Informational

Affinity His-Tag Purification

Troubleshooting Guide

Introduction

Possible causes of problems that could appear during the affinity His-tag purification protocol of biomolecules are listed below. The causes described are theoretical and it is always advisable to contact our team with your specific problem.

The table delineates the potential problems at each step in the protocol that might explain poor performance.

Table 1. Sample applications.

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
HIGH VISCOSITY SAMPLE	Presence of DNA in the sample.	- Increase sonication time until viscosity is reduced.
	Steric hindrance of the substrate.	- Dilute the sample before its application in the column. In this case, sometimes, it is preferable to carry out the purification in batch format instead of the column format.
		- Consult "tailor made resins" for high viscosity samples.
HIGHLY DILUTE OR CONCENTRATED SAMPLE	Highly diluted sample.	- It is preferable to concentrate the sample before its purification in the column.
		- Another solution is to carry out an adsorption step in batch format and pack the column with the resultant resin of the adsorption step.
	Highly concentrated sample.	- It is preferable to make a previous dilution of the sample before its purification in the column.

Table 2. Adsorption

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN NOT BOUND TO THE COLUMN	His-tag is not present or has been degraded.	- Check it. If it has been degraded, make the purification at lower temperatures (4°C) reducing the

		degradation. Try to reduce the purification step times. Add protease inhibitors. (See chemical compatibility table).
	His-tag is not exposed (inaccessible).	- Purify in denaturing conditions or add the tag in other site (N-terminus, C-terminus, or in both positions).
	Inadequate binding conditions.	- Check the buffer and binding pH. - If the binding has been done in presence of imidazole, reduce its concentration or eliminate it in this step. - Verify if some of the reagents used in the adsorption step interfere with the binding reaction. e.g.: A Zinc resin can lose its metal due to the presence of chelating agents in the sample and therefore, the protein will not bind. Since the presence/ absence of Zinc cannot be visualized by a change of color, it would difficult to determine this phenomenon. So, in case of doubt, it is advisable to regenerate the column and observe if the target protein is bound to the regenerated resin.
TARGET PROTEIN BINDS ONLY PARTIALLY TO THE COLUMN	Column capacity is exceeded.	- Apply less fused protein to the column.
	The resin has been previously used during several purification cycles without regeneration. This causes a diminution of the binding capacity. This diminution varies in each case and increases with the number of purification cycles of the resin.	- Apply a regeneration step in the column when a decrease of the binding capacities is observed.
	Loss of chelating metal in the resin.	- Apply a regeneration step in the column. Avoid use of reducing and chelating agents.
	Histidine tail is not very exposed.	- Try to use slower flow rates or make the adsorption in batch to allow a better contact between resin and fused protein.

		Note: a greater exhibition would be obtained working in denaturing conditions.
	Poor protein expression.	- Optimize bacterial expression conditions
	The fused protein forms inclusion bodies.	- Modify bacterial growth conditions. - Work in denaturing conditions.
	Channels have formed in the column so the sample runs mainly through these undesirable channels	- Re-pack column.
	The resin shows low binding capacity.	- Try a HIGH DENSITY TEST KIT or with a less selective cation.

Table 3. Elution

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
HIGH AMOUNT OF CO-ELUTED PROTEINS (CONTAMINANTS)	Insufficient washing stage.	- Increase volume of washing buffer.
		- Add a low concentration of imidazole (5-10mM) in the buffer during washing and equilibrating steps.
	Inadequate adsorption conditions.	- Check pH.
		- Add or increase saline concentration in the binding buffer to avoid non-specific ionic interactions.
		- Low concentrations of non-ionic detergents can also be added.
		- Add small quantities of ethyleneglycol or glycerol in the binding buffer to avoid non-specific hydrophobic interactions.
		- Increase imidazole concentration in the binding buffer. **Note: In general, higher imidazole concentrations than 20mM are not recommended because it can compete with the binding of the target protein. This concentration can be modified with the type of protein to be purified.**

HIGH AMOUNT OF CO-ELUTED PROTEINS (CONTAMINANTS)	The column is too large.	- Reduce the resin quantity so the fused protein and contaminants will compete for less binding sites, increasing the binding selectivity of the tagged protein.
	The resin used in the purification shows low selectivity to bind the fused protein. In some cases, Nickel resin is not as selective as ones loaded with other metals. It may also bind proteins with histidine, cysteine and tryptophan residues.	- Try a LOW DENSITY TEST KIT or a more selective cation (e.g.: Cobalt).
		- Employ an imidazole concentration gradient to separate the target protein from the rest of retained proteins. Also "Single Step Elution" procedures can be used.
TARGET PROTEIN ELUTES POORLY	Too smooth elution conditions.	- Increase imidazole concentration or reduce pH in the elution step.
		- Try, if possible, an elution at a higher temperature.
	Sometimes protein binding with chelating metals is too strong. **Note: Also the position of the histidine tail can influence the strength of the binding of the target protein.**	- Make an elution with a chelating agent such as EDTA.
		- Make an elution reducing pH (pH 4.0) in the presence of imidazole.
		- Purify with other chelating resins as the requirements with each cation are different and the binding strength is different with each one. Also, in many cases, using a Low Density resin gives better desorptions of the fused protein.
		- Increase imidazole concentration up to 1M in the elution buffer.
		- Reduce the flow in the elution step or make this step in batch format to increase contact time.
		- Elute in denaturing conditions.
	Fused protein cannot be precipitated.	- Add solubilizing agents (see compatibilities).
		- Incubate the column with the elution buffer for 8-10 h and elute with the elution buffer.
		- Run binding and elution steps in batch format to avoid local concentration of protein and therefore its potential precipitation.

ELUTION PROFILE IS NOT REPRODUCIBLE IN DIFFERENT CYCLES OF PURIFICATION	Sample's nature could have been modified. The histidine tail could have been lost due to protease action.	- It is necessary to prepare a fresh sample. Run the protocol at 2-8°C. Add protease inhibitors (see chemical compatibilities table).
	Proteins or lipids could have precipitated.	- Regenerate the resin.
	The pH or ionic forces could have been modified.	- Prepare new buffers.
	The sample to apply could be different than the first one.	- Keep all the parameters and same conditions.
	Loss of binding capacity is observed.	- It is recommended to regenerate the column.

Table 4. Changes in resin.

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
LOSS OF COLOR OF THE RESIN	Presence in the sample of chelating agents that could have caused the diminution of the content of the metal.	- Eliminate the chelating agents in the sample (e.g. by gel filtration) and after regenerate the column. **Note: This is easy to see in colored resins (Cobalt, Nickel or Copper).In other cases such as Zinc the loss of the cation is not so evident by color changes and could be the cause the non-binding of the protein.**
CHANGE OF COLOR (BROWN) OF THE RESIN	Reducing agents in the sample.	- Eliminate these reducing agents and regenerate the resin.