compatibility

Reagent	Acceptable Concentration
ß-Mercaptoethanol[a]	10 mM (with caution)
CHAPS[b]	1% (with caution)
Ethanol[c]	30%
Ethylene glycol	30%
HEPES 50 mM	50 mM
Glycerol	20%
Guanidinium[a]	6 M
Imidazole[d]	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[b]	1% with caution
TRIS[e]	50 mM
Triton-X 100	<1%
Urea	8 M

[a] 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.

[b] 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.

[c] Ethanol may precipitate proteins, causing low yields and column clogging.

[d] 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.

[e] TRIS coordinates weakly with metal ions, causing a decrease in capacity.

B. Incompatible Reagents

These reagents are incompatible at any concentration:

- DTT (dithiothreitol) and DTE (dithioerythritol)

  NOTE: Using strong reducing agents will interfere with cobalt metal ion binding 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 Resins.

VIII. References

Bradford, M. M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.

Chaga, G., Widersten, M., Andersson, L., Porath, J., Danielson, U. H. & Mannervik, B. (1994) Engineering of a metal coordinating site into human glutathione transferase M1-1 based on immobilized metal ion affinity chromatography of homologous rat enzymes. *Protein Eng.* **7**(9):1115–1119.

Froelich, C. J., Turbov, J. & Hanna, W. (1996) Human perforin: rapid enrichment by immobilized metal affinity chromatography (IMAC) for whole cell cytotoxicity assays. *Biochem. Biophys. Res. Commun.* **229**(1):44–49.

Hochuli, E., Döbeli, H. & Schacher, A. (1987) New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residues. *J. Chromatogr*. **411**:177–184.

Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. & Stüber, D. (1988) Genetic approach to facilitate purification of novel recombinant proteins with a novel metal chelate adsorbent. *Bio/Technology* **6:**1321–1325.

Porath, J., Carlsson, J., Olsson, I. & Belfrage, G. (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**:598–599.

Stephens, L. R., Eguinoa, A., Erdjument-Bromage, H., Lui, M., Cooke, F., Coadwell, J., Smrcka, A. S., Thelen, M., Cadwallader, K., Tempst, P. & Hawkins, P. T. (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* 89(1):105–114.

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