Guidelines for protein purification at low temperature

Introduction

The main reason for performing protein purification at low temperature is to preserve the integrity of proteins and whole samples during the process. Working at lower temperature reduces proteolytic activity and prevents unwanted degradation of the target protein. The tendency of the target protein to aggregate should also be monitored and can be impacted by factors such as temperature and protein concentration. There are many factors to consider when working with proteins at low temperature. This document will provide you with general guidelines on how to successfully purify proteins under such conditions.

In most cases, ÄKTA™ systems, chromatography resins, and prepacked columns available from Cytiva can be used at low temperature (4°C to 8°C) with performance results similar to those at room temperature (20°C to 25°C). To maintain performance in the cold environment, it might be necessary to adjust buffer, sample, and running conditions.

Information concerning the suitability for use at low temperature is usually provided in the user documentation for the specific product. This document complements information found in these sources with guidelines and tips on handling ÄKTA™ systems, purification resins, and prepacked columns in low-temperature conditions to achieve optimal results and to avoid potential problems. However, it is strongly recommended that you read the user documentation for the system or column to be used for the purification before following the instructions described here.

Working with buffers and samples at low temperature

Before starting the low-temperature purification, there are a few steps that need to be taken to ensure optimal performance:

1. If the buffers have been prepared at room temperature, they need to be refrigerated together with the ÄKTA™ system, chromatography resin, or chromatography columns at least 12 h before start to stabilize the temperature.

   Note! Check buffer pH when the temperature has stabilized. It might be necessary to adjust the pH. This is particularly important when using Tris buffers.

   Note! After use at low temperature and before equipment is used again at room temperature, place buffers and all equipment in room-temperature conditions before use to avoid formation of air bubbles.

2. If sample has not been prepared at low temperature, and especially if larger samples (measured in milliliters or higher) are to be loaded, it might be necessary to refrigerate for optimal performance.

3. Buffer viscosity increases as the temperature decreases: a flow rate at 4°C will have a higher operating pressure than the same flow rate at room temperature.

   a. Make sure not to exceed the pressure limit of the column by decreasing the flow rate (20% to 50% reduction depending on chromatography medium and column format).

   b. When working at low temperatures with 20% ethanol or other viscous solutions, a further reduction in flow rate (40% to 60% reduction depending on chromatography medium and column format) may be required.

Using columns at low temperature

The resin and column type used determine how chromatography columns are used at low temperature. Columns have three important specifications that need to be taken into account in terms of column protection:

1. Max. flow rate
2. Max. pressure drop over the packed bed
3. Column hardware pressure limit

Note! Max. flow rate will be lower, but column hardware pressure limits will not change in the cold room compared with in room temperature.

The first two points are important for protecting the packed bed from collapsing. The third point is important for safety, especially with glass columns, as excessive pressure can damage the column hardware. In general, all this information can be found in the instructions for your product.

Here are some things to consider when purification is performed at low temperature:

1. For some affinity resin, binding of proteins can be affected by low temperature and a lower flow rate during sample application might be required.
    a. The flow rate during binding for GSTrap™ columns may be reduced due to slow binding kinetics. Low temperatures can also cause broader elution peaks due to slow release of GST-tagged protein.
    b. For affinity columns such as HisTrap™ FF, a ~ 20% reduction in flow rate might be needed when more viscous solutions are used.
2. For size exclusion chromatography columns, up to 50% reduction in flow rate might be needed.

An example of the relationship between column pressure and flow rate for Superdex™ 200 Increase 10/300 GL size exclusion chromatography column at different temperatures is shown in Figure 1. The curves show how temperature changes the viscosity of the buffer, which affects the column pressure.

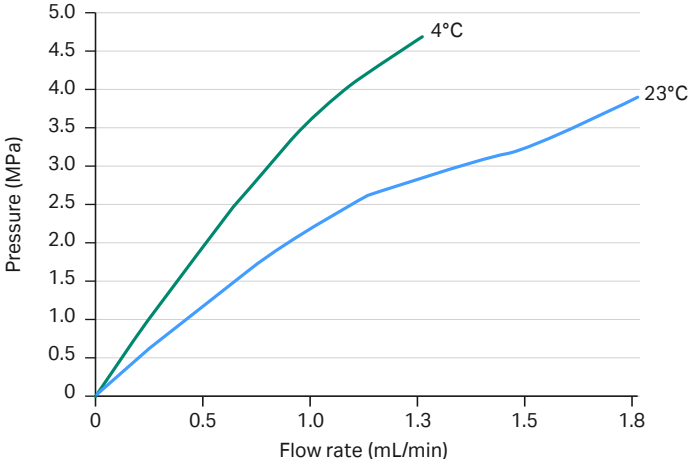

Fig 1. Column pressure at different flow rates and different temperatures on Superdex™ 200 Increase 10/300 GL column in water at two different temperatures.

Using chromatography systems at low temperature

1. Place the ÄKTA™ system into the low-temperature environment (e.g., cold room or cabinet) at least 12 hours before use. Turn on the system and allow all elements to become equilibrated to the environment.
2. When using an ÄKTA™ system where all buffers and sample have reached the desired low temperature, make sure the pressure offset are calibrated (refer to user documentation for the ÄKTA™ system being used).
3. Fittings and connections should be checked as soon as the system is moved between different temperature environments. It is important to tighten all tubing connectors as well as the inlet manifold connectors to avoid air entering the flow path. This procedure should always be performed, as the thickness of the tubing decreases when it is moved from room temperature to a lower temperature.
4. Use a low-temperature compatible computer or place the computer outside the cold room/cabinet and use the Ethernet or USB cable, delivered with the instrument, to connect to the computer.
5. To avoid overheating when the cold room/cabinet containing the ÄKTA™ system is switched off, ensure that the ÄKTA™ system is also switched off and keep the cold room/cabinet open to avoid overheating.
6. Systems such as ÄKTA™ pure have a broad flow rate range and perform well in cold environments. High-viscosity buffers needing lower flow rates (< 0.5 mL/min) can easily be used with all columns on these systems.
7. ÄKTA™ start has a lower flow rate limit of 0.5 mL/min. Running size exclusion chromatography columns at low temperature with high-viscosity buffers can be difficult if flow rates lower than 0.5 mL/min are needed to complete the purification run or to clean the column. To complete the run, it might be necessary to bring the instrument and column to room temperature. High back pressures are more likely to occur with HiPrep™ Sephacryl™ columns than with HiTrap™ columns.

Note! Chromatography equipment should remain in powered ON mode in the cold room. Turning off the equipment can result in condensation on the warm electronic parts, which in turn can result in equipment damage. The UV module of an ÄKTA™ system may be turned ON or OFF separately based on requirements.

Additional information

1. ÄKTA™ Systems Column protection, Instructions.
2. ÄKTA™ Laboratory-scale Chromatography Systems, Instrument Management Handbook.
3. ÄKTA™ pure, User Manual.
4. ÄKTA™ avant, User Manual.
5. ÄKTA™ start, Operating Instructions.
6. ÄKTA™ start, Maintenance Manual.
7. Good ÄKTA™ system practice, Cue Cards.
8. ÄKTA™ start, Maintenance Cue Card.
9. Strategies for Protein Purification, Handbook.

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