Monoclonal Antibody Production:
Building the Platform

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CONSULTING • DEVELOPING • MANUFACTURING

Questions

Questions are encouraged throughout the presentation and can be asked by using the email address provided within your webcast viewer.

Eden Biodesign

"Designing and developing valuable biopharmaceutical medicines by the application of good science from day one"

DESIGN • DEVELOP • DELIVER

Eden Biodesign

- **CMO offering:**
  - Expression system development
  - Process and analytical development
  - Cell banking
  - cGMP production services
- **Consultancy services in CMC issues, regulatory support, cGMP training, technical trouble shooting and clinical trial supply logistics**
- **World class microbial, mammalian and viral process development and cGMP facilities located in Liverpool, UK**
- **US subsidiary located in Research Triangle Park, NC**

Presentation overview

- Challenges facing antibody manufacturers
- An overview of Antibody purification
- Case study into the development of a purification methodology for the clinical production of an IgG_4 monoclonal antibody

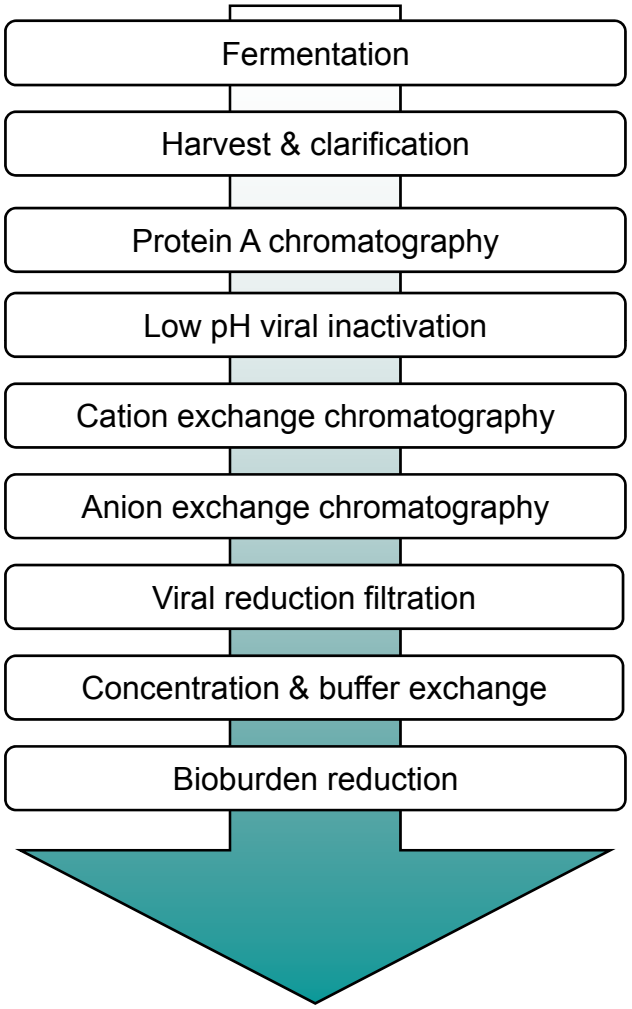
Challenges in Antibody Production

- **High dosing requirements:**
  - Large amounts of product required
  - Continuity of supply
- **Increasing titres:**
  - Typical: 2 – 5 g/L
  - Column and resin limitations
- **Low viability on harvest:**
  - Clarification issues
  - Increased contaminant levels

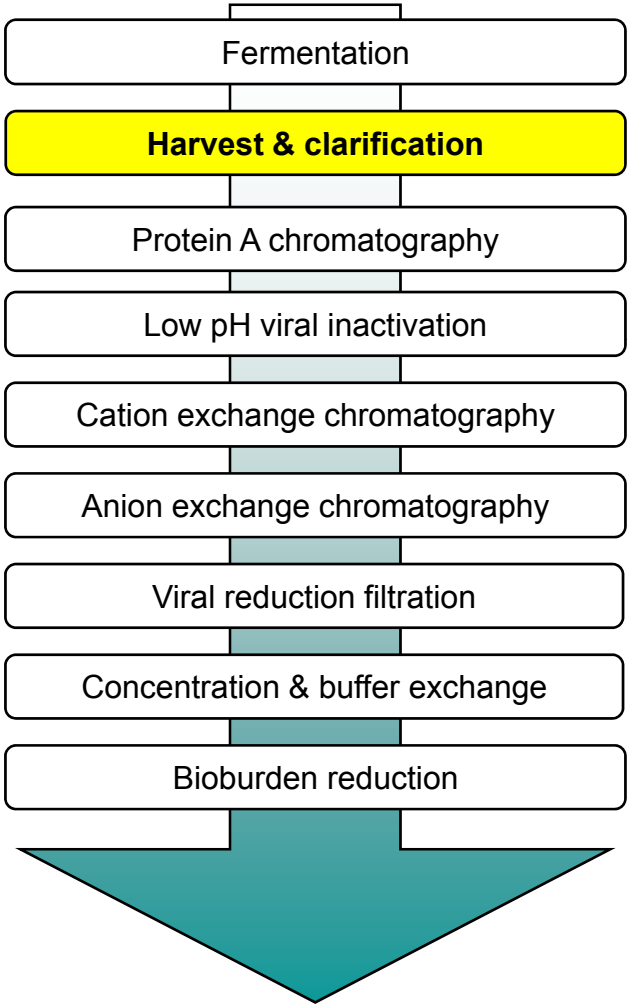
Challenges in Antibody Production

- **High throughput processing:**
  - Increased capacity resin
  - High flow rates
- **Disposable technology:**
  - Pre-packed columns
  - Disposable membrane adsorbtion technology
- **Other options:**
  - Cation exchange primary capture
  - Simulated moving bed
  - Two phase extraction

Typical mAb Purification Process



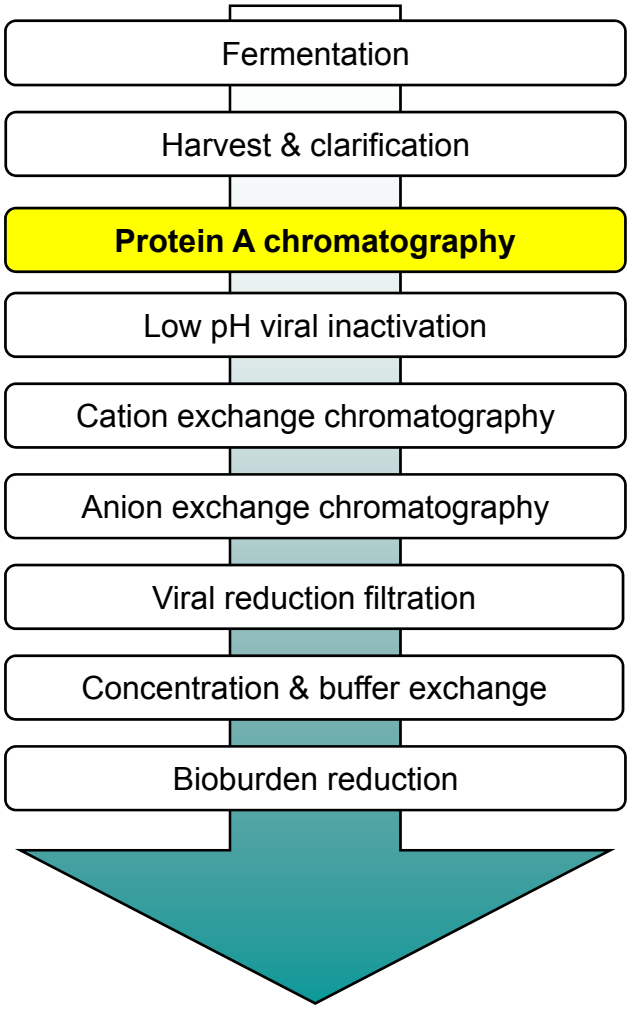
Harvest & Clarification



Harvest & Clarification

- **Direct depth filtration**
  - Ideally suited for volumes (< 1000 L)
  - Disposable options
- **Centrifugation → Depth filtration**
  - Most practical for large volumes (> 1000 L)
  - High shear
  - Difficult to develop and control
  - Equipment and cleaning validation requirements
  - High operating costs
- **Microfiltration**
  - Suited at all scales
  - Equipment and cleaning validation requirements
  - High buffer and utility consumption

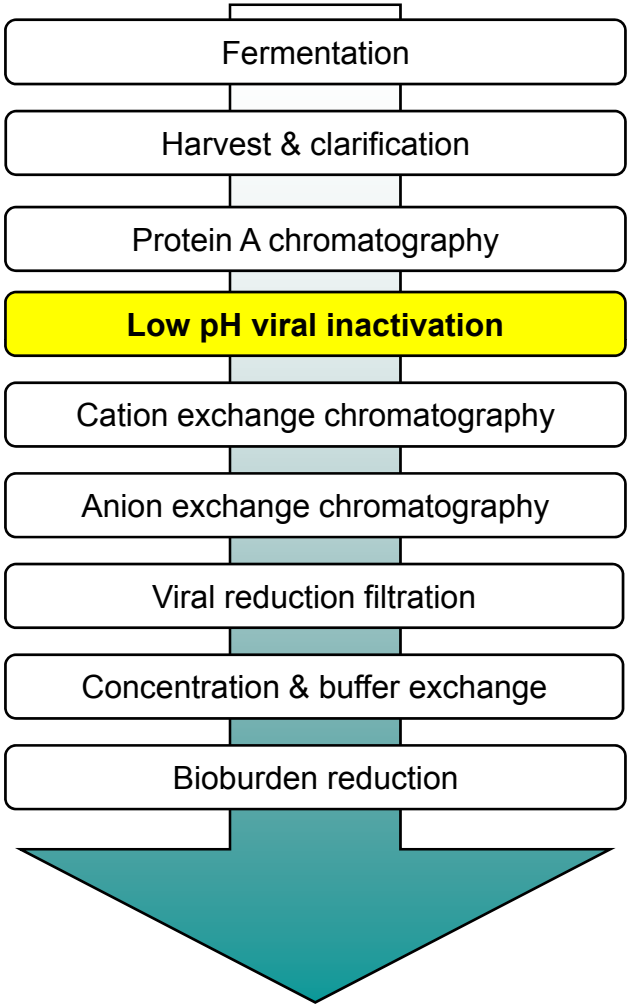
Protein A Chromatography



Protein A Chromatography

- **Industry standard**
- **Robust**
- **Efficient**
  - High purity in singe step
  - Volume reduction
- **High initial capital outlay**
- **Column limitations**
  - Binding capacity
  - Hardware

Low pH Viral Inactivation

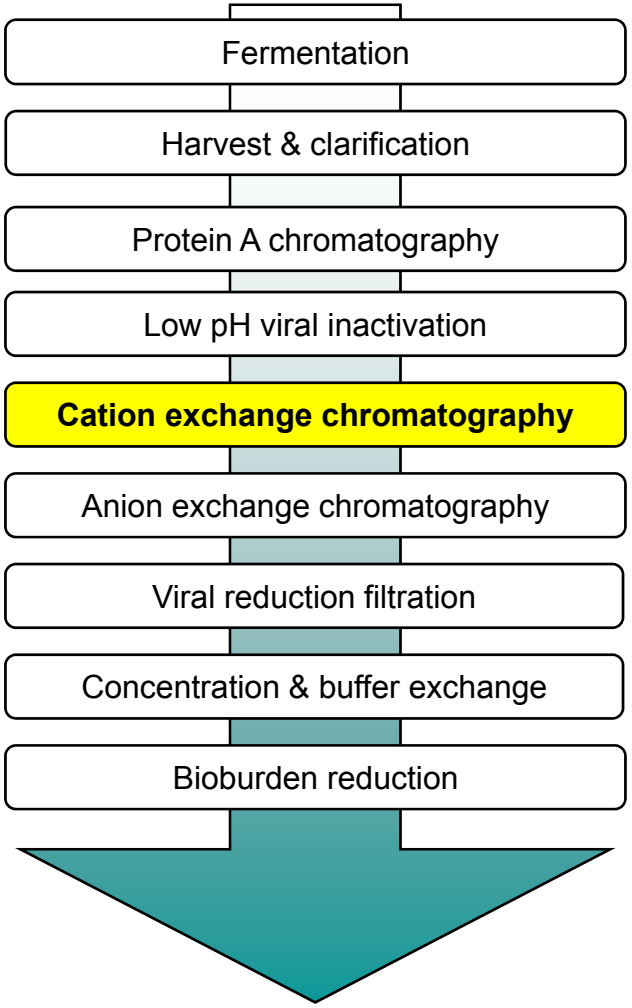


Low pH Viral Inactivation

- **Inactivation of enveloped viruses**
- **Cheap, simple and effective**
- **Minimal hardware requirements**
- **Precipitation of host cell proteins**

- **Product stability**
  - Aggregation
  - Precipitation

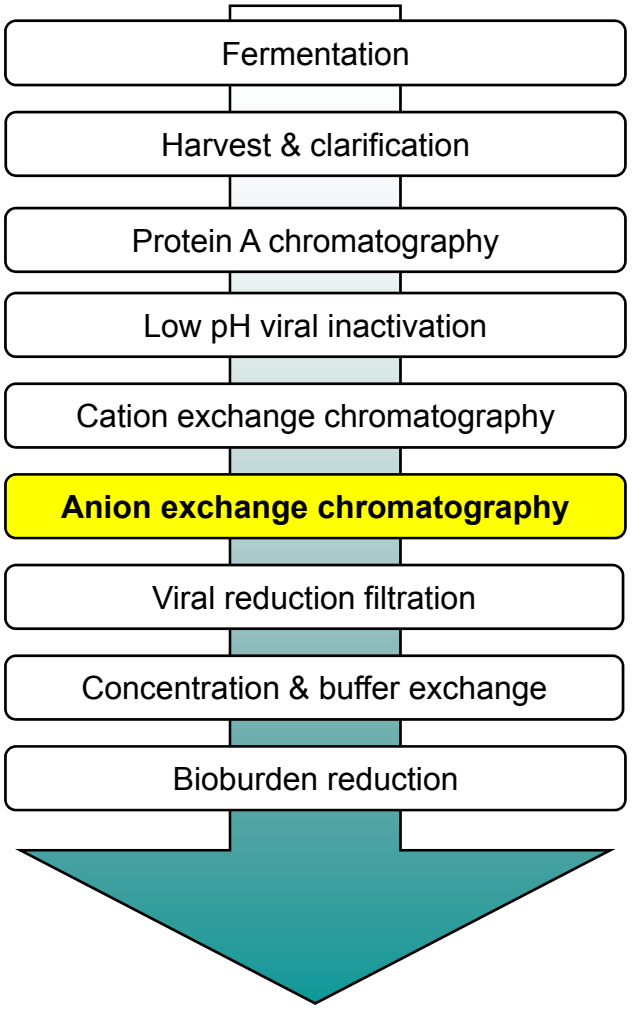
Cation Exchange Chromatography



Cation Exchange Chromatography

- **Intermediate purification:**
  - Aggregate removal
  - Host cell protein reduction
  - Leached Protein A reduction
- **Packed bed chromatography**
- **Bind and elute mode**
- **Possible viral reduction step**

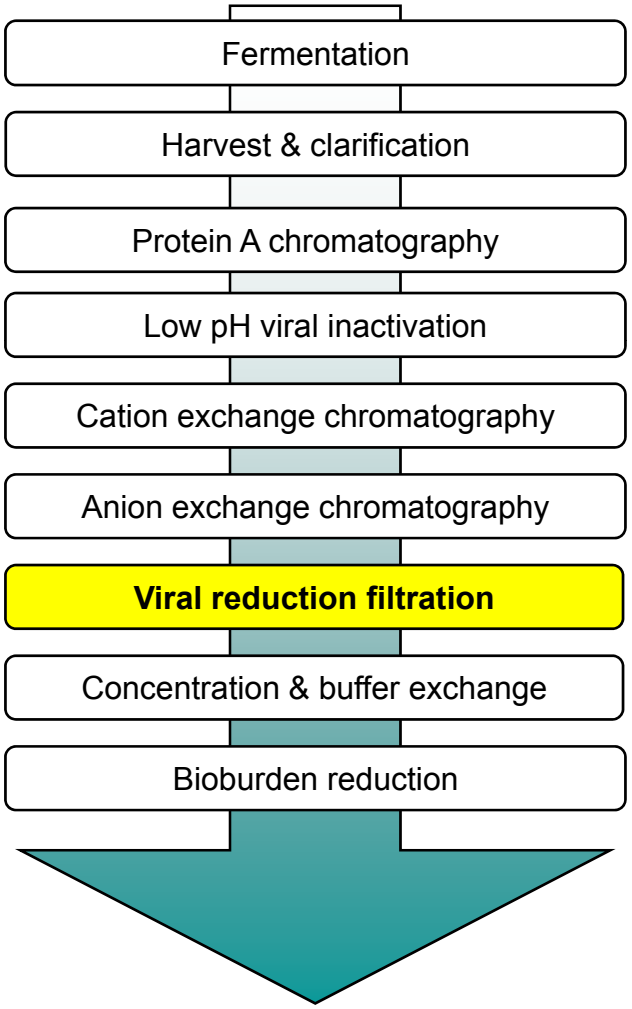
Anion Exchange Chromatography



Anion Exchange Chromatography

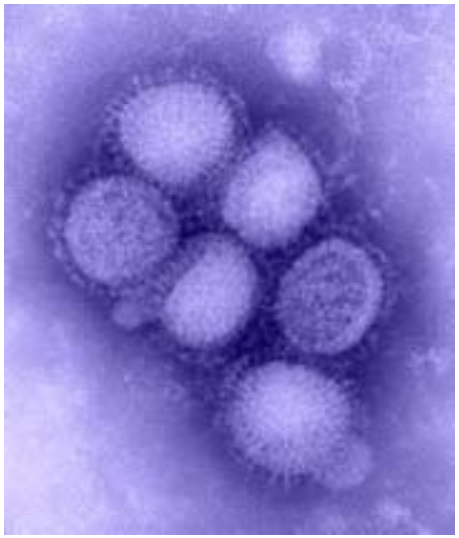
- **Polishing purification:**
  - Residual host cell protein removal
  - Endotoxin reduction
- **Flowthrough mode:**
  - Disposable membrane technology
- **Viral reduction**

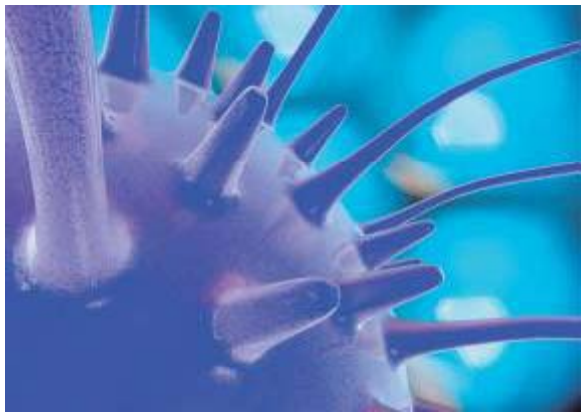
Viral Reduction Filtration



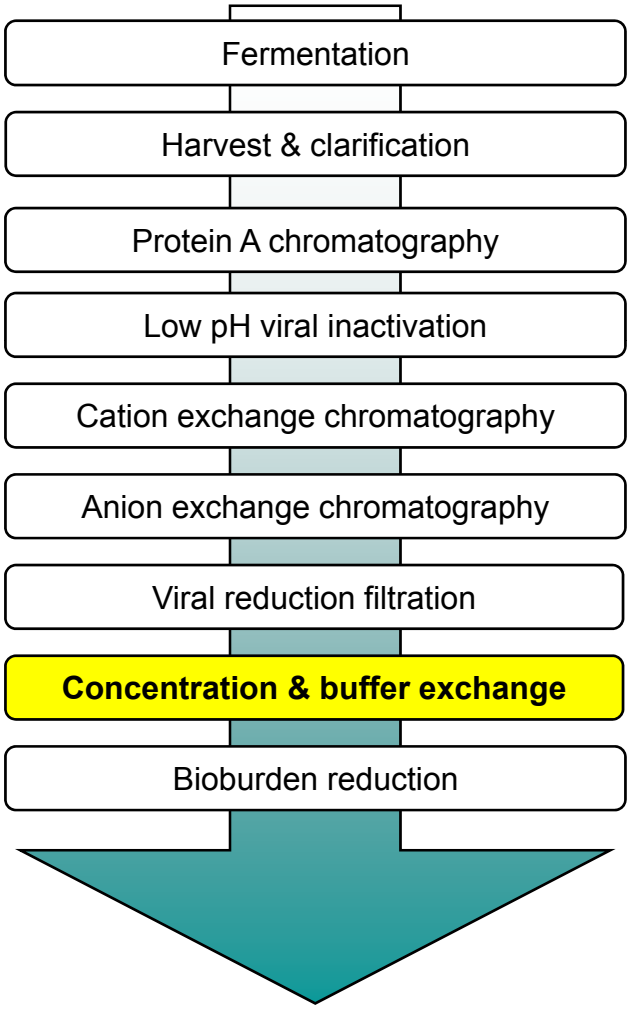
Viral Reduction Filtration

- **Nano-filtration**
  - 100 nm Pre-filter → 20 nm Final filter
- **Small non-enveloped viruses**
- **Disposable capsule format**





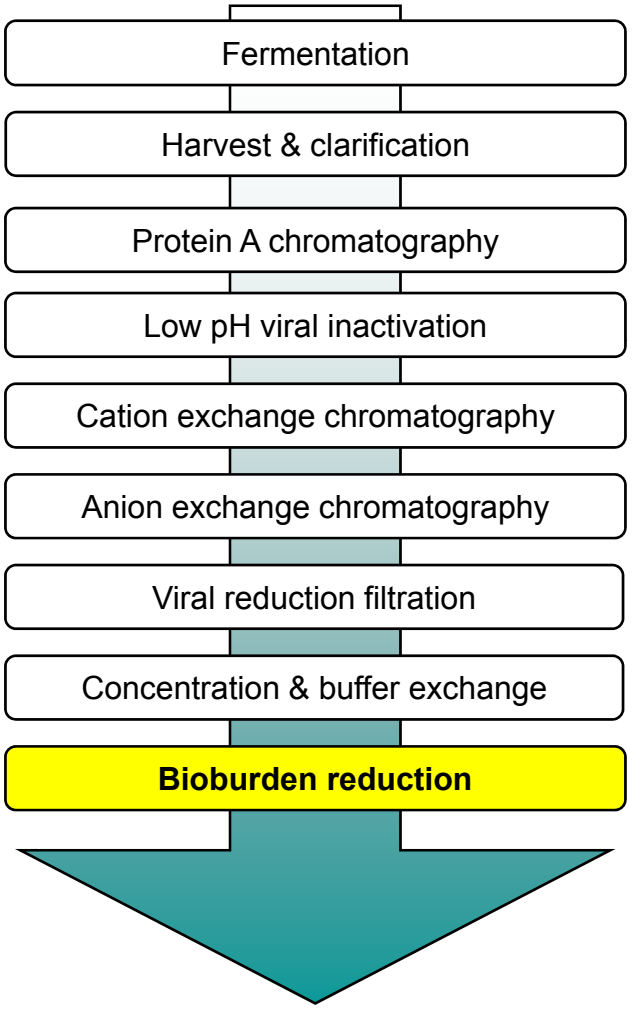
Concentration & Buffer Exchange



Concentration & Buffer Exchange

- **Ultrafiltration / diafiltration**
  - Crossflow filtration
- **Volume reduction**
- **Buffer exchange**
- **Various options**

Bioburden Reduction Filtration



Bioburden Reduction Filtration

- **0.22 µm filtration**
- **Pre-filter → Final filter**
- **Disposable capsules**
- **Bioburden reduction**
  - Patient safety
  - Reduce microbial spoilage

Monoclonal antibody purification: Case Study

Case Study: Process Development

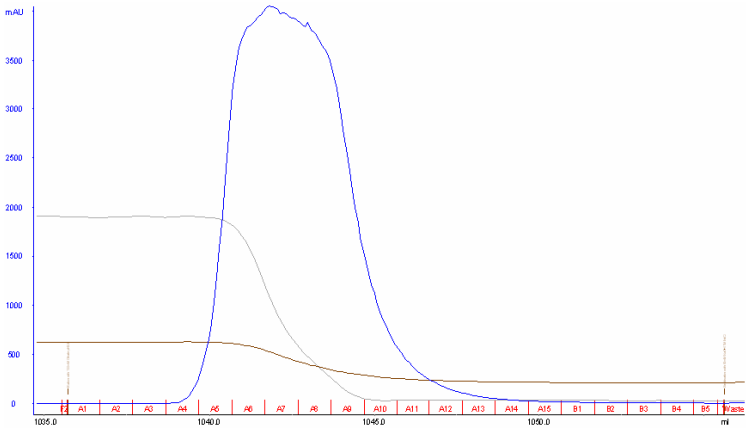
- IgG_4
- ***CHO* cell line:**
  - Enabling cell expression technology
  - Stirred tank Bioreactor
  - Chemically defined media
- **Target = 0.5 g/L expression**

Case Study: Process Development

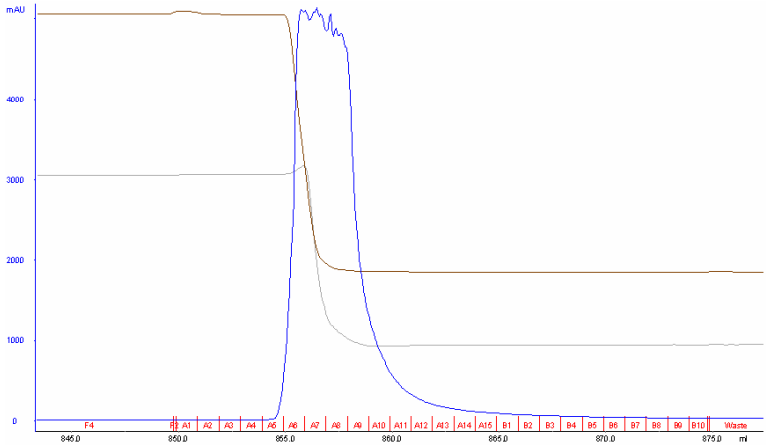
- **Depth filtration**
  - Gross cellular debris removal
  - Direct bioreactor clarification
  - Fully disposable
- **Normal flow filtration**
  - 0.22 µm filtration
  - Bioburden reduction
  - Fine particulate removal
  - Disposable capsule format

Protein A – Resin Screening

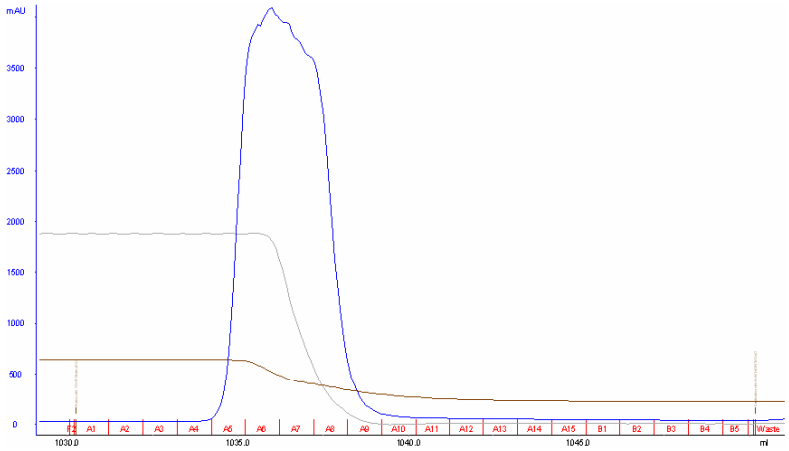
MabSelect A: GE Healthcare



ProSep A: Millipore

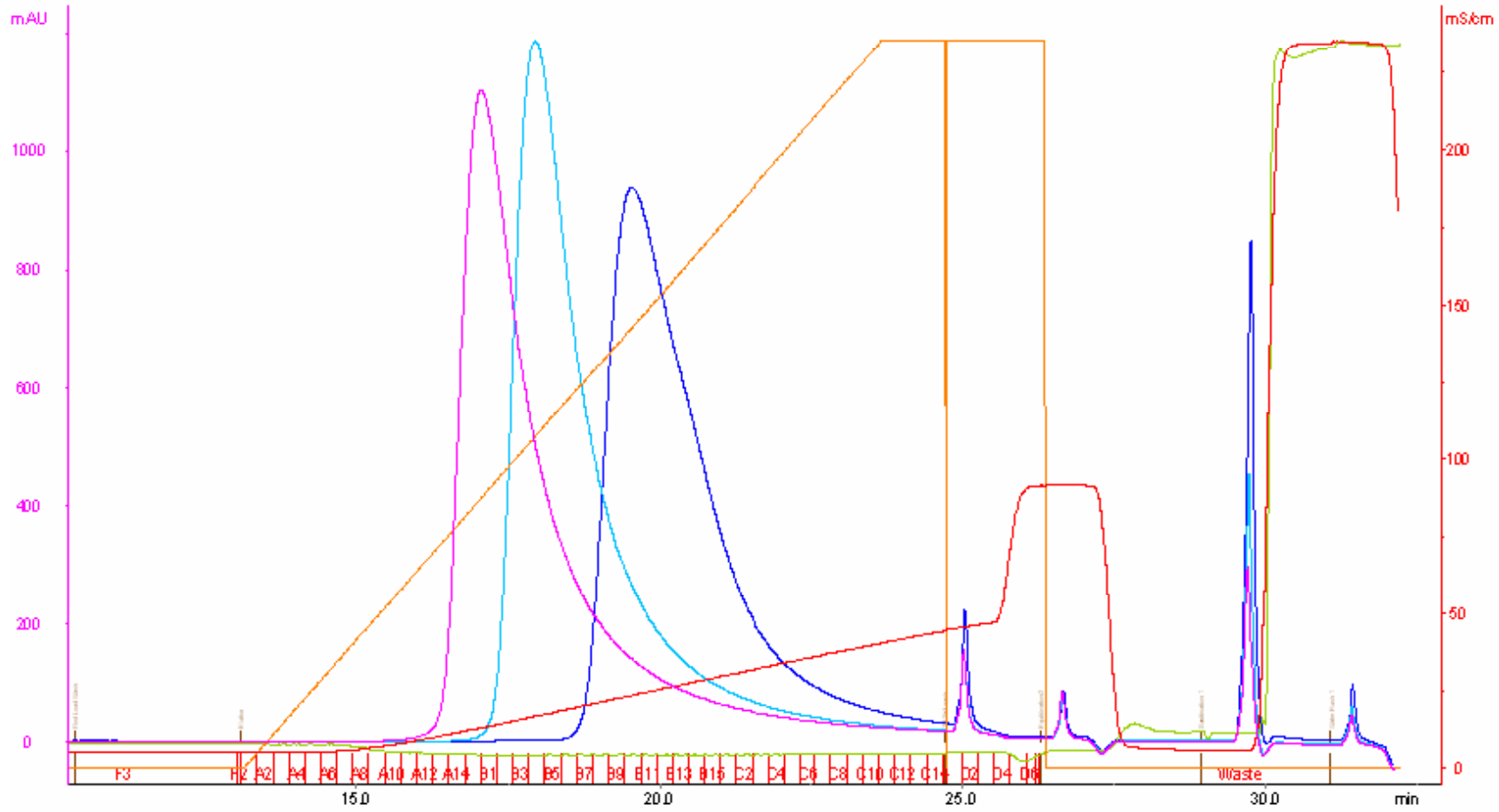


MabCapture A: Applied Biosystems



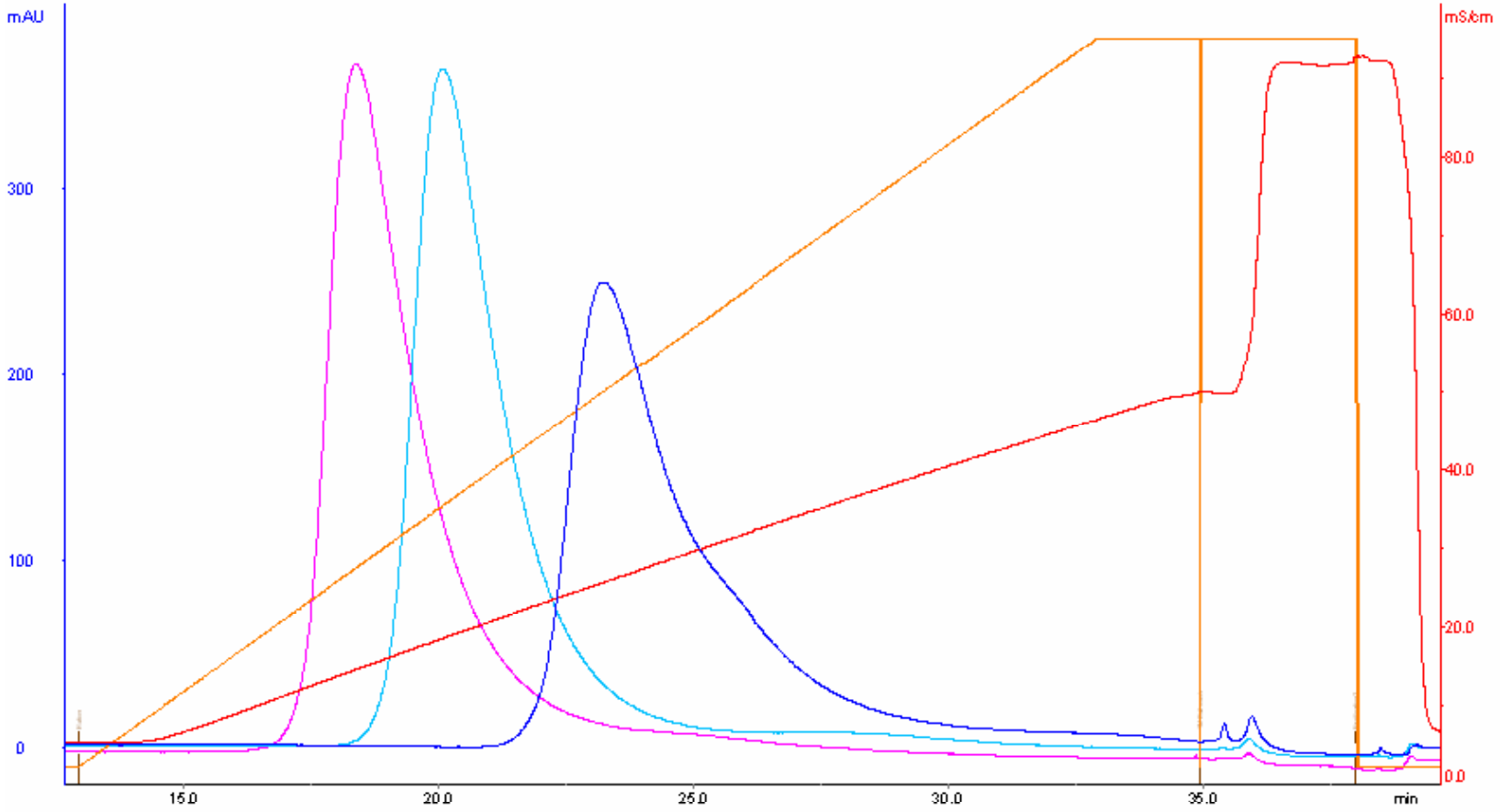
Cation Exchange resin screening: Capto S – GE HealthCare

Run 1 – pH 5.5 **Run 2 – pH 5.0** **Run 3 – pH 4.5**



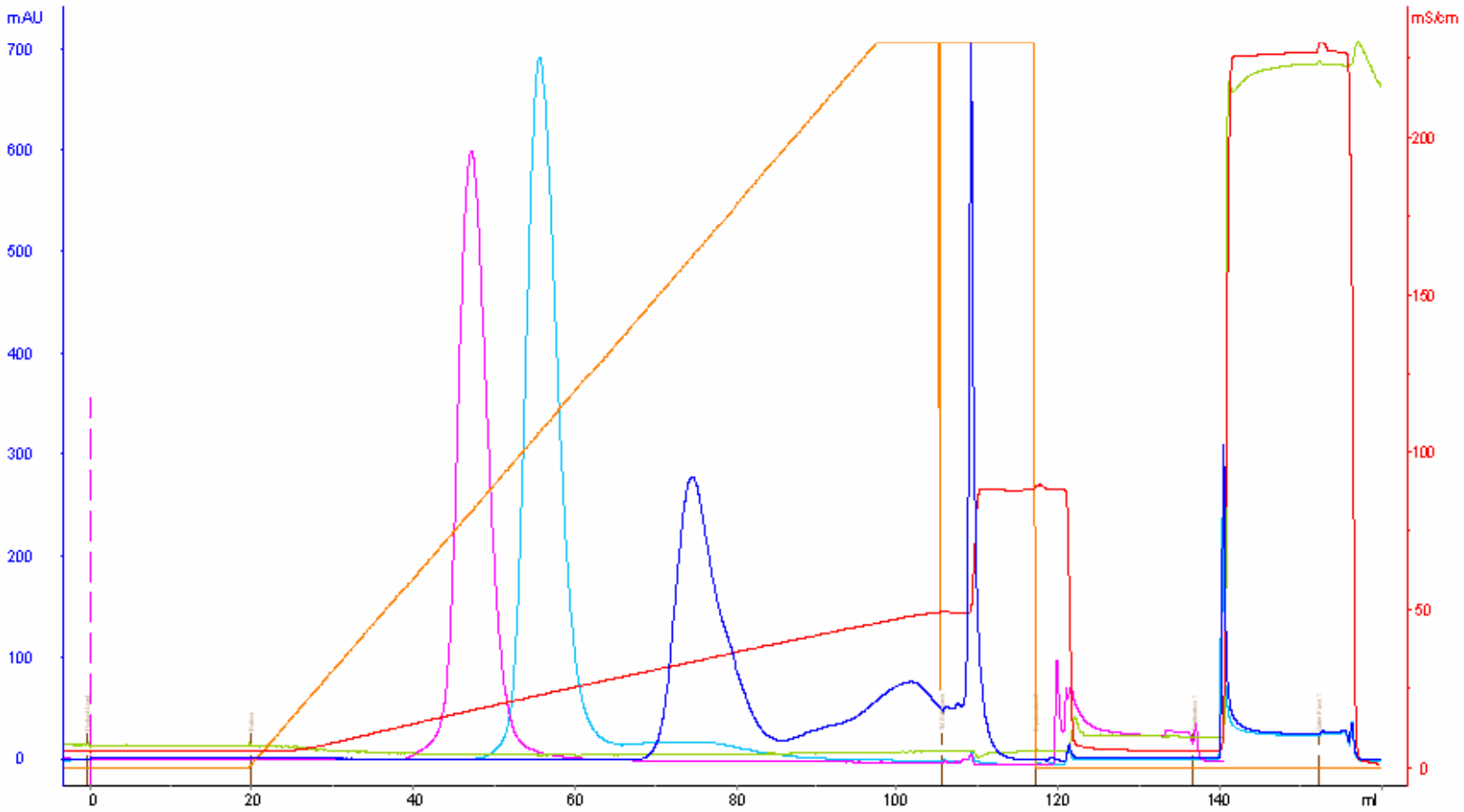
Cation Exchange resin screening: GigaCap S – TosoH

Run 1 – pH 5.5 **Run 2 – pH 5.0** **Run 3 – pH 4.5**



Cation Exchange resin screening: Poros HS – Applied Biosystems

Run 1 – pH 5.5 **Run 2 – pH 5.0** **Run 3 – pH 4.5**



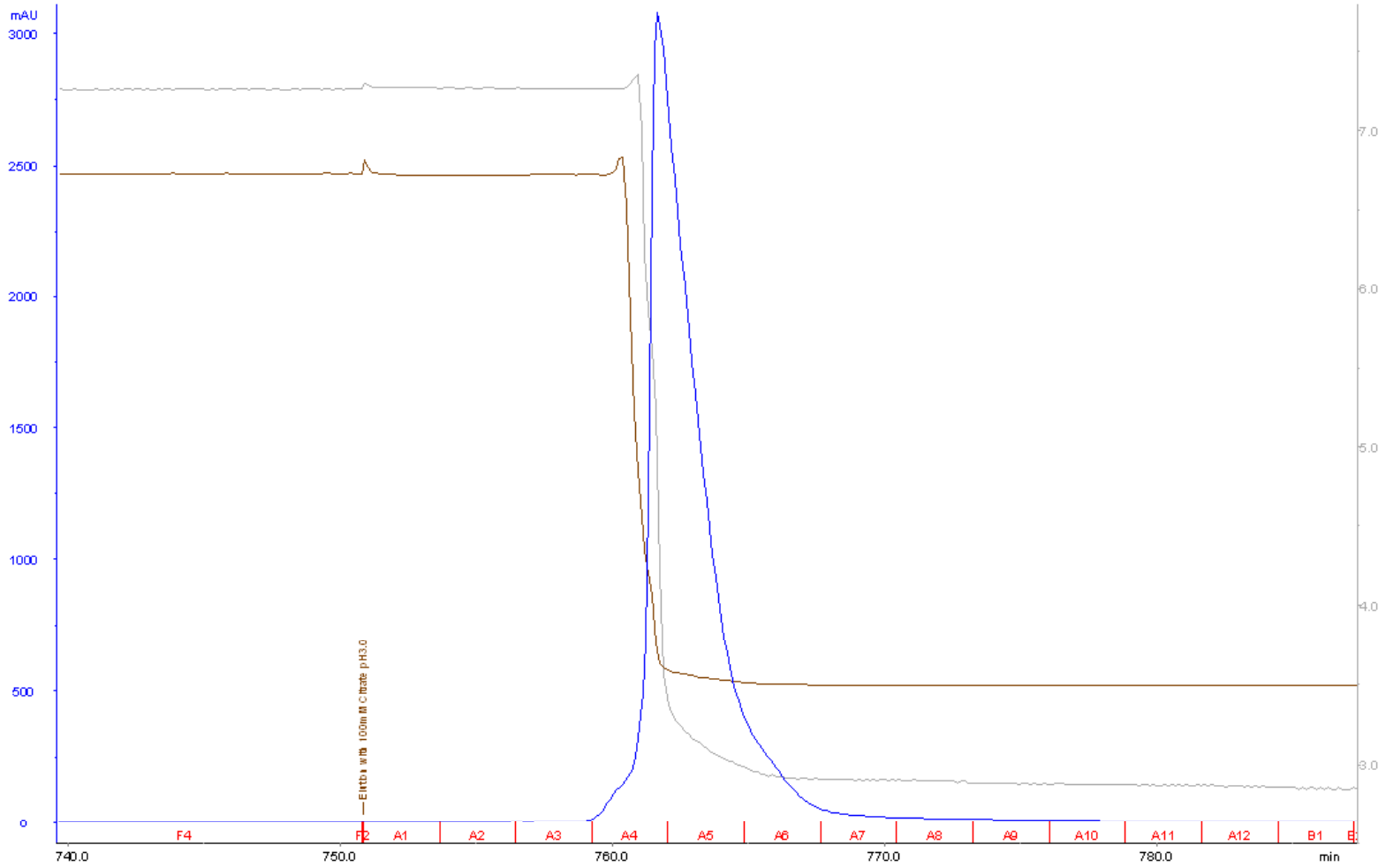
Anion Exchange resin screening

- **Packed bed:**
  - Poros HQ – Applied Biosystems
  - Capto Q – GE Healthcare
- **Membrane adsorbtion:**
  - Sartobind Q – Sartorius
  - Adsep – Natrix Separations
  - Chromasorb – Millipore

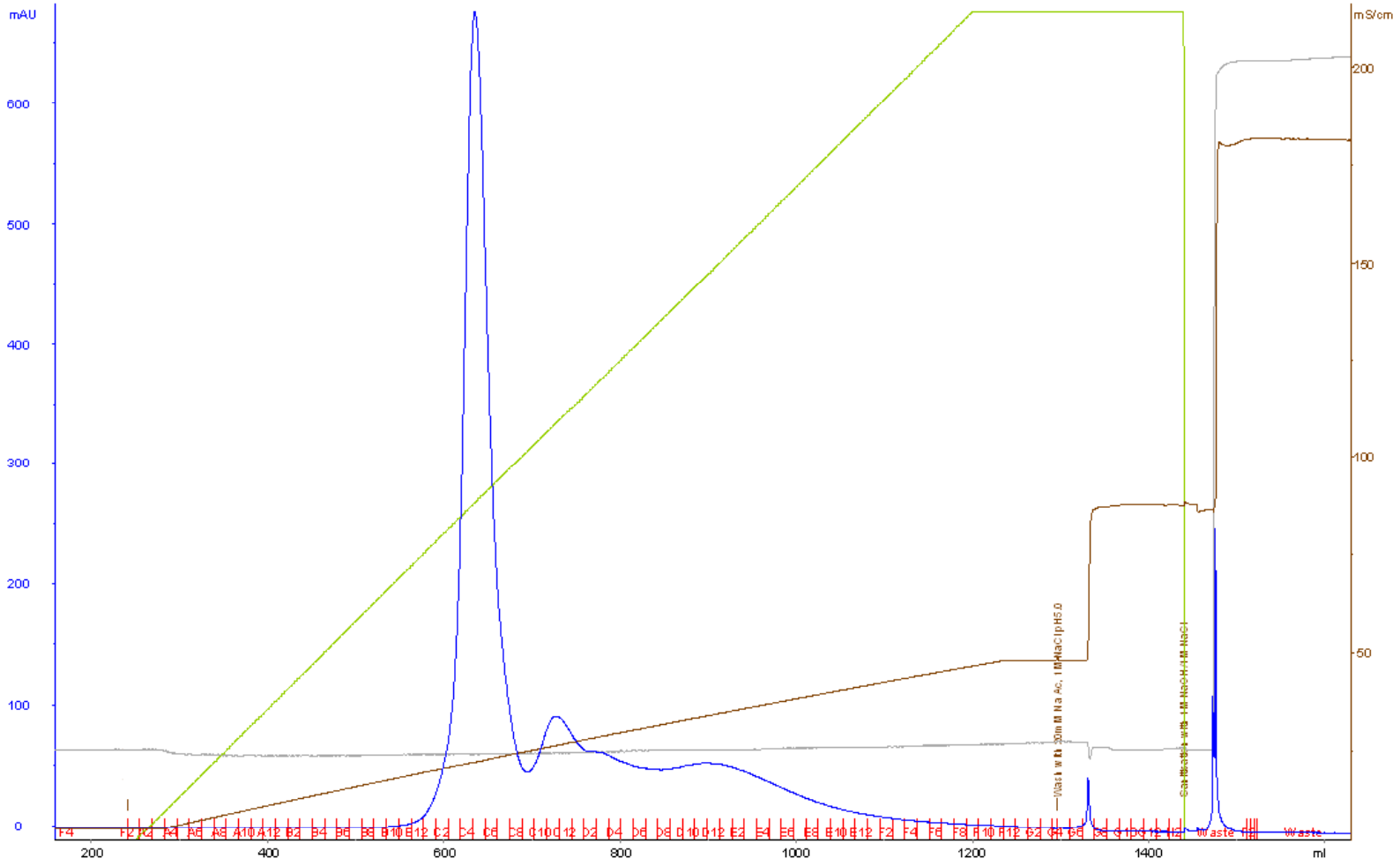
Case Study: 2 L Bioreactor

- 2 L bioreactor
- PowerCHO growth media
  - Feed strategy applied
- 0.5 g/L expression
- 2 x 10^6 cells per mL (on harvest)
- < 50 % viability (on harvest)

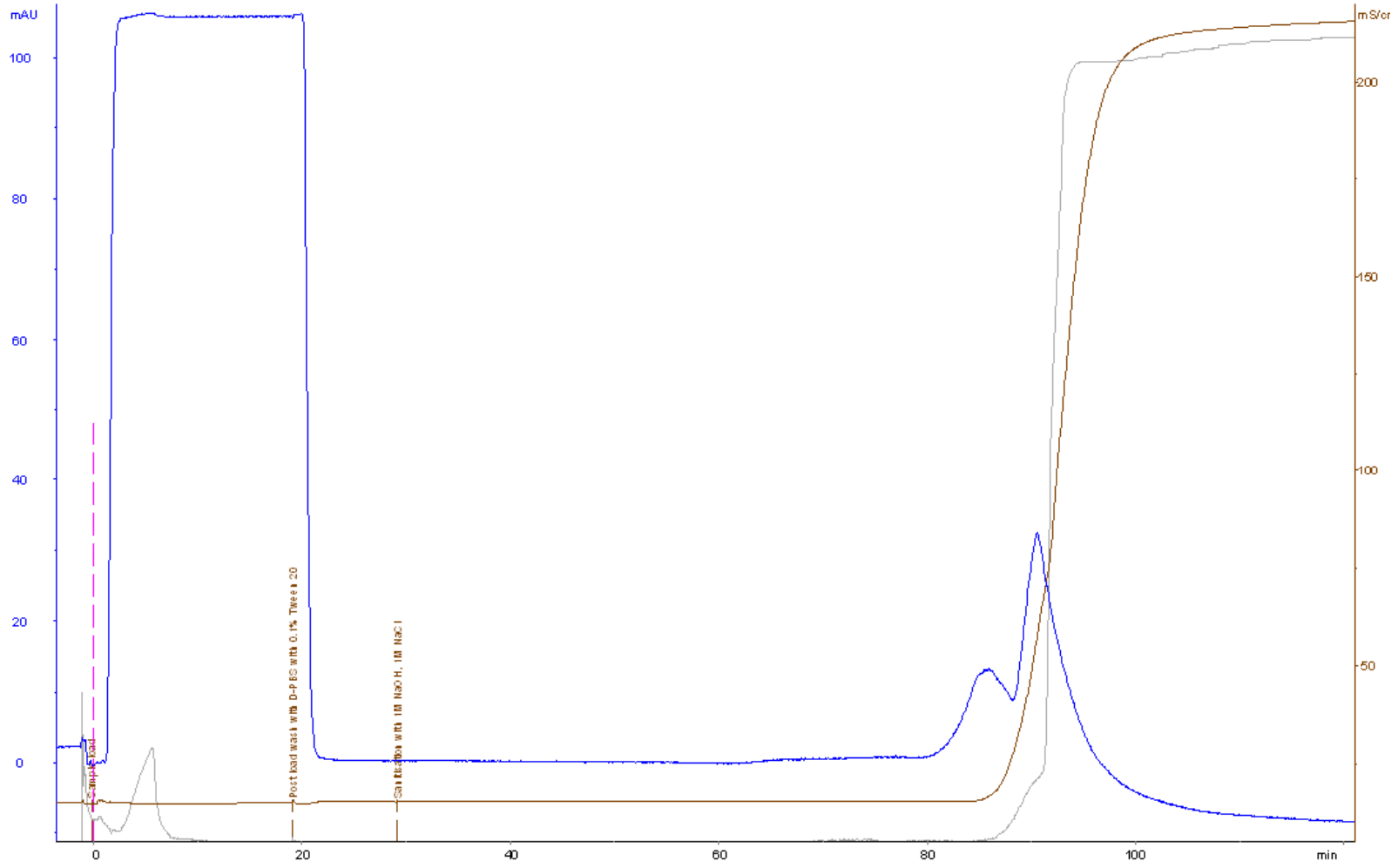
Protein A: MabCapture A Applied Biosystems



Cation Exchange: Poros HS
Applied Biosystems

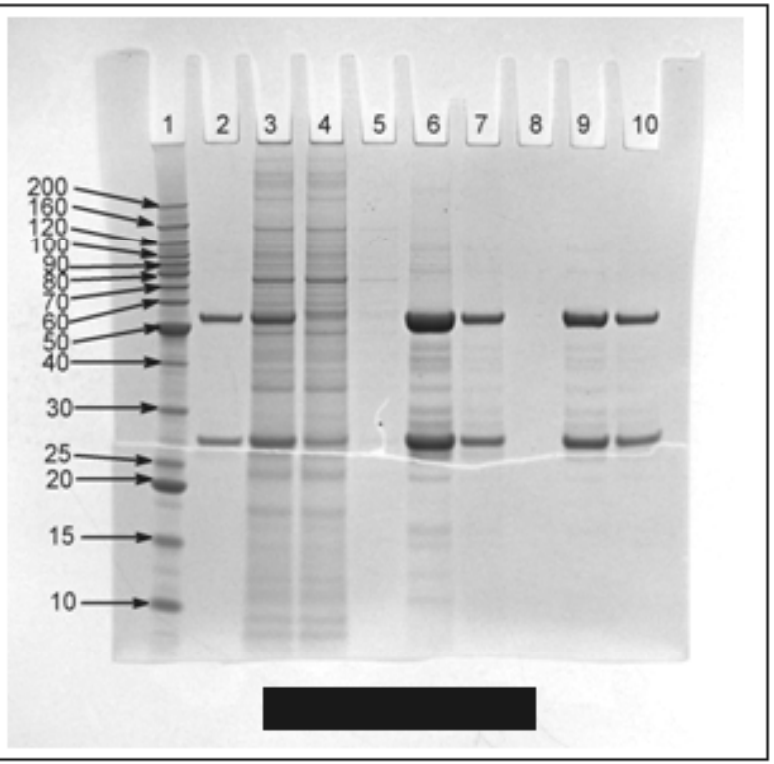


Anion Exchange: Poros HQ
Applied Biosystems



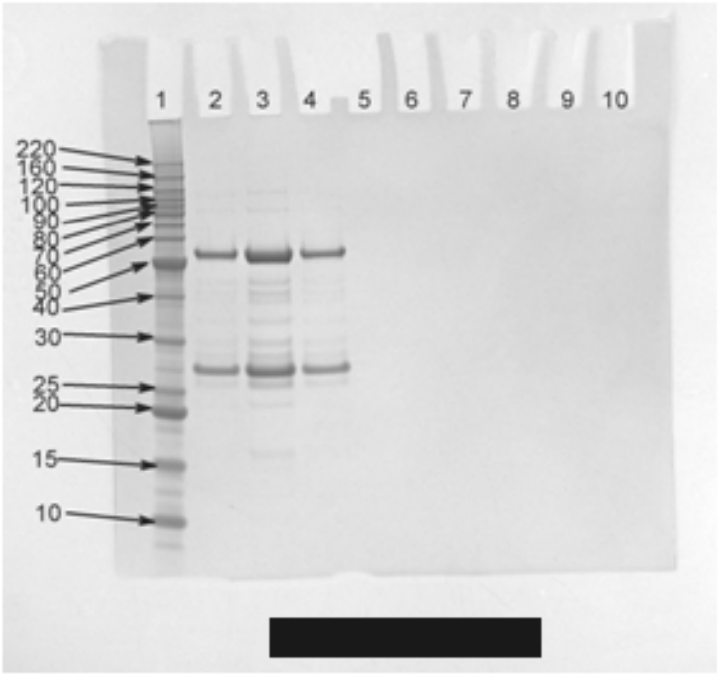
Analytical Results: SDS-PAGE

Lane	Sample	Vol. loaded (µL)
1	Benchmark Protein Ladder	5
2	Anti-XXXXXX (0.1 mg/mL)	20
3	Protein A Load (MabCapture A)	10
4	Protein A Flow Through (MabCapture A)	10
5	Protein A Post Load Wash (MabCapture A)	10
6	Neutralised Protein A Eluate (MabCapture A)	3
7	CEX Load (POROS HS)	5
8	CEX Flow Though (POROS HS)	10
9	CEX Eluate - Fractions B12-L8 (POROS HS)	5
10	AEX Load / GS Pool (POROS HQ)	5

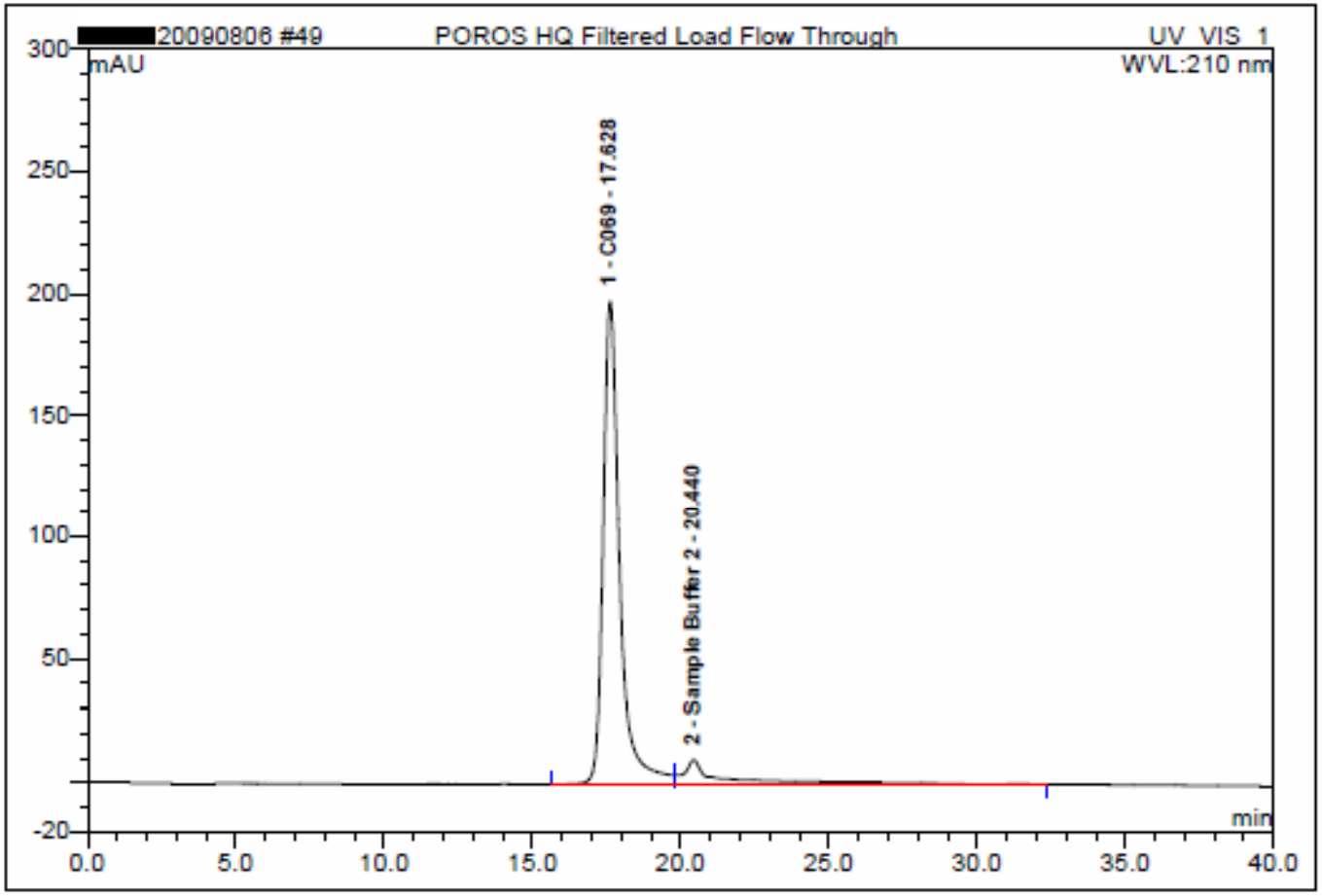


Analytical Results: SDS-PAGE

Lane	Sample	Vol. loaded (µL)
1	Benchmark Protein Ladder	5
2	Anti-XXXXXX (1.6 mg/mL)	20
3	AEX Flow Through (POROS HQ)	10
4	Final Product	3
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-



Analytical Results: Size exclusion



Analytical Results

Analysis	Technique	Result
Endotoxin	LAL	< 0.5 EU/mL
DNA	qPCR	16 pg/mL
Purity (SEC)	SEC	100 %
Host cell protein	ELISA	In development
Recovery	ELISA	In development

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