Chromatography Success Guide

Tips and Tricks for Successful Preparative Chromatography and Protein Purification

BIO-RAD

This Chromatography Success Guide provides practical advice on preparative chromatography and protein purification.

Read on to discover key tips, secrets, and recommended resources every chromatographer should know about.

Your Sample Seems a Little Mixed Up

A popular way to clarify lysates prior to capture step is to centrifuge at 45,000 rpm, 30 min, with a 45 Ti rotor or equivalent.

When filtering proteins, be sure to select a low protein binding membrane such as PVDF or PES. Nylon filters often bind protein and will cause a loss of yield.

Samples should be 0.45 or 0.2 µm filtered to remove particulates and small contaminants. If your sample does not flow easily through one of these filters, try a 0.8 µm filter. This size often works well for proteins with visible particulates and proteins not amenable to smaller pore filtration.

When preparing your sample for ion exchange, dilute or desalt it to bring its conductivity below 10 mS/cm. This will ensure optimal binding.

Use proper protein storage methods after purification to protect proteins.

- **Short term (24 hr or less): 4°C**
- **Long term: –80°C. Include 5–50% glycerol, albumin (10 mg/ml), and/or reducing agents like DTT or BME in the storage buffer. Freeze protein in small aliquots in microcentrifuge tubes. Flash freeze tubes using liquid nitrogen, or dry ice/ethanol bath**
- **Can also store protein as ammonium sulfate precipitate at 4°C**

Mark One in the Win Column

Check your system pressure when first running a column and record it in a place that can be easily referred to. You can use this reading for comparison should you observe abnormal performance.

Height equivalent theoretical plate (HETP) count and asymmetry factor (A_s) are dependable measures of column performance. Note these parameters before first column use to enable monitoring over time.

Always make sure to take your column out of line or put your valves in column bypass prior to purging.

Replace worn frits and O-rings in manually packed columns when they are observed.

When attaching a column to your system, you should use the drop-to-drop method to avoid introducing air onto your column. This is a method of filling the column inlet with fluid prior to final attachment.

Use of an inline precolumn filter (20 µm) between your inject valve and column is an effective way to increase column lifetime.

Your System
Just Wants To
Be Loved

Occasionally check your UV detector lamp life to ensure it meets recommended output parameters.

Tubing on your chromatography system should be short enough to minimize dead volume while allowing component positioning flexibility.

Buffer lines should be cleaned with purified water at the end of the day. This is especially important when working at low temperatures where salts can precipitate.

Always degas or vacuum filter buffers prior to use to minimize air and particulates in your mobile phase.

Occasionally inspect buffer bottles/bags for signs of bacterial growth. Solutions that appear cloudy upon shaking or stirring should be discarded. Treatment with a bacteriostatic agent such as 0.02% sodium azide will prolong solution storage.

It is recommended to replace your piston washing solution weekly with fresh 20% EtOH or isopropyl alcohol. At minimum, regularly inspect for fluid level depletion or bacterial growth.

Sample inlet lines and sample loops should periodically be cleaned with 1 N NaOH solution, followed by water or neutralizing buffer, to eliminate sample buildup in the tubing.

Periodically replace PEEK* tubing that comes in regular contact with sample, especially lines connected to your fraction collector.

Storing all system lines in fresh 20% EtOH when not in use is an effective way to avoid bacterial growth. It is recommended that this is done at the end of each work day.

Injection needles are best used with volumes of 1 ml or less. Larger volumes can be more easily managed with a luer fitting attached to your injection port.

Calibrate your pH probe daily for most accurate results. Electrodes should always be stored in their designated storage solution or a neutral buffer. 20% EtOH should not be used, as it changes the concentration of KCl in the probe.

Pre-equilibrate the selected column prior to running and observe UV/conductivity behavior stability. Many automated systems allow you to advance to sample injection once complete equilibration is observed.

Periodically check system tubing for cracks and bends. Tubing bends can restrict flow and increase system backpressure.

For best results, occasionally sanitize your entire system with 1 N NaOH to clear the flow path from pump to fraction collector.

Your Detectors Are Talking… Are You Listening?

Sawtooth UV signals can be an indication of air trapped in your flow cell. You can often chase the bubble out of your system with 1 N NaOH.

Random spikes in a UV trace that coincide with negative conductivity spikes most often indicate presence of air. Recurring instances of these spikes can often be corrected by purging your system with 1 N NaOH.

Signs of pulsation in your gradient may indicate that you are not using the correct size of mixer for your flow rate.

S-shaped conductivity gradient curves often indicate a mismatch between mixer size and flow rate.

Hardware for Hard Times

Column switching valves save setup time when multiple projects are performed on the same chromatography system.

Loading lysate through sample pumps is preferable over using system pumps. The latter increases the chances of sample buildup and contamination in your system.

Buffer blending is an effective way to reduce both solution prep and method development time.

To achieve optimal gradient performance, choose the mixer size that corresponds with the operating flow rate.

Proteins and nucleic acids can be monitored together with a multi-wavelength detector. You can also analyze their relative proportions using software for automated chromatography systems.

There's a Method to Our Madness

Know relevant properties of your protein: pI, molecular weight, hydrophobicity. This will assist in developing a purification strategy.

If using IMAC as a capture step, do not add EDTA to your buffers. These will strip the active metals and preclude protein binding.

When creating methods try to minimize redundant steps for clarity of protocol and ease of monitoring. Creating method templates saves future setup time and eliminates the need to monitor entire runs.

Tris-based buffers should not be used in IMAC purification, as they will outcompete the protein of interest for the binding sites.

Do not use phosphate buffer systems when using hydroxyapatite resins, as they will interfere with protein binding.

Always name your runs by date or notebook number to facilitate easy data access in the future.

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Notes:

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