

Testa  
CHALLENGE  
2022

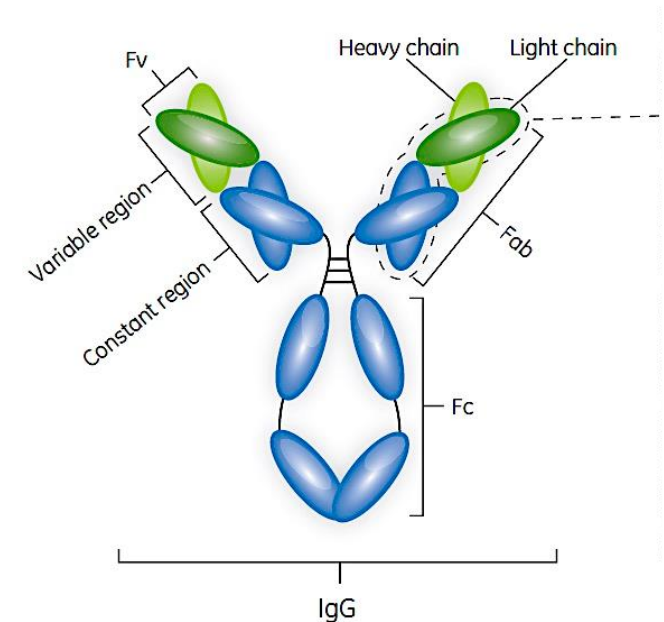


# Technical description

A start to finish Bioprocess – Producing and purifying a monoclonal antibody (mAb).

## Process outline:

- Production of mAb with CHO cells using fed-batch technique in single-use bioreactor
- Clarification of the culture using depth filtration
- Purification of mAb using affinity chromatography followed by additional polishing step with multimodal chromatography resin



# Process overview

Cultivation in rocking  
or stirred bag single  
use bioreactor (30L)

Clarification of culture  
by depth filtration

Purification of product  
on Affinity and  
Multimodal  
Chromatography  
resins

# Timeline

Installation of  
bioreactor and  
media preparation  
Thawing of high cell  
density seed culture

Day 1

Fed-batch cultivation  
including sampling  
and analytics

Day 2-10

Termination of the  
cultivation  
Product conc  
analysis  
Clarification of cell  
culture

Day 11

Affinity  
Chromatography  
Multimodal  
Chromatography  
SEC and HCP  
analysis

Day 11-15

# Upstream process

## 1. Reactor preparation

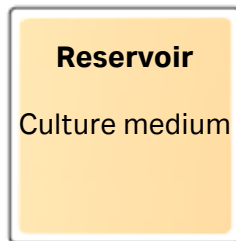
Installation of reactor bag, insertion of sterile probes. Preparation of feed solutions

## 2. Thawing high cell density seed culture

Previously prepared seed culture ( $10^6$  VCD/ml) in cryobag stored deep-frozen

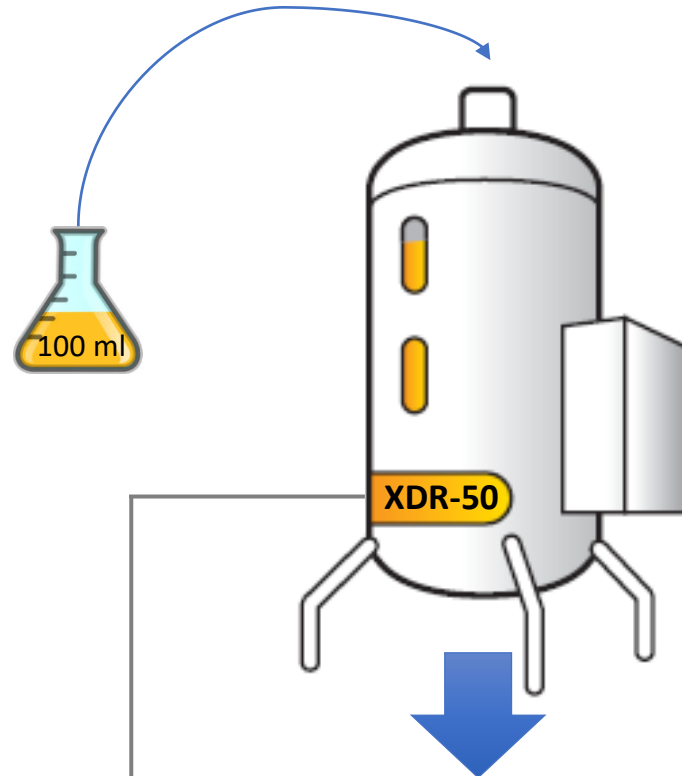
## 3. Sterile filtration of culture medium

30 L of culture medium pumped through 0.2  $\mu\text{m}$  NF capsule filter into the Bioreactor.



ULTA Pure HC  
filter 0.2  $\mu\text{m}$  6"

WM 520 peristaltic pump with ReadyCircuit  
Pumpsil jumper 3/8" x 9/16"



## 4. Inoculation of Bioreactor

100 mL of high cell density seed suspension is inoculated into 30 L culture medium in bioreactor via transfer vessel. Tube from transfer vessel is welded to an appropriate tube at bioreactor.

## 5. Fed-batch culture in Bioreactor

Cultivation performed approx. 10 days with daily addition of feed solutions and regular analysis of critical process parameters.

## 6. Aseptic transfer to harvest line

Aseptic transfer of cells from bioreactor via 3/8" x 5/8" C-flex drain tube connected via ReadyMates to ReadyCircuits harvest line.

Harvest using depth  
filtration

Affinity Chromatography  
using MabSelect Prisma resin

# Clarification process

## Harvest using depth filters

### Material to process:

- 30 L CHO culture
- Conc.: ~3 g mAb/L

### Depth filters:

- Pall Stax PDP8 & PDE2 single-use discs



Peristaltic pump



Depth filter discs



Storage bag

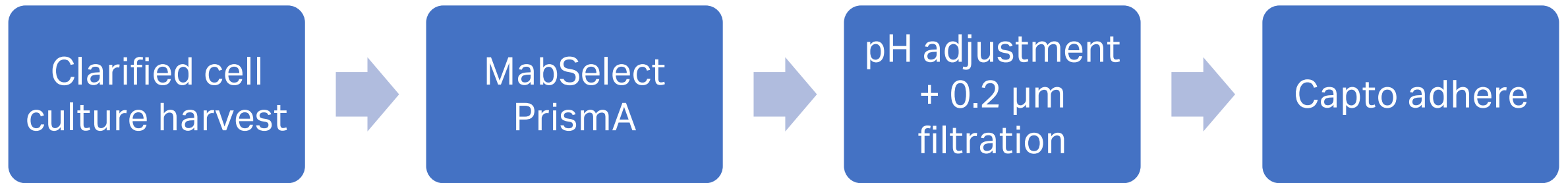
### Clarification conditions:

- Filters flushed with purified water followed by conditioning with phosphate buffer
- Process runs in constant flow mode

### Clarified solution

- Sterile filter to a 30 L bag via Pall Supor EKV capsule

# Downstream process flow chart



# Downstream process: Step 1

## Capture step using Affinity Chromatography: MabSelect Prisma

### Chromatography step 1:

- MabSelect Prisma chromatography resin
- Binding capacity: 48 g/L resin (6 min residence time, 80% load)
- 41 g mAb is processed in 2 runs with a 427 mL column

### ÄKTA pilot 600



### AxiChrom 70/300



### AxiChrom 70/300:

- 11.1 cm bed height
- 427 mL MabSelect Prisma

### Chromatographic method:

Step	Solution	CV	Comment
Eq.	20 mM phosphate, 0.15 M NaCl, pH 7.0	3	
Sample appl.	mAb sample	7 L	6 min res.time
Wash 1	20 mM phosphate, 0.15 M NaCl, pH 7.0	5	
Wash 2	20 mM phosphate, 0.5 M NaCl, pH 7.0	1	
Elution	50 mM acetate, pH 3.5	3	Peak fractionation
Strip	100 mM acetic acid, pH 2.9	2	
CIP	0.5 M NaOH	2	15 min contact time
Re-eq.	20 mM phosphate, 0.15 M NaCl, pH 7.0	5	

**Total run time: ~240 min.**

### Eluate:

- Collect eluates in Duran bottles
- pH adjust pooled eluates with 2 M Tris base to pH 5.



# Downstream process: Step 2

## Polishing step using multimodal chromatography: Capto adhere resin

Capto adhere can remove key contaminants such as:

- host cell DNA (hcDNA)
- host cell proteins (HCP)
- leached protein A
- mAb dimers and larger aggregates
- viruses

### Chromatography:

- Capto adhere resin
- Flow-through mode
- Sample load: 250 g mAb/L resin
- 0.22 µm filtered prior to chromatography run
- 1 run with a 160 mL column

### ÄKTA pure 150



### Chromatographic method:

Step	Solution	CV	Comment
Eq.	100 mM acetate, 0.13 M NaCl, pH 5.0	7	4 min res.time
Sample appl.	mAb sample	2.5 L	
Wash *	100 mM acetate, 0.13 M NaCl, pH 5.0	7	
Rinse	water	3	8 min res.time
CIP	1 M NaOH	3	
Re-eq.	100 mM acetate, 0.13 M NaCl, pH 5.0	10	4 min res.time

\* Fraction collection to be stopped at 100 mAU and the process is continued with rinse.

**Total run time: ~210 min.**

### AxiChrom 50/300:

- 8 cm bed height
- 160 mL Capto adhere

### Eluate:

- Sterile filtered into 1L flasks

# Analytics

## Upstream Process Analytics

- Cell concentration and viability (Vi-Cell XR)
- Nutrients and metabolites concentration (Cedex Bio)
- Culture osmolarity (Osmo1)

## Product Yield and Purity

- mAb concentration on protein A column
- Aggregates and fragments (SEC column)
- mAb size and impurity profile (PAGE)
- mAb identity - peptide mapping (Reversed-Phase chromatography)
- Host cell protein content (Gyrolab)

# Instruments and consumables

# Single-use bioreactors



XDR-50 bioreactor



Wave rocking bioreactor

# Stax™ Disposable Depth Filter Systems



<https://shop.pall.com/us/en/biotech/depth-filtration/zidgri78m6c>

# Chromatography systems and columns

ÄKTA pilot 600



<https://www.cytivalifesciences.com/en/us/solutions/bioprocessing/products-and-solutions/downstream-bioprocessing/akta-pilot-600>

ÄKTA pure 150



<https://www.cytivalifesciences.com/en/us/shop/chromatography/chromatography-systems/akta-pure-p-05844>

AxiChrom 50/300



<https://www.cytivalifesciences.com/en/us/shop/chromatography/columns/process-columns/axichrom-50-to-200-mm-chromatography-columns-p-06215>

AxiChrom 70/300



# Instruments

Instrument	Manufacturer and model	Comment
XDR-50 single-use bioreactor system	Cytiva	With filter condenser and built-in addition pumps
XDM/XDUO 100 Mixer	Cytiva	With Heating/cooling jacket, load cells
Temperature control unit (TCU) to XDR bioreactor	Polyscience	Heating/cooling capacity: 9 kW/ 1.5 HP
ReadyToProcess Wave 25 system	Cytiva	Including gas supply unit (CBCU) and pump unit
Peristaltic pump	Watson Marlow, 520 & 630	For filtration of medium to the vessel & clarification of culture
Pressure Monitor with disposable pressure sensors	SciLog	To monitor pressure during medium filtration & clarification steps
Shaking Incubator	Infors, Ecotron	For cultivation of culture in shake flasks
Cedex Bio	Roche	Nutrients and metabolite analysis
Vi-Cell XR	Beckman Coulter	Measuring cell concentration and viability
Osmo1	Advanced Instruments	Measuring osmolarity of solutions
pH-meter	–	For bioreactor probe calibration and solution preparation
Sterile tube sealer	Cytiva	To seal thermoplastic tubing (Sizes: 1/4 to 1 inch OD)
Sterile tube fuser	Cytiva	To fuse thermoplastic tubing (Sizes: 1/4 to 1 inch OD)
ÄKTA Pure 150	Cytiva	Affinity chromatography using Capto L resin
ÄKTA Pure 25 or HPLC	Cytiva	For analysing mAb titer
Gyrolab Workstation	Gyros	For analysing HCP content

# Consumables

Consumable part	Cat. No.	Comment
XDR-50 Pro cell culture bag	888-0086-C	50 L Single-Use bioreactor bag
XDM-100 BASIC	888-0164-C	Mixer bag for medium preparation in XDUO-100.
ULTA Pure HC filter (5-inch single-use NFF, 0.2 µm)	12410095	For sterile filtration of culture medium
Disposable 20 L hanging bag for permeate	12410224	Sterile bag for collecting filtrate from culture clarification
ReadyMate DAC 375 with 3/8" barb	28936687	Disposable Aseptic Connectors 3/8" Barb, for aseptic connections.
ReadyMate DAC 750 Mini TC	28936695	Disposable aseptic connector converting ReadyMate to TC.
pH probe standard	817-00144	For XDR-50 bioreactor; can be used up to 12 autoclavation cycles.
DO probe standard	817-00145	For XDR-50 bioreactor; can be used up to 12 autoclavation cycles.
Probe sheaths	888-0138	Four probe sheaths pcs included in one package.
Capto adhere, 200 mL	17544401	Multimodal IEX resin
HiTrap MabSelect Prisma	17549852	Column with affinity resin for mAb capture
HiScreen MabSelect Prisma	17549815	Column with affinity resin for mAb capture
HiTrap Capto adhere	28405844	Column packed with multimodal IEX resin
AdvanceBio SEC (Agilent)	L1980-3250PK	Column for SEC analysis of mAb samples
PeptidMap column UPLC BEH130 C18 (Waters)	186003555	Column for analysis of trypsin digested mAb samples
Stax™ large depth filter capsule PDP8 & PDE2 (PALL)	SXLPDP8408SP SXLPDE2408SP	Clarification of cell culture
Supor® EKV membrane capsules (PALL)	KA1EKVP1G	Sterile filtration of mAb solutions



# Chemicals

Chemical	Cat. number	Brand	Comment
ActiPro powder medium for 25L	SH31037.05	Cytiva	Cell culture medium
Cell Boost™ 7a powder medium for 10L	SH31026.02	Cytiva	Cell culture feed
Cell Boost™ 7b powder medium for 5L	SH31027.02	Cytiva	Cell culture feed
D-Glucose	SH30371.04	Cytiva	Cell culture feed
NaHCO <sub>3</sub>	S5761	Sigma	Base, pH control of culture
Antifoam C, 30% suspension	A8011	Sigma	Antifoam agent for cultivation
Na-acetate	1.06268	Merck	Buffer component
Acetic acid	1.00062	Merck	Buffer component
Na <sub>2</sub> HPO <sub>4</sub>	1.06586	Merck	Buffer component
NaH <sub>2</sub> PO <sub>4</sub>	1.06342	Merck	Buffer component
NaCl	1.06404	Merck	Buffer component