

AFFINITY His-TAG PURIFICATION

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TROUBLESHOOTING GUIDE



Problems and Solutions

Possible causes of problems that could appear during the 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.

1. SAMPLE APPLICATION

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
HIGH VISCOSITY SAMPLE	- Presence of DNA in the sample /lysate.	- Lysate may remain viscous. Add 5 μg /ml DNase I and incubate on ice for 10 min.
	- Presence of insoluble material in the sample /lysate.	- Use centrifugation or filtration (0.45 μm membrane) to avoid clogging of the column.
HIGHLY DILUTED 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.
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).
	- It is not exposed (inaccessible).	- Purify in denaturing conditions or add the tag in other site (<i>N-terminus</i> , <i>C-terminus</i> , 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 interferes with the binding reaction. So, in case of doubt, it is advisable to regenerate the column and observe if the target protein is bound to the regenerated resin.
HIGH AMOUNT OF CO-ELUTED PROTEINS (CONTAMINANTS)	- Insufficient washing stage.	- Increase volume of washing buffer. - Increase the concentration of imidazole in the buffer during washing and equilibrating steps.
	Inadequate adsorption conditions.	- Binding and wash conditions are too mild. Use 10 – 20 mM imidazole in the binding and washing buffers. - 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.

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		<ul style="list-style-type: none"> - Add small quantities of glycerol in the binding buffer to avoid non-specific hydrophobic interactions. - Increase imidazole concentration in the binding buffer. <p><i>Note:</i> In general, higher imidazole concentrations than 20 mM 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.</p>
TARGET PROTEIN BINDS ONLY PARTIALLY TO THE COLUMN	Column capacity is exceeded.	<ul style="list-style-type: none"> - 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.	<ul style="list-style-type: none"> - Apply a regeneration step in the column when a decrease of the binding capacities is observed.
	Loss of chelating metal in the resin.	<ul style="list-style-type: none"> - Apply a regeneration step in the column. Avoid use of reducing and chelating agents.
	Histidine tail is not very exposed.	<ul style="list-style-type: none"> - Try to use slower flow rates or make the adsorption in batch to allow a better contact between resin and fused protein. <p><i>Note:</i> a greater exhibition would be obtained working in denaturing conditions.</p>
	Poor protein expression.	<ul style="list-style-type: none"> - Optimize bacterial expression conditions.
	The fused protein forms inclusion bodies.	<ul style="list-style-type: none"> - Modify bacterial growth conditions. - Work in denaturing conditions.
	Channels have formed in the column so the sample runs mainly through these undesirable channels.	<ul style="list-style-type: none"> - Re-pack column.
HIGH AMOUNT OF CO-ELUTED PROTEINS (CONTAMINANTS)	Column too large.	<ul style="list-style-type: none"> - 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.	<ul style="list-style-type: none"> - 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	Too smooth elution conditions.	<ul style="list-style-type: none"> - Increase imidazole concentration or reduce pH in the elution

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POORLY

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.

Fused protein can be precipitated.

step.

- Try, if possible, an elution at a higher temperature.
- Make a elution with a chelating agent such as EDTA.
- 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.
- 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.

Proteins or lipids could have precipitated.

PH or ionic forces could have been modified.

The sample to apply could be different than the first one.

Loss of binding capacity is observed.

- It is necessary to prepare a fresh sample. Run the protocol at 2-8°C. Add protease inhibitors (see chemical compatibilities table).

- Regenerate the resin.

- Prepare new buffers.

- Keep all the parameters and same conditions.

- It is recommended to regenerate the column.

WARNINGS TO THE USER

Activated Agarose Beads are for laboratory use only. Not for use in diagnostic or therapeutic procedures.