Introduction to purification strategy and purification optimization

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Why purify proteins?

Functional Studies

Structural Studies

Diagnostics

Therapeutics

Purified Protein

Basic Research

Therapeutics
- Antibody
- Vaccine
- Antibiotic
- Hormone
- Cytokines
- Enzyme
- Inhibitors
- Test reagents
- Biomaterial

Imagination at work
The trend of Biopharmaceutical?

In 2013, there were over 300 approved biopharmaceutical drugs on the market. A quarter of these drugs were related to monoclonal antibodies (MAbs).
Content

Introduction to protein purification
purification strategy
Application examples: Antibody purification
Purification tools ÄKTA™ System
Introduction to UNICORN™ software
Purification optimization for ÄKTA™ System
Summary
Introduction to protein purification
Chromatography

Crude sample

Buffer

Column

Gel media

Collection

Monitors

UV Absorption

Time / Volume
Chromatography principle

- **Buffer**
- **Sample**
- **Medium**
- **Target protein**
- **Other molecules**

Steps:
- **Equilibrate**
- **Load Sample**
- **Wash**
- **Elute**
Protein properties that matter for protein purification

- Hydrophobicity
- Net charge
- Size
- Ion exchange chromatography (IEX)
- Affinity chromatography (AC)
- Hydrophobic interaction chromatography (HIC)
- Size exclusion chromatography (SEC) or gel filtration

Biospecific affinity (e.g., a tag)
Schematics of common chromatography techniques

- Watch AC principle video
- Watch IEX principle video
- Watch SEC (or GF) principle video
- Watch HIC principle video
The principles of chromatography techniques

- **Affinity Chromatography (AC)**
  - Bind – elute principle
  - Requires specific elution conditions
  - Concentrating effect

- **Ion exchange Chromatography (IEX)**

- **Hydrophobic interaction Chromatography (HIC)**

- **Size exclusion Chromatography (SEC)**
  - Diffusion – no binding
  - Any elution conditions
  - Diluting effect
purification strategy
Purification strategy - key elements for success

• Define the purification objectives

• Build a purification scheme with different purification steps
  Capture, intermediate purification and polishing

• Combine techniques
  Maximize separation power and minimize sample treatment

• Use relevant analytical assays
  Protein ID, purity, quantity (activity, homogeneity)
Goals: successful protein purification

• Maintained biological activity
• Sufficient purity and quantity
• Good economy
Defining the purification objectives

<table>
<thead>
<tr>
<th>Amount</th>
<th>Mass spectrometry</th>
<th>Antigen for immunization</th>
<th>Functional studies</th>
<th>Structural studies</th>
<th>Therapeutic proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg</td>
<td>ng</td>
<td>µg</td>
<td>mg</td>
<td>g</td>
<td>kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Purity</th>
<th>Moderate &gt; 80%</th>
<th>High &gt; 95-99%</th>
<th>Very high &gt; 99%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Activity</th>
<th>Often not necessary</th>
<th>Rigid requirements</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Homogeneity</th>
<th>Often not necessary</th>
<th>Rigid requirements</th>
</tr>
</thead>
</table>
Different Scales, Various equipments

BIOPROCESS SCALE

LAB SCALE

SIMPLE PURIFICATION

LAB SCALE

LOW THROUGHPUT ADVANCED PURIFICATION

INCREASED THROUGHPUT LAB SCALE

KTAprime™ plus
KTAexplorer™
KTAprocess™
KTApilot™
KTAxpress™
KTAstart™
KTApure
KTAavant

KTAprime™
KTAprocess™
KTAxpress™
KTAavant

GE
imagination at work
Purification strategy - key elements for success

• Define the purification objectives

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  Capture, intermediate purification and polishing

• Combine techniques
  Maximize separation power and minimize sample treatment

• Use relevant analytical assays
  Protein ID, purity, quantity (activity, homogeneity)
Introduction to CiPP purification strategy

Purification strategy combining multiple steps

- **Sample prep**
  - Isolate, concentrate and stabilize

- **Capture**
  - Remove bulk impurities

- **Purification**
  - Remove final contaminants

- **Polishing**

Protein recovery plotted against the number of purification steps

Yield (%) vs. Number of purification steps
Important performance factors

Resolution

Speed

Binding capacity

Recovery
CiPP: Capture

**Technique:** Affinity chromatography

**Column:** rProtein A Sepharose™ FF, XK16/20 (9.6 ml)

**Sample:** 88 mg of IgG2 in 600 ml clarified cell culture
CiPP: Intermediate purification

**Technique:** Hydrophobic interaction chromatography
**Column:** Butyl Sepharose™ 4 FF, XK16/20
**Sample:** 5 ml partially purified rec. annexin
CiPP: Polishing

**Technique:** Ion exchange chromatography

**Column:** Mono S™ (8 ml column)

**Sample:** Zap-70 kinase
Which type of chromatography resin provides the desired performance?

Objective: High resolution
Small, uniformly sized beads (e.g., 8-40 µm bead diameter).

Objective: Speed
Large, rigid and uniformly sized beads provide the highest speed (e.g., 50-100 µm, highly cross-linked agarose).

Objective: High binding capacity
Porous beads with high ligand density and directed ligand coupling.

Objective: High recovery
Recovery is mostly dependent on buffer conditions and on how peaks are cut.
## Recommendations: techniques in each CiPP step

<table>
<thead>
<tr>
<th>Affinity chromatography</th>
<th>Ion exchange chromatography</th>
<th>Hydrophobic interaction chromatography</th>
<th>Size exclusion chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate Purification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polishing</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Capture**: Star ratings for each technique
- **Intermediate Purification**: Star ratings for each technique
- **Polishing**: Star ratings for each technique

![Affinity chromatography](image)

![Ion exchange chromatography](image)

![Hydrophobic interaction chromatography](image)

![Size exclusion chromatography](image)
How to combine purification techniques

**A good separation scheme**

- Anion exchange chromatography
- Hydrophobic interaction chromatography
- Size exclusion chromatography

- Uses three different separation principles: charge, hydrophobicity and size.
- Requires little sample handling between steps.

**A poor separation scheme**

- Anion exchange chromatography (again)
- Cation exchange chromatography

- Uses only one separation principle.
- Requires extensive sample handling between steps: dialysis or buffer exchange.
Purification strategy - key elements for success

- Define the purification objectives
- Build a purification scheme with different purification steps: Capture, intermediate purification and polishing
- Combine techniques: Maximize separation power and minimize sample treatment
- Use relevant analytical assays: Protein ID, purity, quantity (activity, homogeneity)
Analytical assays to monitor purification

- **Protein identity**
  (Western blotting; mass spectrometry)

- **Purity**
  (SDS-PAGE)

- **Quantity**

- **Size-homogeneity**
  (Analytical SEC)

- **Activity**
Example: Quick size-homogeneity analysis
Aggregate analysis of protein A purified monoclonal antibodies

Column: Superdex™ 200 Increase 5/150 GL
Run time: 4 min (0.75 ml/min)
Application examples
A single capture step is sometimes sufficient

Monoclonal antibody purification

Column: HiTrap™ protein A 1 ml

His-tagged protein purification

Column: HisTrap™ FF 1 ml
Example: Purification of a tagged protein*

1. Capture: Affinity chromatography

Column: MBPTrap™ HP 5 ml
Sample: 15 ml MBP-MCAD in *E. coli* lysate, Mr ~85 500

2. Polishing: Size exclusion chromatography

Column: HiLoad™ 16/60 Superdex 200 pg
Sample: 2 ml eluted fraction from AC

*On all application examples shared in this presentation, different ÄKTA™ systems from GE Healthcare have been used for purification.
Example: Purification of an untagged protein

1. Capture: IEX

Column: HiPrep™ Q XL 16/10
Sample: 40 ml clarified E. coli extract with DAOCS

2. Intermediate purification: HIC

Column: SOURCE™ 15ISO, packed in HR column 16/10
Sample: 40 ml DAOCS pool from IEX

3. Polishing: SEC

Column: HiLoad™ 16/60 Superdex™ 75 pg
Sample: 3 ml concentrated DAOCS pool from HIC

Purity check (SDS-PAGE)
How to combine purification techniques

<table>
<thead>
<tr>
<th>Capture</th>
<th>Intermediate purification</th>
<th>Polishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>AC</td>
<td>SEC</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td>IEX</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td>SEC</td>
</tr>
<tr>
<td>IEX</td>
<td></td>
<td>IEX</td>
</tr>
<tr>
<td>HIC</td>
<td></td>
<td>SEC</td>
</tr>
</tbody>
</table>

Use orthogonal techniques and minimize sample handling

(NH₄)₂SO₄ precipitation
Application examples: Antibody purification
What is the goal of the purification?

- Purity check and functional analysis
- Importance and properties of remaining impurities

**Purity required for final application**

**Physico-chemical characteristics**
- Size
- Charge
- pI
- Stability

**Scale of purification**
- μg
- mg
- g

**Source**
- Sample preparation

**Economy**
- Time and expense
A platform approach for the purification of antibody or antibody fragments (Fabs)
## Sample preparation
### Sources and their associated contaminants

<table>
<thead>
<tr>
<th>Source: native</th>
<th>Molecular types</th>
<th>Significant contaminants</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>Polyclonal IgG, IgM, IgA, IgD, IgE</td>
<td>albumin, transferrin, (\alpha_2)-macroglobulin, other serum proteins</td>
<td>IgG 8–16 mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM 0.5–2 mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgA 1–4 mg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgE 10–400 ng/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgD up to 0.4 mg/ml</td>
</tr>
<tr>
<td>Hybridoma: cell culture supernatant with 10% foetal calf serum</td>
<td>Monoclonal</td>
<td>Phenol red, water, albumin, transferrin, bovine IgG, (\alpha_2)-macroglobulin, other serum proteins, viruses</td>
<td>Up to 1 mg/ml</td>
</tr>
<tr>
<td>Hybridoma: cell culture supernatant serum free</td>
<td>Monoclonal</td>
<td>Albumin, transferrin (often added as supplements)</td>
<td>Up to 0.05 mg/ml</td>
</tr>
<tr>
<td>Ascites fluid</td>
<td>Monoclonal</td>
<td>Lipids, albumin, transferrin, lipoproteins, endogenous IgG, other host proteins</td>
<td>1–15 mg/ml</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>IgY</td>
<td>Lipids, lipoproteins and vitellin</td>
<td>IgY 3–4 mg/ml</td>
</tr>
</tbody>
</table>

| Source: recombinant                                  | Tagged antibodies, antibody fusion proteins, Fab or F(ab\(^\prime\))\(_2\) fragments | Proteins from the host, e.g. *E. coli*. General low level of contamination | Depends upon expression system |
|                                                     | Proteins from the host, e.g. *E. coli*, phage                                         |                                                                 | Depends upon expression system |
| Intracellular protein expression                     |                                                                                       |                                                                 |                           |
What is your associated contaminants?

Common contaminants after initial purification are albumin, transferrin, DNA, immunoglobulins, antibody aggregates and leached protein A.

- Protein purity – defined by Resolution
- Amount of target protein – defined by recovery
- Ability to purify more protein if needed – defined by reproducibility
Multistep purification in two different ways

Affinity
GST  His  Ab

Gel filtration

Desalting

Size exclusion

Multistep purification in two different ways

Affinity
GST  His  Ab

Gel filtration

Desalting

Size exclusion

Multistep purification in two different ways

Affinity
GST  His  Ab

Gel filtration

Desalting

Size exclusion
Example: Aggregate removal using size exclusion
Mouse IgG₁ Purification

Affinity chromatography
HiTrap™ Protein G HP 1 ml

Size exclusion chromatography
HiLoad™ 16/60 Superdex™ 200 pg
- Removal of dimers and aggregates
- Buffer exchange

Low pH

Remember: aggregates are invisible in reduced SDS-PAGE. The aggregates are found in the small peak that appears immediately before the main peak.
Screening of pH and ion strength conditions for optimal homogeneity and stability of a detergent-protein complex. Chromatogram A–F represent the results from the different screening conditions.
New generation Size Exclusion Chromatography (SEC) columns - Increase

**Superdex 30 Increase**
- Peptides and other small biomolecules
- Fractionation range $M_r \sim 100$ to 7000

**Superdex 75 Increase**
- Recombinant tagged proteins
- Fractionation range $M_r \sim 3000$ to 70000

**Superdex 200 Increase**
- mAb and other antibodies
- Fractionation range $M_r \sim 10000$ to 600000

**Superose™ 6 Increase**
- Larger proteins and protein complexes†
- Fractionation range $M_r \sim 5000$ to 5 000 000

Resolution (Rs) data

<table>
<thead>
<tr>
<th>Peak</th>
<th>Superdex Peptide</th>
<th>Superdex 30 Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>2.19</td>
<td>3.41</td>
</tr>
<tr>
<td>2-3</td>
<td>3.24</td>
<td>4.84</td>
</tr>
<tr>
<td>3-4</td>
<td>3.88</td>
<td>4.94</td>
</tr>
<tr>
<td>4-5</td>
<td>1.33</td>
<td>1.82</td>
</tr>
<tr>
<td>5-6</td>
<td>1.16</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Flow rate: 0.5 mL/min
Example: Application: Endotoxins, DNA Cleaning

LPS Molecule

- O-specific polysaccharide chain
- Core glycolipid
- O-specific oligosaccharide subunit
- Core oligosaccharide

10~20 KD ~ 4 × 105 KD
< 0.25 EU/ml
Remove associated contaminants

Two-step process

1st
Protein A/G or Mabselect

2nd
SEC or AIEX

Alternative two-step process

1st
MabSelect SuRe
MabSelect SuRe LX

2nd
Capto adhere (FT)
Capto adhere ImpRes (FT)

Efficient clearance of aggregates, HCP, DNA, protein A, and viruses; high yield

Three-step process

1st
Protein A/G or Mabselect

2nd
HIC or CIEX

3rd
AIEX

Alternative three-step process

1st
MabSelect SuRe
MabSelect SuRe LX

2nd
Capto S ImpAct (B/E)

3rd
Capto Q (FT)

For increased resolution, removal of more challenging aggregate levels, fragments, and other MAb isoforms

For more challenging purifications
Example: Application: Mix model gel

- aggregates
- DNA, viruses
- HCP
- endotoxin

**Capto adhere**

```
OH
O

N^+ OH
```

**Diagram:***

- Cell culture
- Cell removal
- MabSelect SuRe
  - MabSelect SuRe LX
  - Virus inactivation and filtration
    - Capto SP ImpRes
    - Capto S
    - Capto Q
    - Capto Q, Capto adhere, Capto Q ImpRes
    - Capto adhere
  - Virus filtration and final formulation

**Column:** Superdex 200 10/300
**Sample:** Flowthrough fraction (red) and eluate (blue) from the Capto adhere step
**Sample load:** 50 µL each
**Loading buffer:** 0.01 M sodium phosphate, 2.7 mM potassium phosphate, 137 mM sodium chloride, pH 7.4
**Flow rate:** 0.5 mL/min
**System:** ÄKTA chromatography system
How to choose media and column
SEC: Important performance factors

- Particle size
- Flow rates
- Column efficiency
- Sample volume
- Separation range
How to choose media and column - GF

- Superdex Peptide
- Superdex 75
- Superdex 200
- Superdex 30 prep grade
- Superdex 75 prep grade
- Superdex 200 prep grade
- Superose 6
- Superose 12
- Superose 6 prep grade
- Superose 12 prep grade
- Sephacryl S-100 HR
- Sephacryl S-200 HR
- Sephacryl S-300 HR
- Sephacryl S-400 HR
- Sephacryl S-500 HR
- Sephacryl S-1000 SF

High resolution

Log scales: $10^2$ to $10^8$
**Sample:**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>(M_r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgM</td>
<td>970,000</td>
</tr>
<tr>
<td>2</td>
<td>Thyroglobulin</td>
<td>669,000</td>
</tr>
<tr>
<td>3</td>
<td>Ferritin</td>
<td>440,000</td>
</tr>
<tr>
<td>4</td>
<td>BSA</td>
<td>66,000</td>
</tr>
<tr>
<td>5</td>
<td>Myoglobin</td>
<td>17,600</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin B</td>
<td>1,300</td>
</tr>
</tbody>
</table>

---

**Superdex 200 Increase 10/300 GL**

**Superose 6 Increase 10/300 GL**
New generation Size Exclusion Chromatography (SEC) columns - Increase

Superdex 30 Increase
Peptides and other small biomolecules
Fractionation range $M_r \sim 100$ to 7000

Superdex 200 Increase
mAb and other antibodies
Fractionation range $M_r \sim 10000$ to 600000

Superdex 75 Increase
Recombinant tagged proteins
Fractionation range $M_r \sim 3000$ to 70000

Superose™ 6 Increase
Larger proteins and protein complexes‡
Fractionation range $M_r \sim 5000$ to 5000000

Resolution (Rs) data

<table>
<thead>
<tr>
<th></th>
<th>Superdex Peptide 10/300 GL</th>
<th>Superdex 30 Increase 10/300 GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1-2</td>
<td>2.19</td>
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<tr>
<td>Peak 4-5</td>
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<td>1.82</td>
</tr>
<tr>
<td>Peak 5-6</td>
<td>1.16</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Flow rate: 0.5 mL/min

Superdex Peptide 10/300 GL
60 min (0.4 mL/min)

Superdex Peptide 10/300 GL
20 min (1.2 mL/min)
How to choose media and column - GF

Superdex™ Peptide column: 10/30_24ml

Desalting and buff exchange  25% CV
PD-10 (gravity) loading capacity 2.5 ml
HiTrap™ 5 ml loading capacity 1.5 ml
HiPrep™ 53 ml loading capacity 15 ml

Polishing–Fractionation  0.5% ~ 5% CV
µl – analytical column  1% CV
- 10/300 loading capacity 250 µl
-  5/150 loading capacity 50 µl

ml – preparative column  5% CV
- 16/60 loading capacity 5 ml
- 26/60 loading capacity 13 ml
## How to choose media and column - column

<table>
<thead>
<tr>
<th>Description</th>
<th>Fractionation Range (kD)</th>
<th>Bed volume (ml)</th>
<th>Particle size</th>
<th>Sample volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superdex Peptide 10/300 GL</td>
<td>0.1~7</td>
<td></td>
<td>13</td>
<td>25~250 ul</td>
</tr>
<tr>
<td>Superdex 75 10/300 GL</td>
<td>3~70</td>
<td>24</td>
<td>13</td>
<td>25~250 ul</td>
</tr>
<tr>
<td>Superdex 200 10/300 GL</td>
<td>10~600</td>
<td></td>
<td>13</td>
<td>25~250 ul</td>
</tr>
<tr>
<td>HiLoad 16/60 Superdex 30 pg</td>
<td>up to 10</td>
<td></td>
<td>34</td>
<td>≤ 5 ml</td>
</tr>
<tr>
<td>HiLoad 16/60 Superdex 75 pg</td>
<td>3~70</td>
<td>120</td>
<td>34</td>
<td>≤ 5 ml</td>
</tr>
<tr>
<td>HiLoad 16/60 Superdex 200 pg</td>
<td>10~600</td>
<td></td>
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<td>up to 10</td>
<td></td>
<td>34</td>
<td>≤ 13 ml</td>
</tr>
<tr>
<td>HiLoad 26/60 Superdex 75 pg</td>
<td>3~70</td>
<td>240</td>
<td>34</td>
<td>≤ 13 ml</td>
</tr>
<tr>
<td>HiLoad 26/60 Superdex 200 pg</td>
<td>10~600</td>
<td></td>
<td>34</td>
<td>≤ 13 ml</td>
</tr>
<tr>
<td>HiPrep 16/60 Sephacryl 100</td>
<td>1~100</td>
<td></td>
<td>50</td>
<td>≤ 5 ml</td>
</tr>
<tr>
<td>HiPrep 16/60 Sephacryl 200</td>
<td>5~250</td>
<td>120</td>
<td>50</td>
<td>≤ 5 ml</td>
</tr>
<tr>
<td>HiPrep 16/60 Sephacryl 300</td>
<td>10~1500</td>
<td></td>
<td>50</td>
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<td></td>
<td>50</td>
<td>≤ 13 ml</td>
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<td>10~1500</td>
<td></td>
<td>50</td>
<td>≤ 13 ml</td>
</tr>
</tbody>
</table>
How to choose media and column - IEX

Titration curves

- Attached to cation exchangers
- Attached to anion exchangers

Isoelectric point

Range of stability
How to choose media and column - IEX

Matrix (particle size)
- MiniBeads (3)
- MonoBeads (10)
- Source 15 (15)
- Source 30 (30)
- Sepharose High Performance (34)
- Capto™ ImpRes (40)
- Capto™ ImpAct (40)
- Sepharose Fast Flow (90)
- Capto (90)

Resolution increases

Anion exchanger
- Q (strong)
- ANX (weak)
- DEAE (weak)

Cation exchanger
- SP (strong)
- CM (weak)
How to choose media and column - IEX

**strong ion exchangers:** capacity is constant over a wide range of pH

- Q Sepharose™ Fast Flow
- SP Sepharose Fast Flow
- DEAE Sepharose Fast Flow
- CM Sepharose Fast Flow

**weak ion exchangers:** capacity varies with pH
Use HiTrap selection kit to Quick screening

- HiTrap AR20 FF (High Subl) 1 ml
- HiTrap Q XL, 1 ml
- HiTrap Q FF, 1 ml
- HiTrap DEAE FF, 1 ml
- HiTrap SP XL, 1 ml
- HiTrap SP FF, 1 ml
- HiTrap CM FF, 1 ml

HiTrap IEX selection kit 7 x 1 ml
HiTrap Capto IEX selection kit 5 x 1 ml
HiTrap HIC selection kit 7 x 1 ml
Purification tools
 ÄKTA™ System
Purification tools

Manual purification

Automated purification
Comparison: manual vs LC system purification

**Manual purification**

- Little training or start-up time required.
- Easy to do parallel runs for increased throughput.

**LC system purification**

*e.g. ÄKTA™ instruments*

- Automation – Free up time
- Gradient elution – high resolution
- Reproducibility – Minimize human error
- Documentation
ÄKTA system purification

**ÄKTA™ AKTApure 25**
- 23.6 ml column

**ÄKTA™ process**
- 9.4 L column

**Linear scale-up in the same AKTA platform**

10 mm column diameter

200 mm column diameter

400x scale up
Proven technology
Continues ÄKTA™ tradition

• Incorporates over 50 years of experience in protein research
• Backed by 30 years of experience in developing protein purification systems
• ÄKTA systems are used by 100,000 researchers globally

1959—Sephadex™, the world’s first gel filtration medium
1982—FPLC™ system released, predecessor to ÄKTA systems
2012—ÄKTA pure: protein purification your way
1997 - ÄKTApurifier™, single platform for all chromatography techniques
ÄKTA Development from 1996 to 2017
The Market Leader

1996 - ÄKTAmicro™
1997 - ÄKTApurifier™
1998 - ÄKTA FPLC™
1999 - ÄKTApurifier™
2000 - ÄKTA OligoPilot
2001 - ÄKTA add on’s
2002 - ÄKTA Crystal and ÄKTApilot™
2004 - ÄKTA Express™
2005 - ÄKTAcrossflow™
2006 - New ÄKTApurifier
2009 - ÄKTAmicro™
2012 - ÄKTAPure
2013 - ÄKTA Start
2014 - ÄKTA Pure

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UNICORN 5
UNICORN 7
ÄKTA prime plus
ÄKTA™ Pure

New ÄKTA platform

Co-promoting solution with software and column packages

• UV wavelength: 280 / 190-700 nm

ÄKTA™ Pure

Flow rate (ml/min) 0.001-25
Max. pressure/MPa 20

• Fraction collect
Max: 576 fractions

Classicism System

ÄKTAprime™ plus
ÄKTApurifier™ UPC
ÄKTApurifier™
ÄKTAexplorer™
ÄKTA™
ÄKTAFPLC™

2018/7/16
New Generation of ÄKTA pure

Flexible
Intuitive
Reliable

Intuitive viewing and new interactive process picture

Valve options to simplify basic tasks or automate extensively

Choose your scale: High precision pumps that go to 25 ml/min or 150 ml/min

Triple- or single-wavelength UV monitors

• High-quality, prepacked columns to ensure reproducibility

Modular design

Flexible design
Module options

**Core modules**
- System pump P9 A
- System pump P9 B
- System pressure monitor R9
- Mixer M9
- Injection valve V9-Inj
- (ICU I9n – internal module)
- (ICP B9 – internal module)

**Optional modules**
- Inlet valve V9-IAB
- Inlet valve V9-IA
- Inlet valve V9-IB
- Inlet valve X1
- Inlet valve X2
- Mixer valve V9-M
- Column valve V9-Cs
- Column valve V9-C
- pH valve V9-pH
- Outlet valve V9-Os
- Outlet valve V9-O
- Versatile valve V9-V
- Versatile valve V9-V, 2nd
- UV monitor U9-M
- UV monitor U9-L
- UV monitor U9-L, 2nd
- Cond. monitor C9n

**Optional modules (external)**
- Air sensor L9, 1
- Air sensor L9, 2
- Air sensor L9, 3
- Air sensor L9, 4
- I/O-box E9
- I/O-box E9, 2nd
- Fraction collector F9-R
- Fraction collector F9-R, 2nd

**Module naming**

```
V9-X
```

- Optional sub-type
- “9” = New ÄKTA™ platform
  (”900” = classic ÄKTA)

Letter to identify module type

Improved vs ÄKTApurifier™
Flexibility to match your applications
Introduction to UNICORN™ software
Fast method setup

Predefined methods

✓ Drag-and-drop programming
Conditional programming
adopt run according to actual experimental conditions

Equilibrate based on monitor input

Watch functions: flexible control options
Software
– default System Control view

Run data Default
System flow
PreC pressure
UV
Cond

Curves Default
UV
Cond
Conc B
PreC pressure
Fraction
Injection
Run Log

Process picture

Active flow path green
Purification optimization for ÄKTA™ System
Repeatability of automation method tested on initial set up using ion exchange (IEX) step followed by desalting step

Flow rate accuracy ±1.2 %
MAb purification example

The process steps

1. Flow Through Polishing
   - Capto™ Q/ Capto adhere

2. Capture (sample loading)
   - MabSelect SuRe™ LX

3. Polishing
   - Capto S ImpAct

4. Capture (non-load)
   - MabSelect SuRe™ LX

All process steps supported in 16 mm columns on ÄKTA pure 25

Capture, polishing and capture non-load steps supported in 50 mm columns on ÄKTA pure 150
Maximize flow rates and protect your columns

Save time on your runs
Pressure sensors within the column valve
Flow control based on precolumn and delta pressure
Advanced continuous control algorithm
Connect up to 5* columns

* Installing optional extra column valves enables simultaneous use of up to 10 columns.

PID control
Cycle time variable loading - Improving productivity

80% of QB10 was loaded for each residence time on MabSelect SuRe™ LX DoE study performed to optimize each residence time.

Maintained recoveries and impurity profile:
- Pool volumes: 1.5 CV
- Recoveries: > 95%
- Aggregates: 1%
- HCP: ~ 1000ppm
- Ligand leaching: 3 to 7 ppm
Cycle time normal loading Vs variable loading strategy

40% productivity increase. Maintained capacity and performance.
ÄKTA system: Linear gradient elution is an efficient approach for obtaining high purity.

The linear gradient in this example was easily created by using an ÄKTA™ chromatography system.
ÄKTA system: Different elution conditions can be used

Total 26 CV

Total 10 CV
SEC: Narrow system tubing and slow flow rate improves resolution

Tubing diameter 0.15 mm 0.25 mm 0.50 mm 0.75 mm

Column: Superdex™ 200 5/150 GL
System: ÄKTA pure 25
SEC: Analysis for pooling decisions

Step 1: Purification step (preparative SEC)

Step 2: Identification of fractions to pool using SEC

Column: Superdex™ 75 Increase 5/150 GL
System: ÄKTA™ pure
Multistep purification in two different ways

Two-step process

Protein A/G or Mabselect

Polishing

Method queue outline

Method 1: Affinity and peak elution to loop(s)

Intermediate storage in loop(s)

Method 2: Desalting or Gel filtration/Size exclusion

Loop configuration

Tandem configuration

Method queue outline

Method 1: Affinity and peak elution to column two

Method 2: Desalting or Gel filtration/Size exclusion
**ÄKTA™ pure applications**

**Tandem MAb purification and neutralization (buffer exchange)**

<table>
<thead>
<tr>
<th>Eluents:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) PBS (10 mM sodium phosphate, 140 mM NaCl, 2.7 KCl, pH 7.4);</td>
<td></td>
</tr>
<tr>
<td>(B) 50 mM citrate, pH 6.0;</td>
<td></td>
</tr>
<tr>
<td>(C) 50 mM citrate, pH 3.5);</td>
<td></td>
</tr>
<tr>
<td>(D) 100 mM NaOH</td>
<td></td>
</tr>
</tbody>
</table>

**Flow:** 1 ml/min and 5 ml/min

**Elution method:** pH step gradient; pH 6.0 and pH 3.5

**UV cell:** 2 mm (both U9-M and U9-L)

**System configuration:** 0.5-mm tubing, UV-monitors U9-M and U9-L, V9-pH (pH/flow restrictor in use).

- **Elution AC:** pH 3.5
  - Watch: Flow through DS and 2nd UV
  - Elution DS: pH 7.4
  - Flow through DS and 2nd UV, bypass AC column

- **Elution DS:** pH 7.4
  - Watch: Flow through DS and 2nd UV

**HiTrap™ MabSelet SuRe™, 1ml**

2 x HiTrap™ Desalting
Summary
Summary

• The purification objectives & the target protein properties
  define the purity requirements and the selection of tools and methods

• The strategy Capture, intermediate Purification and Polishing (CiPP)
  provides a proven framework for developing the purification scheme

• Tools
  Manual purification, LC system purification, analytical

• Keep it simple!
Do you want to know more?
Handbooks from GE Healthcare

For guidance on choosing the right chromatography column, download the Purify App – www.gelifesciences.com/Purify
Protein aggregation

• Low PH
• Stable size homogeneity (PH、Salt)
• Additive
Thank you