Testa CHALLENGE

2020

Technical Description

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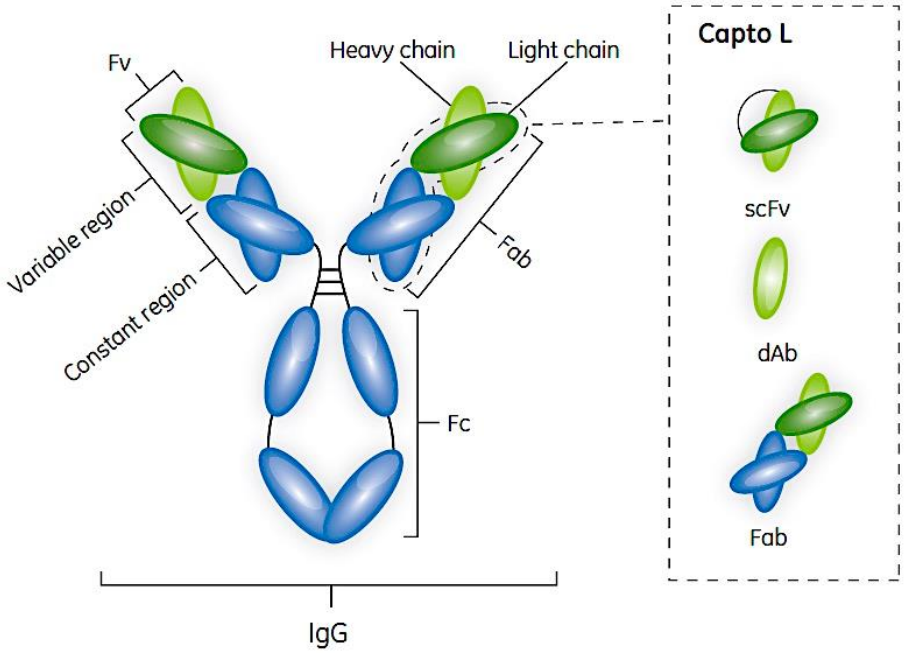
A start to finish Bioprocess – Producing and purifying a domain antibody fragment, dAb.

Process outline:

- Production of dAb in *E.coli* in bioreactor
- Release of dAb to periplasm under heat treatment
- Clarification and concentration of dAb using cross flow filtration
- Purification of dAb using affinity chromatography

More info on antibody fragments, suggested reading:

https://www.mdpi.com/2073-4468/4/3/259/htm



Process overview

Cultivation in 50 L bioreactor

Clarification of harvest – Cross flow filtration

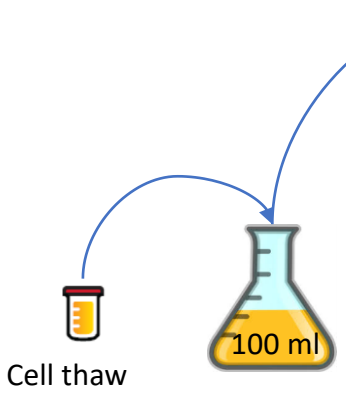
Purification of product – Affinity Chromatography

Timeline

Day 1	Day 2	Day 3	Day 4
Preparation of culture medium Prep and installation of bioreactor. Thawing of cell bank and pre-culture start	Cultivation in XDR-50 Preparation of solutions for filtration Installation and preparation of harvest filter	Heat treatment in Bioreactor Transfer of cell broth to filtration line Clarification of harvest in ÄKTA Ready	Preparations for purification, buffers and method scripting Purification of product using ÄKTA pilot and protein L resin in AxiChrom column

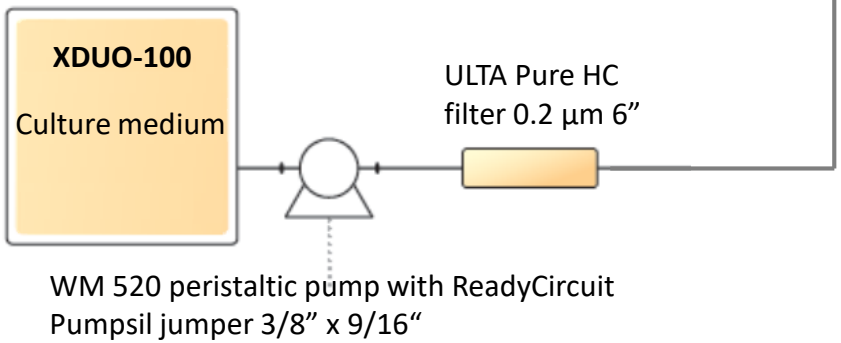
Upstream process flow chart

1. Cell thaw
Thawed cell broth from Cryovial is seeded into Erlenmeyer flask

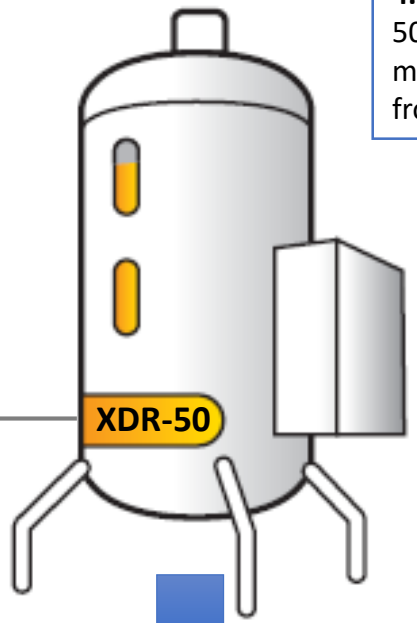


2. Pre-culture
Cells are cultured to appropriate density, OD_{600nm} ≈3-6

3. Sterile filtration of culture medium
30 L of culture medium pumped through 0.2 µm NF capsule filter into the Bioreactor.



4. Inoculation of Bioreactor
50-100 mL cell broth is inoculated into 30 L culture medium in bioreactor via transfer vessel. Tubes from vessel are welded with the bioreactor tubes.



5. Fed-batch culture 50 L Bioreactor
Cultivation performed approx. 16 h including temp shift to 30 °C and 2 x induction with IPTG.

6. Heat treatment in Bioreactor and cold storage
At the end of cultivation, cells are heated to 48 °C for 3 h. Cell suspension is thereafter cooled to 8 °C and held overnight in the bioreactor.

7. Aseptic transfer to harvest line
Aseptic transfer of cells from XDR-50 via 3/8” x 5/8” C-flex drain tube connected via ReadyMates to ReadyCircuits harvest line.

Harvest using Cross Flow Filtration

Affinity Chromatography using Capto-L resin

Clarification process flow chart

Material to process:
- 8.5 L *E.coli* cultivation
- Conc.: ~2 g dAb/L

Cross flow filtration:
- Hollow fiber cartridge
- 750 kD cut off
- 8.5 L *E.coli* cultivation is processed in 1 run with 0.28 m2 filter area
- 10 L recirculation bag

Clarification conditions:
Buffers:
- 20 mM citrate, 0.8 M NaCl, pH 5.0
- 0.5 M NaOH, 300 ppm NaOCl
- 0.1 M NaOH

- 2.5 x concentration
- 3 x wash
- Feed flow 8L/min
- Permeate flow 70 mL/min
- 15 L clarified dAb solution

Harvest using Cross Flow Filtration/Hollow fibers

ÄKTA Ready Flux, with flowkit

UFP-750-E-6A

Clarification method:

Step	Solution	Time (min)	Volume (L)
Rinse+normalized water flux test	Water	35	16.0
Buffer flush	20 mM citrate, 0.8 M NaCl, pH 5.0	15	3.0
Concentration	E.coli cultivation	60	8.5
Diafiltration	20 mM citrate, 0.8 M NaCl, pH 5.0	180	10.2
Empty waste	E. coli cells	10	3.4
Waste flush	20 mM citrate, 0.8 M NaCl, pH 5.0	10	3.0
Flush	Water	10	9.0
CIP	0.5 M NaOH + 300 ppm NaOCl	75	9.0
Rinse+normalized water flux test	Water	35	16.0
Storage	0.1 M NaOH	10	3.0

Total run time: 7 hours

Permeate:
- Sterile filter to a 20 L bag
- 15 L dAb solution for chromatography

Hollow fiber cartridge UFP-750-E-6A
- 0.28 m2 filter area
- 63.5 cm long
- 170 fibers
- 1 mm lumen inner diameter

Downstream process flow chart

Capture step using Affinity Chromatography/ Capto L resin

Material to process:
- 15 L permeat from clarification
- Conc.: 1 g/L
- 15 g dAb to purify

Chromatography:
- Capto L resin
- Binding capacity: 14 g/L resin
- 15 g dAb is processed in 3 runs with a 360 mL column

Chromatography conditions:
- Buffers:
  - 20 mM citrate, 0.8 M NaCl, pH 5.0
  - 20 mM citrate, pH 5.0
  - 20 mM citrate, pH 2.8
  - 15 mM NaOH
- Residence time during sample application: 4 minutes

ÄKTA pilot 600

AxiChrom 50/300

Chromatographic method:

Step	Solution	CV	Comment
Eq.	20 mM citrate, 0.8 M NaCl, pH 5.0	1	300 cm/h
Sample appl.	dAb sample	5 L	4 min res.time
Wash 1	20 mM citrate, 0.8 M NaCl, pH 5.0	5	
Wash 2	20 mM citrate, pH 5.0	1	
Elution	20 mM citrate, pH 2.8	5	Peak fractionation
Wash 3	20 mM citrate, 0.8 M NaCl, pH 5.0	1	
CIP	15 mM NaOH	2	250 cm/h
Re-eq.	20 mM citrate, 0.8 M NaCl, pH 5.0	5	300 cm/h

Total run time: ~120 min.

Eluate:
- Neutralize eluate …?
- Collection bottle/bag ?

AxiChrom 50/300:
- 18.3 cm bed height
- 360 mL Capto L chromatography resin

Potential Analytics (TBD)

Offline cell count and viability (Vi-Cell XR)

Offline pH and pCO2 (ABL9)

Glycerol and Acetate concentration (Cedex Bio)

dAb concentration (ÄKTA Pure 25)

Instruments and consumables

Testa Challenge

XDR MO single-use stirred-tank fermenter

https://www.cytivalifesciences.com/en/us/shop/cell-culture-and-fermentation/microbial-fermentors/microbial-fermentation-systems/xcellerex-xdr-mo-single-use-stirred-tank-fermentors-p-06352

ÄKTA Ready Flux, Ultra filtration HF cartridge

150 cm

ÄKTA Ready Flux, with flowkit

https://www.cytivalifesciences.com/en/us/shop/bioprocessing-filtration/tangential-flow-filtration/filtration-systems/akta-readyflux-p-07377

UFP-750-E-6A

https://www.cytivalifesciences.com/en/us/shop/bioprocessing-filtration/tangential-flow-filtration/hollow-fiber-cartridges/laboratory-and-pilot-scale-ultrafiltration-hollow-fiber-cartridge-p-00452

ÄKTA pilot, AxiChrom 50/300

ÄKTA pilot 600

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

AxiChrom 50/300

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

Instruments

Instrument	Manufacturer and model	Comment
XDR-50 MO system	Cytiva	With baffles and filter condenser
XDM/XDUO 100 Mixer	Cytiva	With Heating/cooling jacket, load cells
Temperature control unit (TCU)	Polyscience	Heating/cooling capacity: 9 kW/ 1.5 HP
Peristaltic pump	Watson Marlow, 520	For feed & filtration of medium to the vessel
Pressure Monitor with disposable pressure sensors	SciLog	To monitor pressure during medium filtration Optional
Shaking Incubator	Infors, Ecotron	For cultivation of seed culture.
Moisture analyser	Mettler-Toledo, HB 43	To determine dry content of the culture
Spectrophotometer	–	For OD measurements
Centrifuge	Sorvall	Sample prep for dry content measurements. Optional
Nutrient and Metabolite analyzer	Cedex bio (Roche diagnostics)	Measurements of Glycerol and acetate in culture. Optional
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 Ready Flux	Cytiva	Used for clarification of harvest
ÄKTA Pure 150	Cytiva	Affinity chromatography using Capto L resin
ÄKTA Pure 25 or HPLC	Cytiva	For analysis of dAB titer. Optional
HiScale 16/40 column	Cytiva	Affinity chromatography using Capto L resin

Consumables

Consumable part	Cat. No.	Amount	Comment
ULTA Pure HC filter (5 inch single-use NFF, 0.2 µm)	12410095	2 pc	For sterile filtration of culture medium and clarified dAb.
Disposable 10 L hanging bag for recirculation	12410222	1	Bag needed for the ÄKTA readyflux system
Disposable 20 L hanging bag for permeate	12410224	2	Sterile bag for collecting product
ReadyMate DACs hose barb	28936687	1 pack	Disposable Aseptic Connectors 3/8" Barb, for aseptic connections.
ReadyMate DACs with Mini-TC	28936695	1 pack	Disposable aseptic connector converting ReadyMate to TC.
pH probe standard	817-00144	1 pc	For XDR-50 bioreactor; can be used up to 12 autoclavation cycles.
DO probe standard	817-00145	1 pc	For XDR-50 bioreactor; can be used up to 12 autoclavation cycles.
Probe sheaths	888-0138	1 pack	Four probe sheaths pcs included in one package.
XDM-100 BASIC	888-0164-C	1 pc	Mixer bag for medium preparation in XDUO-100.
XDR-50 Microbial BBA	888-0235	1 pc	50 L Single-Use microbial bioreactor bag
Capto L, 25 mL	17547801	2 pcs	Affinity resin for Dab capture
Pilot Scale Ultrafiltration Cartridge	UFP-750-E-6A	1 pc	Hollow fiber cartridge for clarification of harvest
Flow Kit plus RM, ÄKTA readyflux	29187382	1 pc	Flow kit for ÄKTA ready Flux