

Q&A

Answers to webinar questions "*Obstacles in protein purification*" by Marianne Carlsson on November 26, 2007



Q Is the Ni Sepharose™ leaching Me ions? What kind of chelator

A The Ni leakage from our Ni Sepharose medium is low compared with media from other suppliers. Please find information regarding leakage in the Datafile (Ni Sepharose HP Code no. 18117440).

Q Avoiding freezing concentrated protein solution. Could you say something about the concentration level, or the range where you prefer to freeze?

A As mentioned in the lecture, every protein purification is an adventure. Each protein has its own properties. Not all proteins tolerate freezing at -20°C. If the aim is to produce and store a large amount of purified protein at a time, it would be worth doing some initial experiments to check both protein concentration as well as buffer conditions that are suitable for freezing protein. 10%-50% glycerol is often used when one wants to retain enzymatic activity.

To get more information regarding protein handling see the link below.

<http://64.233.183.104/search?q=cache:0GXskwxG4KsJ:www.moleculardimensions.com/datasheets/samples.pdf+freezing+concentration+purified+concentrated+protein,+crystallization&hl=sv&ct=clnk&cd=3&gl=se>

or

<http://www.moleculardimensions.com/datasheets/samples.pdf>

Q Can TCEP be used for Ni or IMAC Sepharose instead of DTT, and if so, at what concentration? It seems that we have problems with oligomerization on Ni-IMAC.

A Yes, we have successfully used TCEP at 5 mM with Ni Sepharose (in the sample and in the chromatography buffers). Note that in the literature, there are reports that traces of metal ions have caused oligomerizations via interactions with the His-tags in free solution. With all IMAC media, there will always be traces of metal ions leaking off. A treatment of the eluted oligomerized protein with EDTA will help if metal ions are the cause.

Q Do you have suggestions to express large proteins in E.coli. We suffer from very low expression levels and low solubility of these large proteins?

A We suggest that you try low temperature during cell growth or use a low amount of inducers. In the end if it does not work, you may have to change the strain or the host system.

Q I am working with his-tagged proteins. Which metal ions could be helpful, if nickel does not show the expected result? More specifically, my problem is that proteins bind too strongly to the column?

A With our IMAC Sepharose, we have found that Co^{2+} , and especially Zn^{2+} and Cu^{2+} give weaker protein binding than Ni^{2+} . If a protein cannot be eluted as expected, another reason could be: Namely that at the elution with imidazole (especially at one-step elutions), the concentration of released protein gets so high that the protein will precipitate and stick to the column. In such cases: 1) try a run while loading less protein 2) perform gradient imidazole elution instead of step elution.

Q I am using TAP-tag purification system and my protein complex is being lost after the first flowthrough- it does not attach to IgG column. What can I do?

A It may be that the bait protein you have chosen is not on the surface of the complex and hence the Protein A tag is not accessible for binding to IgG. The interaction between IgG and Protein A is very rapid and therefore if the tag is exposed properly, it would bind to IgG immediately.

One alternative is to use another bait protein from the complex for attaching the tandem tag. I hope you have done some calculations regarding the “expected amount of protein complex”, the volume of starting material and concentration of complex in it, and the volume of IgG Sepharose (in μl or ml) used for the purification.

Q Regarding location of the webinar presentation?

A The presentation is available on our web page:

<http://www6.gelifesciences.com/APTRIX/upp01077.nsf/Content/pure>

Q Do you have any experience as to whether a protein that has a SUMO-MBP fusion will bind to a MBP column with the SUMO tag in front of it?

A We have not tested purification of a SUMO-MBP fusion protein in our laboratories. We have tested other tags in series such as His-Strep-fusion protein on the Streptactin Sepharose column and this has worked fine. One of the reasons it worked well was that histidine tag is relatively small in size and hence would not affect the structure of the next protein.

As both SUMO and MBP are relatively larger in size, there is a risk that the SUMO-tag may change the accessibility or the conformation of MBP, thus preventing the binding to the MBP column.

If one wants to have both the tags on the protein, one can think of using one tag in the N-terminus and the other one in the C-terminus of the protein.

Q What are the pros and cons for expressing a protein in the cytoplasm vs the periplasm?

A The *E. coli* system is well studied and many proteins are expressed in cytoplasm and purified. If you are expressing a tagged protein, it is often easy to purify the cytoplasmic target protein, even when there are many other contaminants or unwanted proteins present. The target protein concentration will be higher in the sample, when the cells are lysed and clarified for further purification; in other words you will have less volume to process on a column.

For periplasmic expression, the extraction of protein is done either by thawing and freezing, or by osmotic effect. Often the amount of protein will be lower than the cytoplasmic expression. There are not many contaminating proteins in the periplasmic space and hence it will be easy to get pure protein upon purification. You have to have a signal peptide and often all molecules will not be cleaved off from the signal peptide.

Advantages of periplasmic expression can be utilized in the following cases:

1. When the target protein gets degraded by proteases in the cytoplasm.
2. If the protein has disulfide bonds, it is better to secrete the protein into the periplasm. Cytoplasm is considered to be of strong reducing nature and thus appropriate disulfide bonding may not be formed.

Q What denaturing conditions do you recommend for HisTrap™ columns for a his tagged protein whose tag may be occluded?

A We have successfully used both 8 M urea and 6 M Gua-HCl, in sample and in chromatography buffers (pH around 7.5 – 8). Urea has the advantage that fractions from the IMAC can be loaded on SDS-PAGE, whereas the Gua-HCl will have an excessively high conductivity for that (you will have to do a buffer exchange). On the other hand, Gua-HCl is considered to be a more efficient unfolders than urea - that is probably of importance only when working with inclusion bodies. If using urea, include also 0.5 M NaCl in all solutions, to give an ionic strength that prohibits unwanted ion-exchange interactions with the medium. Elution is done by applying the urea or Gua-HCl together with imidazole. See the Troubleshooting section of the HisTrap/Ni Sepharose/IMAC Sepharose Instructions.

Q What reagent could be added to samples after elution to minimize aggregation? What could cause long, broad elution peaks of GST fusion protein from the glutathione column?

A The aggregation of GST tagged proteins often occurs on the column leading to difficulty in eluting the protein or elution in broad peak. This is often due to the formation of intra- and inter-molecular disulfide bonds, especially if the concentration of reducing agents is low. The easiest way to elute the protein in this case is to incubate the column in 10-50 mM glutathione or DTT over a period of half an hour. This will reduce the disulfide bonds and the protein will be eluted.

In general, to elute the protein in smaller peaks, try the following:

1. Reduce the flow rate during elution.
2. Increase concentration of glutathione, varying from 10-50mM in elution buffer.
3. Increase salt concentration to 0.1-0.2 M NaCl in the elution buffer.
4. Add non-ionic detergent such as 0.1% Triton™ X-100 or 2% N-octyl glucoside to reduce non-specific interactions with the media.
5. One can also screen the binding and elution of the protein to different media (e.g., Glutathione Sepharose 4B, Glutathione Sepharose Fast Flow, Glutathione Sepharose High Performance).

Q What to do with inclusion bodies?

A Recently we had a webinar regarding refolding. See the link below. You may find lots of tips. In short, if you think your protein is easy to refold, you can take advantage of inclusion bodies and purify the protein and refold. If your protein has to be in soluble form, you may have to change the tag, or lower the temperature during the cell culture or change the bacterial strain.

http://www1.gelifesciences.com/APTRIX/upp00919.nsf/Content/Pure_TechDoc%7Ewebbin_pplr?OpenDocument&hometitle=pureLS



imagination at work

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12/2007