Combine chromatography techniques to optimize your protein purification

Typical purification protocols to obtain the right purity and yield

Click on the sample type you are interested in:

- Antibody purification (Page 3)
- His-tagged protein purification (Page 4)
- Untagged protein purification (Page 5)
Check list before you start

1. Define the level of purity that you need

<table>
<thead>
<tr>
<th>Typical applications</th>
<th>Purity level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass spectrometry</td>
<td>Moderate to high, 80% to 90%</td>
</tr>
<tr>
<td>Antigen for immunization</td>
<td></td>
</tr>
<tr>
<td>Functional studies</td>
<td>Very high, 95% to 99%</td>
</tr>
<tr>
<td>Structural studies</td>
<td></td>
</tr>
<tr>
<td>Structural studies</td>
<td></td>
</tr>
<tr>
<td>Therapeutic proteins</td>
<td>Highest &gt; 99%</td>
</tr>
</tbody>
</table>

2. Make sure that you have developed analytical assays to follow the progress of the purification.

3. Combine techniques in a logical way with minimized number of steps to obtain the expected purity yield balance.


Abbreviations used

AC = affinity chromatography
AIEX = anion exchange chromatography
CIEX = cation exchange chromatography
HCP = host cell proteins
HIC = hydrophobic interaction chromatography
IEX = ion exchange chromatography
IMAC = immobilized metal ion affinity chromatography
PD = Process development
SEC = size exclusion chromatography
**Antibody purification**

### Protein A or protein G for Ab purification?

**Protein G**: good first choice for general purpose capture of antibodies at laboratory scale.

**Protein A**: commonly preferred when purifying human monoclonal antibodies or developing processes for manufacturing scale applications.

### Purifying multiple types of antibodies from different sources or batches?

**HiTrap MabSelect SuRe and HiTrap MabSelect PrismA columns**: efficient cleaning with NaOH (0.5 M for MabSelect SuRe and 1.0 M for MabSelect PrismA) makes reuse of columns possible with minimal risk of cross-contamination. Antibody recovery is unaffected by the NaOH cleaning. MabSelect PrismA delivers higher protein binding capacity compared with MabSelect SuRe.

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**1-step protocol**

- Manual or system use (ÄKTA™ start, ÄKTA pure)

**2-step protocol**

- System recommended (ÄKTA start, ÄKTA pure)

**3-step protocol**

- Protocol for PD/Scale up. System recommended (ÄKTA avant)

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**AC**

**Isolation of antibodies from initial sample (e.g., serum, cell culture)**

- HiTrap™ Protein A HP, HiTrap Protein G HP, HiTrap MabSelect SuRe™, HiTrap MabSelect™ PrismA

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**CIEX (Bind/Elute mode)**

**Further removal of HCP leached protein A ligand, mAb aggregates, fragments, and other isoforms**

- HiTrap Capto™ S ImpAct, HiScreen Capto S ImpAct

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**AIEX (Flowthrough modes)**

**Final removal of remaining impurities of HCP, DNA, and viruses**

- HiTrap Capto Q, HiScreen Capto Q

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**SEC**

**Neutralization of low pH elution buffer and removing Ab aggregates and/or fragments**

- Superdex™ 200 Increase, HiLoad™ Superdex 200 pg, HiPrep™ Sephacryl™ S-300 HR

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**B1**

Buffer exchange to neutralize low pH Ab elution buffer.

(PD-10 Desalting, HiTrap Desalting columns)

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**B2**

Buffer exchange to prepare for IEX.

(HiTrap Desalting, HiPrep 26/10 Desalting columns)

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**C**

Concentration for sample volume reduction. May also be performed before SEC.

(Vivaspin™ Sample Concentrators)

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Steps in circles are optional and are applied if necessary.

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1. Superdex Increase columns are not for use with ÄKTA start. ÄKTA pure and ÄKTA avant are recommended.
His-tagged protein purification

### Imidazole concentration: it is all about the purity/yield balance

- **To increase purity**: use a high imidazole concentration (> 20 mM) in the sample and binding buffer.
- **To increase yield**: use no or a low imidazole concentration (0 to 5 mM) in the sample and binding buffer.

### Did the color of your nickel column change from green to a very light color?

Nickel stripping agents might be present in your sample. Choose HisTrap excel column, which contains a resin with strongly bound nickel ions.

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<tr>
<th>1-step protocol</th>
<th>2-step protocol</th>
<th>3-step protocol</th>
</tr>
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<tr>
<td>Manual or system use (AKTA™ start, AKTA pure)</td>
<td>System recommended (AKTA start, AKTA pure)</td>
<td>System recommended (AKTA pure, AKTA avant)</td>
</tr>
</tbody>
</table>

### IMAC

Isolation of target protein from initial sample (e.g., lysate, cell culture)
- HisTrap™ HP
- HisTrap excel
- HisTrap FF crude
- HiTrap TALON® crude

### IEX

Removal of impurities such as HCP that were co-purified in the first step
- HiTrap™ Q HP or HiTrap SP HP
- HiTrap Capto™ Q ImpRes, or
- HiTrap Capto SP ImpRes

### SEC

Final removal of salt and remaining impurities
- Superdex 75 Increase®, Superdex 75 pg,
- HiPrep Sephacryl S-100 HR,
- HiPrep Sephacryl S-200 HR

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Steps in circles are optional and are applied if necessary.

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1. Superdex Increase columns are not for use with AKTA start. AKTA pure and AKTA avant are recommended.
Untagged protein purification

**2-step protocol**
- System recommended (ÄKTA™ start, AKTA pure)

**3-step protocol option 1**
- System recommended (AKTA pure, AKTA avant)

**3-step protocol option 2**
- System recommended (AKTA pure, AKTA avant)

### IEX
- **Isolation of target protein from initial sample** (e.g., lysate, cell culture)
  - HiTrap™ Q HP or HiTrap SP HP,
  - HiTrap Capto™ Q ImpRes,
  - or HiTrap Capto SP ImpRes

### HIC
- **Isolation of target protein from initial high-salt sample**
  - HiTrap Phenyl HP,
  - HiTrap HIC Selection Kit

### SEC
- **Final removal of remaining impurities**
  - Superdex Increase columns,
  - Superose Increase columns,
  - HiLoad Superdex pg columns,
  - HiPrep Sephacryl™ columns

**Ammonium sulfate precipitation**

**Buffer exchange to prepare for IEX.**
(HiTrap Desalting, HiPrep 26/10 Desalting columns)

**Concentration for sample volume reduction.**
May also be performed before SEC.
(Vivaspin™ Sample Concentrators)

Steps in circles are optional and are applied if necessary.

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**Why use ammonium sulfate precipitation in the 3-step protocol?**
Ammonium sulfate is used for initial sample concentration and cleanup. It stabilizes proteins with no denaturation. The sample will contain a high salt concentration and may be applied directly to a HIC column with little or no additional preparation.

**What about combining IEX techniques?**
IEX is a method that offers different selectivity by using either cation (CIEX) or anion (AIEX) exchangers. A purification protocol can thus be designed to include a combination of CIEX and AIEX. The order of the IEX columns is dependent on the isoelectric point (pI) of the target protein and which contaminants you want to remove. Consider using the first column for binding and concentration of the target protein and the second column for binding of remaining contaminants (target protein flows through).

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1. Superdex Increase and Superose Increase columns are not for use with ÄKTA start. AKTA pure and AKTA avant are recommended.
Recommendation for ÄKTA system users

Use predefined methods for protocol guidance

1. **Create method**
   Select a predefined method based on application, in *Method editor* by selecting *File: New method*.

2. **Adapt method**
   Adapt method to your conditions in *Phase properties* tab by selecting:
   - Column in *Method settings* phase
   - Column position in *Method settings* phase
   - Way of fractionating in *Elution* phase

3. **Prepare ÄKTA**
   Set up ÄKTA system with column(s), sample, buffers, tubing, and components.

4. **Run method**
   - Select the created method in *System control* in the *Method navigator*.
   - Click the *Run* button to run your method.

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1 Relevant for ÄKTA pure and ÄKTA avant. For ÄKTA start, same steps apply but software terms are named differently.
More protein purification resources

Visit gelifesciences.com/ProteinResearch

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Interactive column selector
gelifesciences.com/Purify

How to videos
gelifesciences.com/ProteinVideos

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