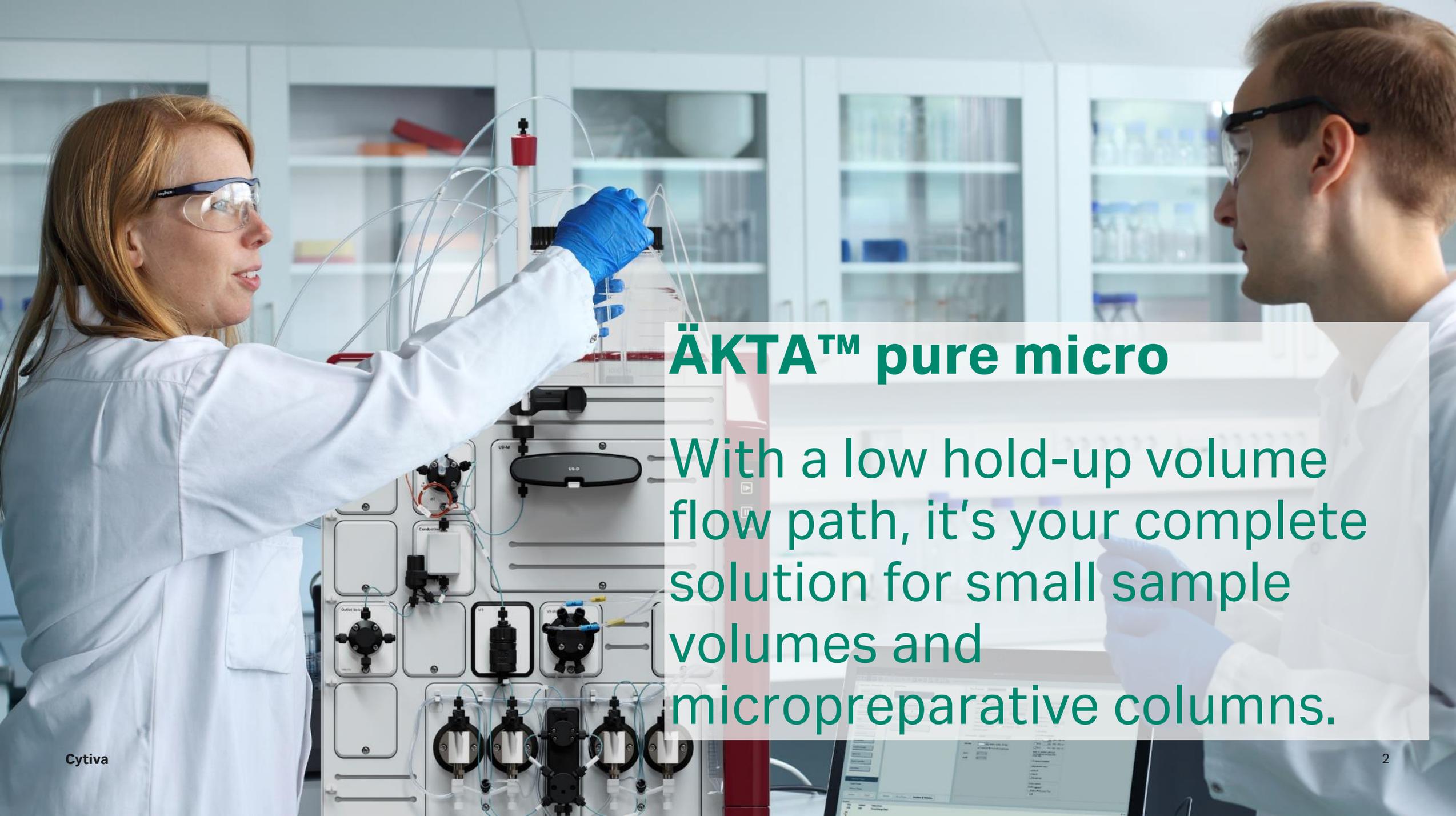




ÄKTA™ pure micro

Enabling small sample volumes and
micropreparative columns





ÄKTA™ pure micro

With a low hold-up volume flow path, it's your complete solution for small sample volumes and micropreparative columns.

1

Introduction

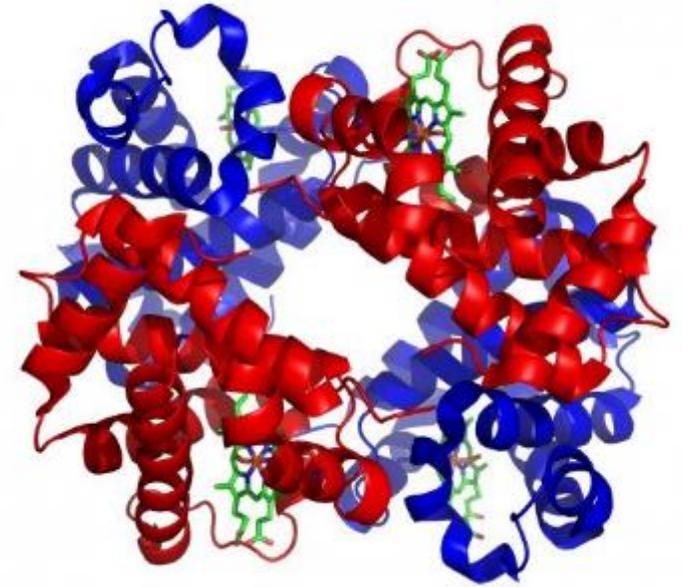
Knowing the 3D structure is essential to understanding the function of a protein

In recent years, technical development has led to cryo-EM becoming the preferred technique in structural biology.

Cryo-EM enables structural determination of difficult targets and captures the native state of the molecules.

Top challenges when purifying proteins for cryo-EM experiments include:

- Limited sample volume in microliter (μL) scale.
- The inability of the chromatography system to handle limited sample volumes.



Hemoglobin

What is required for a successful cryo-EM experiment?

- Only a few microliters (μL) of sample required
- Sample concentration in the range of 0.1 to 5 mg/mL
- Compositional and conformational homogeneity
- Modification/ treatment for increased stability (complexes, membrane proteins)

Ensuring sample quality puts high demand on protein characterization and purification.



Single particle reconstruction of the intramembrane protease γ -secretase at 3.4Å (nm) resolution.

2

Introducing ÄKTA™ pure micro

ÄKTA™ lab-scale systems: added functionality to ÄKTA™ pure supporting microscale purification

ÄKTA™ start

- Transition from manual to automated purification
- Educational tool
- Affordable and easy-to-use



ÄKTA™ go

- Achieve desired purity with ease
- Routine purifications
- Make the most of valuable bench/cold room space
- Quick method creation



ÄKTA™ pure

- Flexible to match current and future purification challenges
- Advanced automation setups
- Automated multistep
- Microscale purification



ÄKTA™ avant

- Productivity in PD
- Secure
- Scale-up



ÄKTA™ pure micro

- **High performance** for increased resolution with minimized internal volumes
- Allows for **fraction collection in small droplets**
- Together with UNICORN™ 7 software and fraction collector F9-T provides a **complete solution for micropreparative purification**
- Equip your ÄKTA™ pure 25M for micropreparative runs using our **Micro kit***

*Requires update to UNICORN™ 7.6 or higher and ÄKTA™ pure 25 Instrument Configuration version 2.0 or higher.



ÄKTA™ pure micro with optimized flow path for high performance microscale purification

Tubing kit

Minimize sample dilution and maintain peak resolution with low-volume tubing kit adapted for column size used.

Micro injection valve

Minimize sample dilution between injection valve and column inlet to ensures SEC¹ resolution.

Micro outlet valve

Minimize sample dilution after column for less peak broadening

Maintain peak resolution between outlet and fraction collector.

Column clamp

Hold SEC¹ column mounted directly on UV for minimized system volume

UV microflow cell

Minimize sample dilution after column and gives less peak broadening.

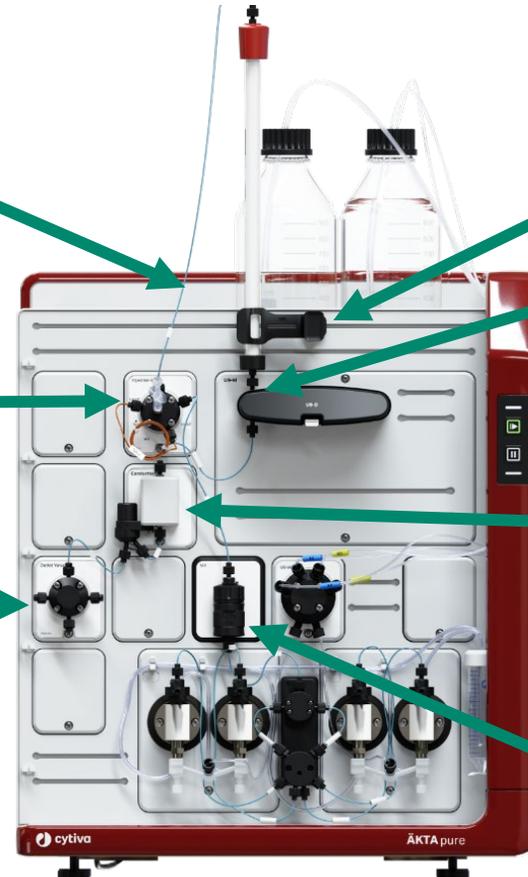
Micro conductivity monitor

Minimize sample dilution after column and gives less peak broadening.

Keep track of salt peak in SEC¹ and gradient formation for IEX²

Mixer chamber

Optimizes gradient for IEX²



¹SEC = size exclusion chromatography. ²IEX = ion exchange chromatography

Fraction collector F9-T for micropreparative purification



- Recommended fraction collector for ÄKTA™ pure micro
- Dual plate fraction collector
 - Microtiter plates
 - Microplate holder
- Micro nozzle creates small drops
- Drop sync for spillage free fractionation
- UNICORN™ 7.6 (or later version) required



Columns, fraction collector, software, service for microscale purification

Cytiva columns for cryo-EM applications:

| Technique | Prepacked columns | Dimensions |
|-----------|--------------------|------------------------|
| SEC | Superdex™ Increase | 10/300, 5/150, 3.2/300 |
| IEX | Capto™ HiRes | 5/50 |

Resins with bead size down to 5 μm^1 can be used.

ALIAS™ Bio autosampler, to handle multiple samples, down to 1 μL .

UNICORN™ 7 software, designed to quickly get started using pre-defined methods for microscale purification.

Service offering will be available for installation.

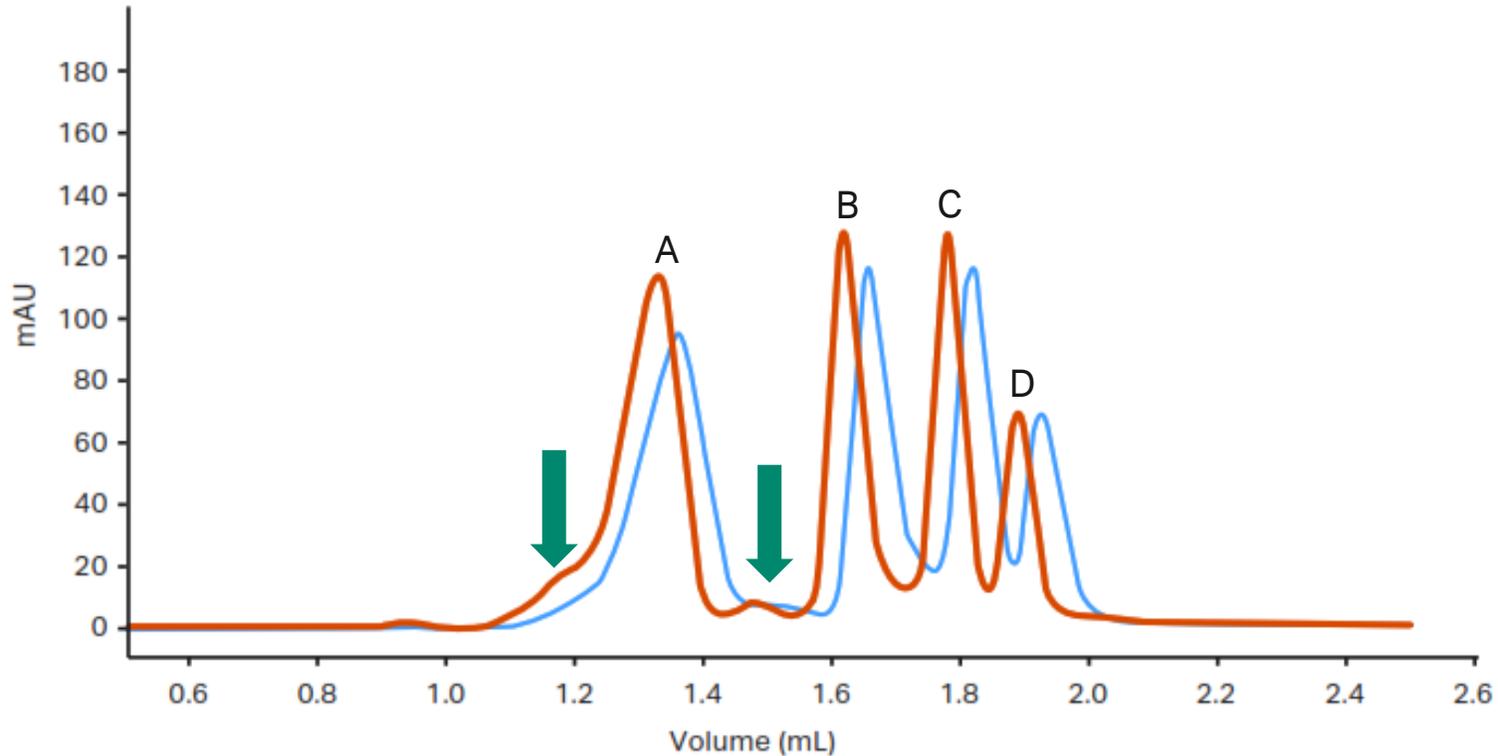
¹ For long columns, back pressure may cause problems and require decreased flow rate.



3

Performance

ÄKTA™ pure micro for improved resolution and sharper peaks



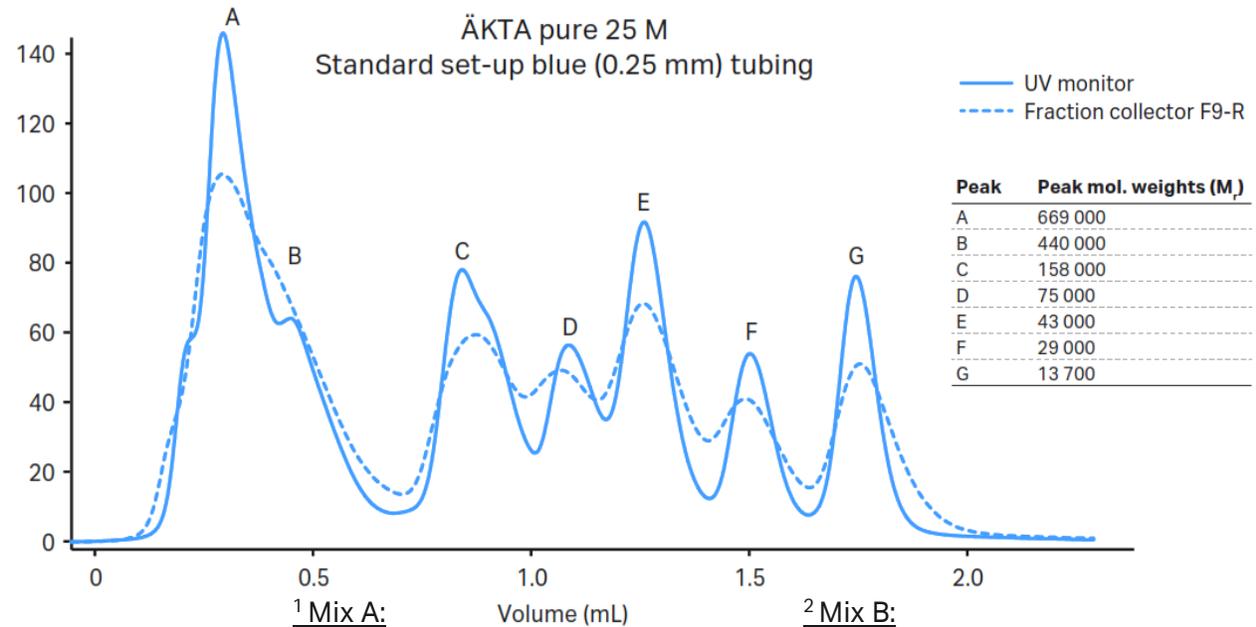
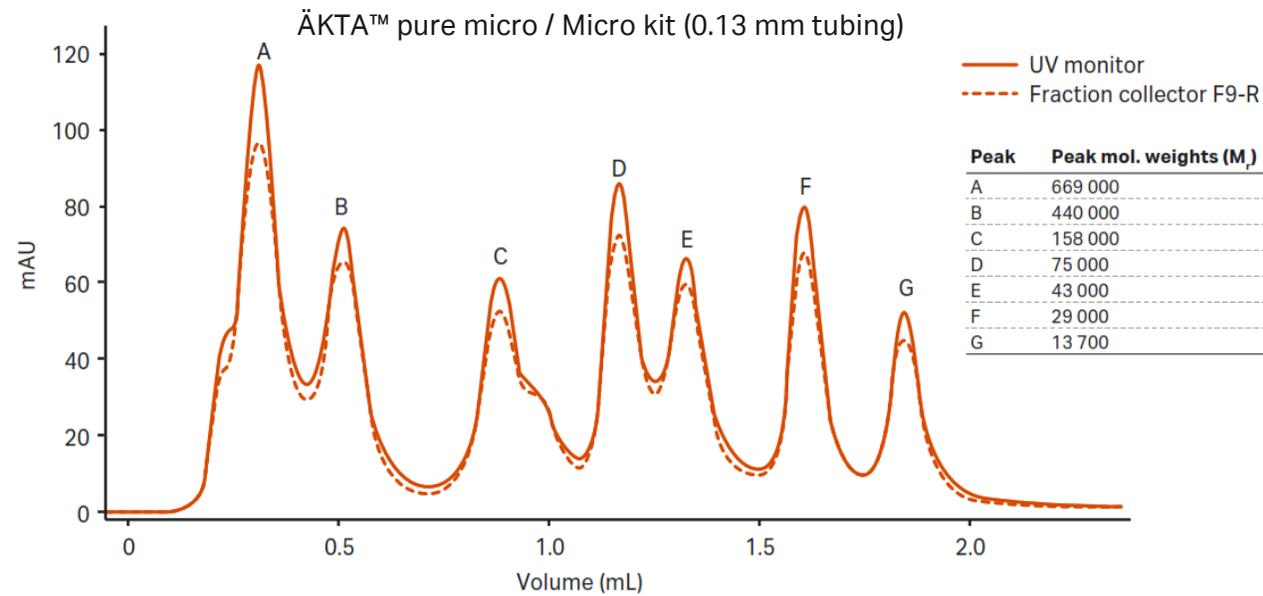
Superdex™ 200 Increase 3.2/300 column

| Peak* | Peak volume at half height (mL) | |
|--------|---------------------------------|-----------------------------|
| | Standard ÄKTA™ pure | ÄKTA™ pure micro/ Micro kit |
| Peak A | 0.113 | 0.098 |
| Peak B | 0.063 | 0.054 |
| Peak C | 0.059 | 0.047 |
| Peak D | 0.054 | 0.049 |

| Peak* | Resolution | |
|--------|---------------------|-----------------------------|
| | Standard ÄKTA™ pure | ÄKTA™ pure micro/ Micro kit |
| Peak A | | |
| Peak B | 1.97 | 2.23 |
| Peak C | 1.55 | 1.87 |
| Peak D | 1.13 | 1.35 |

*Peak A: Ferritin; Peak B: Conalbumin; Peak C: Carbonic anhydrase; Peak D: RNase

Comparing peak resolution at column outlet and fraction collector shows maintained resolution



Overlay Mix A¹ and Mix B² on Superdex™ 200 increase 3.2/300

¹Mix A:

0.3 mg/mL ferritin
3 mg/mL conalbumin
3 mg/mL carbonic anhydrase

²Mix B:

5 mg/mL thyroglobulin
4 mg/mL aldolase
4 mg/mL ovalbumin
3 mg/mL ribonuclease

ÄKTA™ pure micro supports cryo-EM purifications

Standard ÄKTA™ pure:

For larger samples, revert to standard ÄKTA™ pure 25.

ÄKTA™ pure micro :

- Supports IEX runs on Capto™ HiRes 1 mL columns at microliter (μL) scale.
- SEC on Superdex™ Increase 3.2/300 columns at microliter scale.
- Simply change tubing after column valve outlet to fraction collector and run on the same system configuration.

Initial sample

Affinity chromatography of tagged proteins



Ion exchange chromatography (optional)



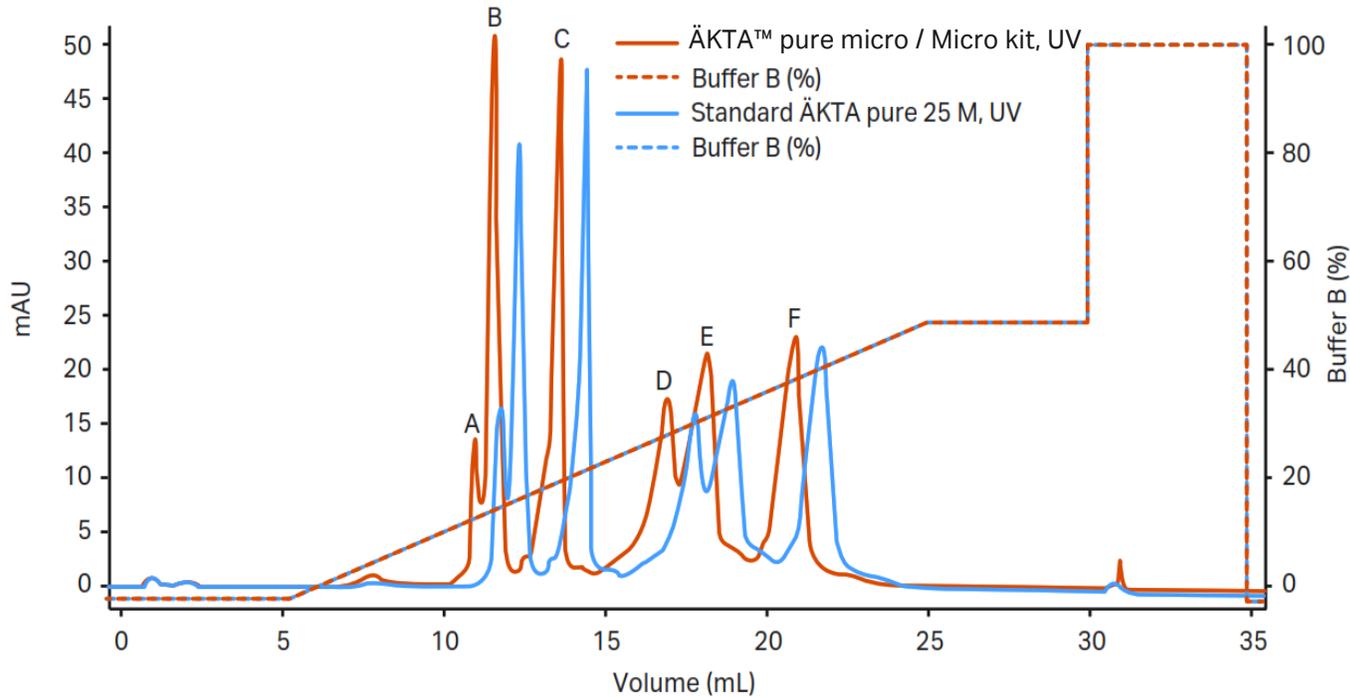
Size exclusion chromatography

Sample volume



Peak resolution – AEX (Capto™ HiRes Q 5/50)

- ÄKTA™ pure micro system works with 1 mL Capto™ HiRes columns.
- Use the blue tubing kit to minimize system back pressure.



- A. apo-Transferrin
- B. apo-Transferrin
- C. α -Lactalbumin
- D. β -Lactoglobulin
- E. β -Lactoglobulin
- F. Amyloglucosidase

| Peak | Peak volume at half height (mL) | |
|--------|--|-----------------------------|
| | Standard ÄKTA™ pure with blue (0.25 mm) tubing | ÄKTA™ pure micro/ Micro kit |
| Peak A | 0.221 | 0.177 |
| Peak B | 0.278 | 0.242 |
| Peak C | 0.222 | 0.221 |
| Peak D | 0.559 | 0.537 |
| Peak E | 1.015 | 0.929 |
| Peak F | 0.837 | 0.831 |

ÄKTA™ pure micro for small sample volumes and micropreparative columns



- Addresses protein purification challenges for cryo-EM samples
- High performance for increased resolution with minimized internal volumes
- Combine with fraction collector F9-T for collection in microtiter plates
- Equip your ÄKTA™ pure 25M for micropreparative runs using our Micro kit



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