A. Protein Expression

Problem	Possible Cause	Solution
1. No expression	• Bad vector construct	Check sequence of the vector.
	• Bad transformation	Make a plasmid miniprep and confirm sequence.
	• No inducing agent added to culture before harvest	Add proper amount of inducing agent
2. Apparent low expression	• Insoluble over-expressed protein	Use denaturing extraction and purification conditions or reduce expression levels by lowering the amount of inducer.
	• Unsuitable expression conditions	Check cell growth and inducer concentration; check for wild-type (nontransformed) or antibiotic-resistant cells.
	• Protein is secreted	Use fermentation liquid or serum (for Thiophilic Resin) as starting sample for purification after proper buffering

B. Loading/Washing

Problem	Possible Cause	Solution
1. Protein elutes in the wash buffer	• Problems with vector construction	Ensure that protein and tag are in frame.
	• Buffer is 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, lower its imidazole concentration, or increase the sulfate concentration (for Thiophilic Resin).*

B. Loading/Washing *...cont.*

Problem	Possible Cause	Solution
2. Protein elutes in the wash buffer ...cont.	• Protein degraded during extraction	a) Perform initial purification step more quickly. b) Use mild extraction conditions in the presence of protease inhibitors (e.g., β-ME and EDTA) at 4°C. Be sure to remove EDTA before applying to TALON Resin. c) For expressed proteins, make a C-terminal construct.
	• Reagent interferes with binding	a) Check Reagent Compatibilities b) Dilute an aliquot of lysate (1:10), or sonicate, and check binding on a small scale. c) Try using a different polyhistidine-tagged protein as a control.
	• Tag is not accessible under native conditions	a) *TALON Resin only:* If the protein fails to bind under native conditions, treat a small aliquot (<1 ml) with 6 M guanidinium and bind to 50 µl of TALON. Then follow the miniscale procedure in Section V. 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. b) *GST only:* GST is denatured.
2. High back pressure during load of sample	• High viscosity due to presence of DNA	a) Use DNase I or b) Dilute sample five-fold before loading on column.

C. Elution

Problem	Possible Cause	Solution
1. High amount of co-eluted impurities	• Insufficient wash	Use larger volumes of Extraction/Wash Buffer
	• Buffer compositions are not optimal	a) Check buffers used for sample preparation and wash steps. b) Check pH. The Extraction/Wash Buffer should be pH 7.0. Contaminants will co-elute in buffers with pH < 7.0. c) Increase volume of wash buffer and continue to wash resin bed until the A_{280} drops to zero. d) Increase counterion concentration up to 0.5 M NaCl or KCl to inhibit nonspecific ionic interactions. e) Add small amounts of nonionic detergent(s); this is particularly important when isolating proteins from a eukaryotic expression system. f) *TALON Resins only:* Add ethylene glycol or glycerol to inhibit nonspecific hydrophobic interactions. g) *TALON Resins only:* add 1–5 mM imidazole to the Extraction/Wash Buffer and use it as a wash step immediately before elution.
	• Proteolytic product	Use mild extraction conditions in presence of protease inhibitors (e.g., β-ME and EDTA) at 4°C. **Remove EDTA before applying to TALON.**
	• Covalent attachment (Cys-Cys, disulfide bonds) of impurities to the protein	Use 5–10 mM of β-ME in the Extraction/Wash Buffer. (Not for Thiophilic Resin)

C. Elution...*cont.*

Problem	Possible Cause	Solution
1. **High amount of co-eluted impurities**...cont.	• Co-purifying histidine rich (for TALON) or sulfone rich (for Thiophilic) proteins	a) For HAT- or His-tagged proteins, use enterokinase to remove HAT tag and re-run IMAC with the mixture. Target protein will pass through the column, while impurities and tag will be adsorbed. *Note: Remove chelating ligands by gel filtration before loading the proteolytic mixture onto TALON Resin.* b) Buffer pH is not optimal. Refer to TALON Resin Section II. c) 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. Also, see the note on reducing sample viscosity after sonication in TALON Resin Section IV.A.8.
2. **Excessive background after TALONspin Column procedure**	• Sample is too viscous	a) Treat sample with DNase I (TALON Section IV.A.8). b) Dilute clarified sample with an equal volume of Extraction/Wash Buffer and load as two aliquots. c) Increase the number of 1-ml washes. d) Use Extraction/Wash Buffer (pH 7.0). e) Add 1–5 mM imidazole to Extraction Buffer, pH 8.0 and use it as an intermediate wash step before elution.

C. Elution...*cont.*

Problem	Possible Cause	Solution
2. Excessive background after TALONspin Column procedure...cont.		re-purify a TALONspin sample, perform the following after performing TALON Purification Section V.B:Step 37. (1) Add 4 volumes of Extraction/Wash Buffer to semi-purified sample. (2) Load sample onto another TALONspin Column. (3) Wash twice with 1 ml of Extraction/Wash Buffer. (4) Elute as before (Section V.B.30–35).
3. 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.
	• Proteins precipitated on the column	Use a mild detergent such as Decanoyl-N-methylglucamide (MEGA-10, Sigma, #D-6277) in the Extraction/Wash Buffer.
	• The lower resin bed support may be clogged with cellular debris	a) Remove resin from clogged column and resuspend. Then wash it in a batch format and transfer to a fresh column b) 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.

C. Elution...*cont.*

Problem	Possible Cause	Solution
4. polyhistidine-tagged proteins do not elute	• Elution Buffer is not optimal	a) Elute with 150 mM imidazole or pH 4.0 buffer. b) *TALON only*: For proteins that will not elute otherwise, you can strip off the protein using 100 mM EDTA (pH 8.0); however, doing so will remove the cobalt from the resin and deposit it in your protein sample. c) *Not for Thiophilic Resin*: Add 1–5 mM β-ME to reduce disulfide linkages. Supplement buffer with 1% nonionic detergent. d) Purify His-tagged protein under denaturing conditions.

D. Changes in Resin

Problem	Possible Cause	Solution
1. Loss of Co^{2+} (TALON only)	• Presence of chelators in sample	Remove chelators from sample by gel filtration and regenerate adsorbent as described in TALON Resin Section VII.D.
2. Gray or brown resin	• TALON Resin was over-exposed 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).
3. Resin particles aggregate or exhibit change in consistency	• DNA crosslinking	a) Increase ionic strength of the buffers by using ≤ 500 mM NaCl or KCl. b) Vigorously sonicate samples before loading to shear DNA. c) Pretreat sample with 100 µg/ml DNase I at 30°C for 30 min. d) Dilute sample 1:5–1:10 with buffer before loading on column.

D. Changes in Resin *...cont.*

Problem	Possible Cause	Solution
3. Resin particles aggregate or exhibit change in consistency ...cont.		e) Avoid long-term storage of resin in denaturants.

E. Analysis

Problem	Possible Cause	Solution
1. High background on silver-stained gels	• Nucleic acid contaminant	a) Supplement buffer with 0.5 M NaCl or KCl. Repeat purification
		b) Shear DNA more vigorously.
		c) Use DNAse I in the extraction procedure.
2. Nonfunctional protein	• Protein was damaged by sonication	a) 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.*
		b) Perform the lysis or sonication procedure on ice.
	• Protein has degraded	a) Keep protein samples at 4°C during purification
		b) Reduce purification time for initial steps.
		c) Add some proteinase inhibitors. Try different proteinase inhibitors.

F. Resin Reuse

Problem	Possible Cause	Solution
1. Binding drops below original capacity	• Lysate contains naturally occurring reducing agent or a nonspecific polyanion may be obscuring the metal binding sites.	a) Use a larger volume of the re-used resin. b) Replace used resin with fresh resin. c) TALON only: 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. These resins are re-usable with proper handling and regeneration. However, they do not last indefinitely. TALON Resins can be reused at least 3–5 times. Thiophilic Resin can be reused more than 10 times if properly maintained. Glutathione Resins can be reused at least 5–10 times.