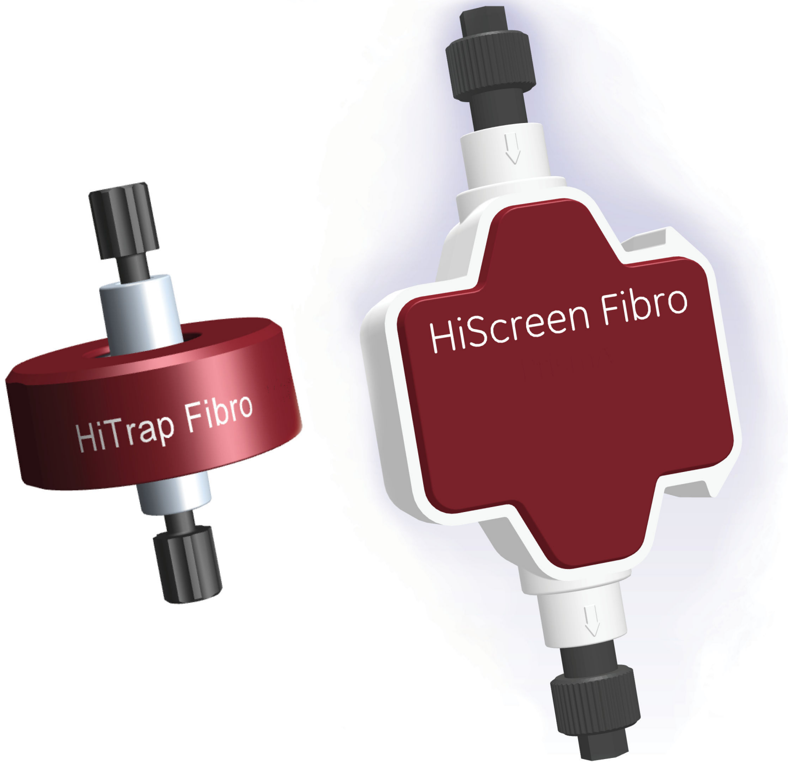
cytiva

HiTrap Fibro PrismA
HiScreen Fibro PrismA

Instructions for Use



1 Introduction

HiTrap™ Fibro PrismA and HiScreen™ Fibro PrismA (Fibro PrismA units) are ready to use units for capturing monoclonal antibodies (mAbs) and Fc-containing recombinant proteins. Single batch disposable format eliminates the need for sanitization. However, the alkali-tolerant protein A-derived ligand allows the use of 0.5 to 1.0 M sodium hydroxide for Cleaning-In-Place (CIP) in case of challenging mAb harvest materials and extended cycling.

The matrix consists of PrismA protein A ligand coupled to cellulose fibers. The binding capacities for the Fibro PrismA units are comparable to the capacity of chromatography beads. However, the Fibro PrismA units have high binding capacity at short residence times. This enables cycle times of less than 5 minutes. Short cycle times makes it possible to screen binding capacities and running conditions in a high throughput manner.

Good capacity, low ligand leakage, plus the rigid matrix, make Fibro PrismA units ideal for the purification of monoclonal antibodies. The ready to use format is well suited for preparative purifications when short cycle times, high productivity, and flexibility is important. The units are especially useful for cell cultures with low expression levels.

The Fibro PrismA units can be used for up to 200 cycles before disposal, depending on the application. A full lifetime study can be conducted in less than 24 hours.

2 Product description

2.1 Fibro technology

The Fibro technology used for HiTrap Fibro and HiScreen Fibro (Fibro units) offers faster protein capture compared to resin-based chromatography, with residence times measured in seconds rather than minutes.

The Fibro technology is based on electrospun cellulose fibers. The porous matrix for the Fibro units has an open and a well-defined structure without dead-end pores, see *Fig. 2.1, on page 3*. The pore size of the matrix has been optimized, the surface of the cellulose fibers modified, and suitable ligand has been coupled.

The high porosity and high mechanical strength of the Fibro matrix allows for operation under high flow rates, only restricted by the size of the pores in the matrix. The large accessible surface area enables high binding capacity, comparable to the binding capacity of chromatographic beads.

The mass transfer in the matrix is governed by convective flow, in contrast to diffusion within chromatography beads. The immediate mass transfer within the Fibro PrismA units leads to high binding capacity at short residence times, see *Fig. 2.2, on page 3*.

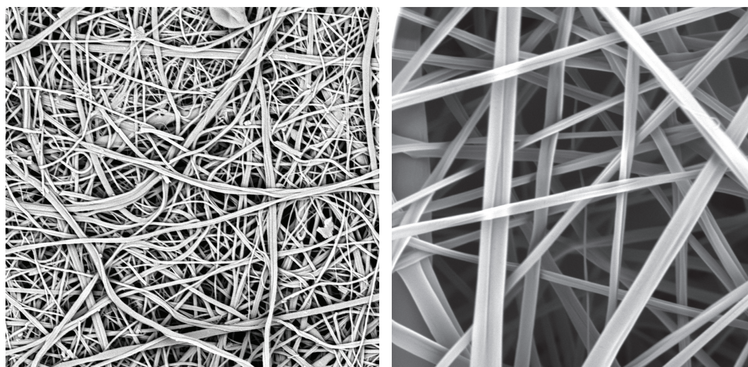


Figure 2.1: The Fibro matrix is shown with a magnification of 4000X and 15000X.

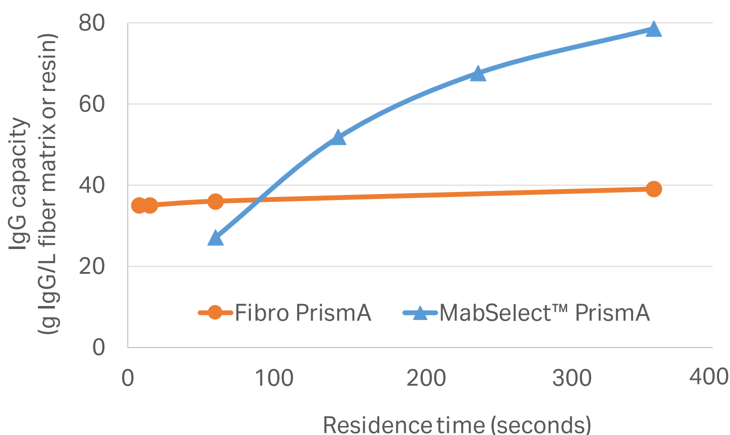


Figure 2.2: Binding capacity vs residence time.

2.2 HiTrap Fibro and HiScreen Fibro

HiTrap Fibro and HiScreen Fibro units (Fibro units) are ready to use units for research and early process development. The Fibro units can be operated with a peristaltic pump or a chromatography system. The HiTrap Fibro unit can also be operated with a syringe.

The HiTrap Fibro unit can be operated using bidirectional flow. For the HiScreen Fibro unit there is a defined inlet and outlet. Elution of the proteins should always be done in the defined flow direction for smallest possible elution pool volume.

The Fibro units are delivered with stoppers at the inlet and outlet. The characteristics of the HiTrap Fibro and HiScreen Fibro units are summarized in the table below.

Table 2.1: Characteristics of HiTrap Fibro and HiScreen Fibro

	HiTrap Fibro	HiScreen Fibro
Matrix material	Derivatized electrospun cellulose fibers	
Housing material	Biocompatible polypropylene[1]	
Plastic cover (red part)	Polyamide	
Matrix volume (MV)	0.4 mL	3.75 mL
Housing pressure limit	1.0 MPa (10 bar)	
Recommended ÄKTA™ systems	Avant/pure 25	Avant/pure 150

[1] Does not interact with biomolecules.

2.3 Fibro PrismA units

HiTrap Fibro PrismA and HiScreen Fibro PrismA (Fibro PrismA units) are ready to use units for capturing monoclonal antibodies and Fc-containing recombinant proteins. The characteristics of the Fibro PrismA units are summarized in the table below.

Table 2.2: Characteristics of HiTrap Fibro PrismA and HiScreen Fibro PrismA

	HiTrap Fibro PrismA	HiScreen Fibro PrismA
Ligand	PrismA ligand (alkali-tolerant, protein A-derived from *E. coli*)	
Coupling chemistry	Single point attachment	
Dynamic binding capacity (DBC), Q_{B10}[1]	~ 30 mg polyclonal IgG/mL matrix	
DBC, Q_{B10}[1] per unit	~ 12 mg polyclonal IgG/ HiTrap Fibro unit	~ 112 mg polyclonal IgG/ HiScreen Fibro unit
Cycle time	~ 3 min	~ 5 min
Recommended operating flow rate	≤ 16 mL/min (40 MV/min)	≤ 30 mL/min (8 MV/min)
Maximum operating pressure	1 MPa (10 bar)	
Chemical stability	Compatible with commonly used aqueous buffers for protein A chromatography	
Chemical compatibility	Compatible with chemical compounds specified in *Chemical compatibility, on page 6*	

	HiTrap Fibro PrismA	HiScreen Fibro PrismA
pH stability[2] Operational[3] CIP[4]	 pH 3 to 12 pH 2 to 14	
Temperature stability Operational Storage	 4°C to 35°C 2°C to 8°C	
Storage solution	20% (v/v) ethanol in water	

[1] Determined at 10% breakthrough by frontal analysis in Tris buffer, pH 7.5.
[2] The pressure increases at pH ≤3 and low conductivity.
[3] pH range where the Fibro PrismA unit can be operated without significant change in function.
[4] pH range where the Fibro PrismA unit can be subjected to CIP without significant change in performance.

PrismA ligand

The matrix is coupled with the PrismA protein A ligand. The protein A-derived ligand is produced in *Escherichia coli*. Fermentation and subsequent purification are performed in the absence of animal products. The ligand has been specially engineered for enhanced alkali and protease stability. The specificity of binding to the Fc region of IgG is similar to that of conventional protein A and provides excellent purification in one step. The PrismA ligand also has affinity for the VH3 chain, which gives the possibility to use it for purification of antibody fragments.

Applications

The Fibro PrismA units have a binding capacity of approximately 30 mg/mL at residence times of 1.5 to 7.5 seconds.

Good capacity, low ligand leakage, plus the rigid matrix, make Fibro PrismA units ideal for the purification of monoclonal antibodies. The ready to use format is well suited for preparative purifications when short cycle times, high productivity and flexibility is important. Because of the high flow rates and the high throughput, the units are especially useful for cell cultures with low expression levels. The disposable/single batch format eliminates the need for sanitization.

Stability

The alkali-tolerant protein A-derived ligand allows the use of 0.5 to 1.0 M NaOH for Cleaning-In-Place (CIP) in each purification cycle. In case of challenging mAb harvest materials even 1.5 to 2.0 M NaOH can be used. The figure below shows the dynamic binding capacity (DBC) for HiTrap Fibro PrismA after CIP with NaOH concentrations of 0.5 to 2.0 M.

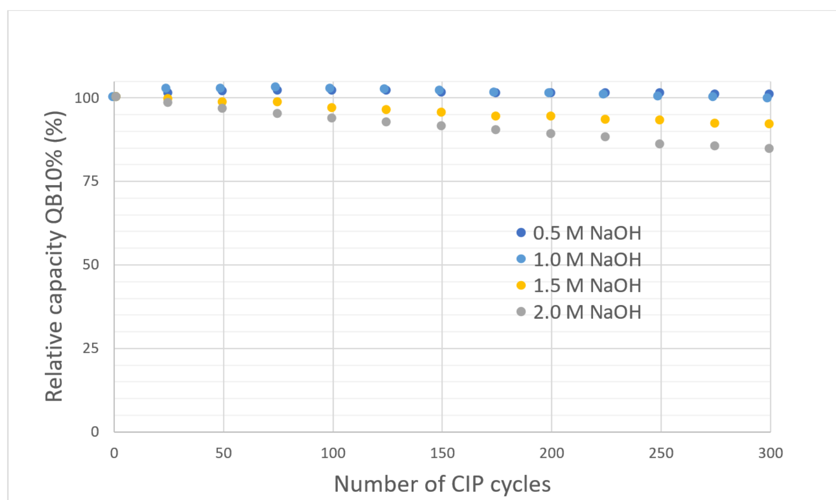


Figure 2.3: Relative DBC for HiTrap Fibro PrismA after CIP for 300 cycles, 1 min contact time per cycle.

The data presented above was obtained by performing the following study.

Binding buffer: 20 mM phosphate, 150 mM NaCl, pH 7.4

Elution buffer: 50 mM sodium acetate, pH 3.5

The unit was equilibrated with binding buffer before start.

Each cycle consisted of:

- 1 mL binding buffer, flow rate 16 mL/min
- 6 mL elution buffer, pH 3.5, 16 mL/min
- 8 mL NaOH (0.5 to 2.0 M.), 8 mL/min, 1 min contact time
- 4 mL binding buffer, 8 mL/min
- 8 mL binding buffer after 0.5 and 1.0 M NaOH, or 13 mL binding buffer after 1.5 and 2.0 M NaOH, 16 mL/min

DBC at 10% breakthrough by frontal analysis, Q_{B10}, for a pre-purified monoclonal antibody (IgG1) was measured every 25th cycle during the study.

Chemical compatibility

HiTrap and HiScreen Fibro PrismA have verified compatibility to the compounds shown in the table below. More than 95% of the dynamic binding capacity (polyclonal IgG) remained after 24 hours storage at 22-24°C in these solutions.

Compound	Concentration
1-propanol	30%
2-propanol	30%
Acetic Acid	1.0 M
Acetone	2.5%
Arginine	0.5 M
Benzyl alcohol	2%
DTT (in PBS, pH 7.4)	100 mM
EDTA	100 mM
Ethanol	20%
Ethanol	70%
Glycine	1.0
Guanidine hydrochloride	6 M
Imidazole	1 M
Pluronic™ F-6800	1%
Polyethylene glycol, PEG 200	5%
Polyethylene glycol, PEG 1500	1%
Polyethylene glycol, PEG 4000	1%
SDS	1%
Sodium acetate	1 M
Sodium chloride	1 M
Sodium citrate	1 M
Sucrose	1 M
Thioglycerol (in PBS, pH 7.4)	100 mM
Tween™ 20	1%
Tween 80	1%
Urea	8 M

3 Operation

3.1 Preparations

Instrument Configuration

For using the predefined chromatography methods for Fibro, make sure that your system is configured with the latest Instrument Configuration available at *cytiva.com/akta*, then select your system, choose ***Related Documents*** →***Software***.

Preparation of the chromatography system

- Disconnect the mixer chamber on the system by connecting the tube from the pump valve directly to the injection valve. If there is a separate mixer valve it can be bypassed in the method.
- Bypass the flow restrictor in the method to keep the system back pressure to a minimum.

An online filter can be installed between the pump valve and the injection valve.

Preparation of buffers

- Use water and chemicals with high purity for buffer preparation.
- Filter buffers through a 0.22 or 0.45 µm filter before use.
- Preferably prepare buffers as concentrated stock solutions that are filtered before dilution in purified water or Water for Injection (WFI).

For examples of suitable buffers see *Table 3.3, on page 11*.

Sample for DBC

Pre-purified mAb is preferably used for determination of DBC by frontal analysis. See the table below for recommended concentrations.

Dilute the sample with buffer with a higher conductivity than the binding buffer. The delay volume of the unit and system can then be determined in the frontal analysis for determination of DBC. This is described in the optimization section, see *Delay volume, on page 16*.

Table 3.1: Recommended concentration of pure protein for frontal analysis

HiTrap Fibro	HiScreen Fibro
0.5 mg/mL	2.0 mg/mL

Prepare the sample as described below:

Step	Action
1	Adjust the sample to the composition of the start buffer with additional salt using one of these two methods: **a.** Dilute the sample with binding buffer with additional salt. **b.** Perform a buffer exchange using a prepacked column for desalting.
2	Filter the sample through a 0.22 or 0.45 µm filter or centrifuge immediately before loading it to the Fibro unit. This prevents clogging and increases the lifetime of the unit when loading large sample volumes.

Cell culture supernatants

Particulates might clog the Fibro unit and increase back pressure over time. If the mAb harvest material is thoroughly clarified, the Fibro PrismA units can be used for up to 200 cycles.

Clarify the cell culture using conventional harvest steps, see suggestions in the table below. For challenging materials, especially with low titer when large volumes need to be processed, a more rigorous harvest can be required. Then add for example charged depth filtration to the harvest procedure.

Sterile filter the mAb cell culture supernatant into a sterile bag or container just previous to the Fibro PrismA run. This will prevent bacterial growth and is especially important for cycling studies that span over a longer time.

Note: *Cell culture supernatant that has been stored either in liquid form or as frozen material must always be sterile filtered into a sterile container just before loading to the Fibro unit.*

Note: *Decrease the flow rate at low temperatures or when viscous solutions are used.*

NOTICE

Do not exceed the maximum operating pressure, 1.0 MPa.

Table 3.2: Clarification of the cell culture

Step	Centrifugation/filtration	Filter train
Cell removal	Centrifugation	Coarse depth filter
Polishing	Fine depth filter	Fine depth filter
Sterile filtration	0.22 µm filter	

Connect the Fibro unit to the system

For HiTrap Fibro PrismA:

- Fingertight Connector 1/16" Male

For HiScreen Fibro PrismA:

- Connector 1/16" Male
- Ferrules for o.d. 1/16" tubing connector

Connect the Fibro unit to the ÄKTA system column valve using PEEK Tubing, i.d. 1.0 mm, o.d. 1/16".

Drop-to-drop connection or venting is not needed, if air has been introduced into the Fibro unit it will be removed when flushing with buffer in the first blank run.

Attach the HiScreen unit in a clamp holder for columns. The HiTrap unit does not need a holder.

Note: *Make sure that the connectors are tight to prevent leakage.*

3.2 Recommended starting conditions

General recommendations

To keep the buffer consumption and time to a minimum, some steps can be avoided in the method. The following three steps can be replaced by switching to the next buffer and chasing previous buffer.

1. System wash
2. Pump wash
3. Priming

Buffers

Table 3.3: Examples of suitable buffers

Binding buffer	20 mM sodium phosphate, 0.15 M NaCl, pH 7.4
Elution buffer	50 to 100 mM sodium acetate, pH 3.0 to 3.6
Post load wash buffer 1	Buffer with 0.5 to 1 M NaCl, pH neutral or close to neutral, e.g., 20 mM phosphate, 0.5 to 1.0 M NaCl, pH 7.0
Post load wash buffer 2	Same buffer system as the elution buffer, but at a higher pH, e.g., 50 mM acetate, pH 5.5 to 6.0

Operating flow rate and volume

Recommended flow rates and volumes for HiTrap Fibro PrismA and HiScreen Fibro PrismA on their recommended systems are shown in the table below.

Table 3.4: Recommended operating flow rates and volumes

Phase	HiTrap Fibro PrismA ÄKTA avant/pure 25		HiTrap Fibro PrismA ÄKTA avant/pure 150		HiScreen Fibro PrismA ÄKTA avant/pure 150	
	Volume (mL)	**Flow rate (mL/min)**	**Volume (mL)**	**Flow rate (mL/min)**	**Volume (MV)**	**Flow rate (mL/min)**
Equilibration	6	16	8	16	6	30
Wash	6	16	8	16	6	30
Elution	6	16	8	16	6	30
CIP	8	8 or 16[1]	8	8 or 16[1]	4	15 or 30[1]
Wash	6	8 or 16[1]	6	8 or 16[1]	2	15 or 30[1]
Re-equilibra-tion	10	16	22	16	10	30

[1] Depending on 1 or 0.5 min CIP.

Before starting a method

A blank run including CIP is recommended before first time use or after storage.

Step	Action
1	Prime inlets and system with buffers.

Step	Action
2	Equilibrate the Fibro unit with loading buffer at 5 mL/min (HiTrap) or 10 mL/min (HiScreen) to remove the storage solution (20% ethanol). The storage solution can also be washed out with distilled water before the equilibration.
3	Run a blank run including all buffers and CIP solution in the method except for sample load. This removes any loosely (non-covalently) bound ligand. *Result:* The Fibro unit is ready for use.

Recommended method for HiTrap Fibro PrismA

Before starting a method, the unit can be manually equilibrated with binding buffer until the UV baseline, pH, and conductivity are stable. If so, step 1 in the method below can be shortened.

For this method for ÄKTA avant/pure 25, the A-pump is used in all steps except for elution, which is done using the B-pump, and for the sample load, where the sample pump is used.

For recommended flow rate see *Recommended flow rate, on page 15*.

Step	Action
1	Equilibrate with 6 mL of binding buffer. Make sure the UV baseline, pH, and conductivity are stable.
2	Load the sample.
3	Wash with 6 mL of binding buffer. Make sure the UV returns to near base line.
4	Elute by a step elution with 6 mL of elution buffer.
5	CIP with 8 mL at 8 mL/min for a contact time of 1 min, or 16 mL/min for a contact time of 0.5 min.
6	Wash with 6 mL binding buffer at the same flow rate as in step 5.

Step	Action
7	Re-equilibrate with 10 mL binding buffer, 16 mL/min. Make sure that pH and conductivity are stable. A typical chromatogram is shown below.

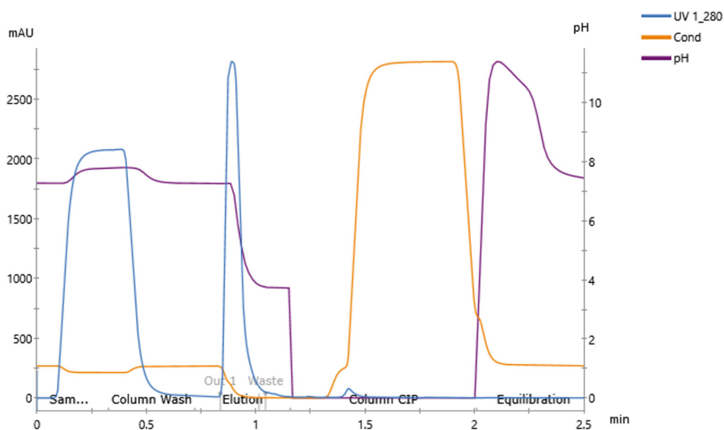


Figure 3.1: HiTrap Fibro PrismA on ÄKTA pure 25.

Recommended method for HiScreen Fibro PrismA

Before starting a method, the unit can be manually equilibrated with binding buffer until the UV baseline, pH, and conductivity are stable. In that case, step 1 in the method can be shortened.

For this method for ÄKTA avant/pure 150, the A-pump is used in all steps except for elution, which is done using the B-pump, and for the sample load, where the sample pump is used.

Step	Action
1	Equilibrate with 6 MV of binding buffer. Make sure the UV baseline, pH, and conductivity are stable.
2	Load the sample.
3	Wash with 6 MV of binding buffer. Make sure the UV returns to near base line.
4	Elute by a step elution with 6 MV of elution buffer.
5	CIP for 4 MV at 15 mL/min for a contact time of 1 min, or 30 mL/min for a contact time of 0.5 min.
6	Wash with 2 MV binding buffer at the same flow rate as in step 5.
7	Re-equilibrate with 10 MV binding buffer, 16 mL/min. Make sure that pH and conductivity are stable. A typical chromatogram is shown below.

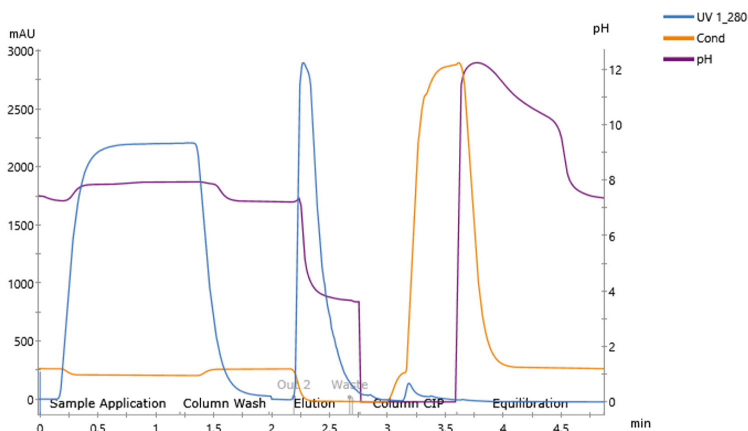


Figure 3.2: HiScreen Fibro PrismA on ÄKTA pure 150.

4 Optimization

This chapter describes procedures for optimizing your process like determination of DBC, and how to optimize conditions for wash and elution.

The Fibro PrismA units should be used for research and early process development.

- HiTrap Fibro PrismA should be used for high throughput purification, screening of buffer conditions, and lifetime studies.

- HiScreen Fibro PrismA should be used for early process development, optimization, and verification of the HiTrap screening.

4.1 Determination of DBC

DBC, typically at 10% breakthrough (Q_{B10}), of the Fibro PrismA unit is determined by frontal analysis in the same way as for resin-based chromatography. The target protein is loaded beyond the breakthrough to be evaluated, and the amount mAb bound/loaded is calculated, see *Evaluation of DBC, on page 17*. The evaluation can be done using the UNICORN extension DBC calculations, refer to *How to determine dynamic binding capacity (DBC) of chromatography resins* at cytiva.com.

The following sections describes how to determine some input parameters needed for the evaluation of DBC.

DBC extension

The *DBC calculation extension* at cytiva.com provides the capability of DBC calculations in UNICORN in an extension. It can be used in UNICORN with version 6.4 or later releases. For help on installing DBC calculation extension, please contact your local ÄKTA system specialist.

Recommended flow rate

The binding capacity is relatively independent of the residence time. Even so, the DBC should be determined at flow rates corresponding to the residence time in the intended application.

Table 4.1: Recommended flow rate

Unit	Flow rate	Residence time
HiTrap Fibro PrismA	16 mL/min (40 MV/min)	1.5 seconds
HiScreen Fibro PrismA	30 mL/min (8 MV/min)	7.5 seconds

Sample

Pre-purified mAb is preferably used for determination of DBC by frontal analysis. High concentration of mAb in the sample results in low volume applications for the Fibro units and makes the determination of DBC difficult. See *Sample for DBC, on page 8* for instructions on preparation and recommended concentrations.

The DBC for the target mAb can also be determined by frontal analysis using real process feedstock. If so, collect fractions during sample application for off-line analysis.

Delay volume

The delay volume of the unit and the system can be determined by a conductivity difference between sample and binding buffer. The volume at a 50% increase in conductivity can be used.

By diluting the mAb sample to the specified concentration with a buffer with higher NaCl concentration than the binding buffer (for example 250 mM NaCl) a shift in conductivity will appear at the beginning of sample load.

Unbound species

If the sample contains species that don't bind to the ligand there will be a 280 nm offset plateau, called ***Contaminant offset*** in the UNICORN extension DBC calculations. This offset is determined from the UV280 nm offset plateau that is reached before the breakthrough.

Max absorbance

Determine the max absorbance of the sample used just before the start of the frontal analysis. This is called ***100% absorbance*** in the UNICORN extension.

Step	Action
1	Bypass the column valve.
2	Run binding buffer through the system. Auto-zero the UV when the signal is stable.
3	Inject the mAb sample.
4	When the UV signal is stable, determine the 100% UV absorbance at 280 nm.
5	Run binding buffer through the system until the UV signal returns to zero.

Frontal analysis

DBC should be determined at flow rates corresponding to the residence time in the intended application.

Step	Action
1	Equilibrate the unit with binding buffer until the UV baseline, pH, and conductivity are stable, and then auto-zero the UV signal.
2	Load the sample to a breakthrough beyond the breakthrough to be evaluated.
3	Wash with binding buffer.

Step	Action
4	Elute the bound protein with elution buffer.
5	CIP with a contact time of 0.5 min.
6	Re-equilibrate with binding buffer. *Result:* The following input parameters needed for the evaluation of DBC can be determined from this frontal analysis: • V_x, the retention volume at selected breakthrough • V_{delay}, delay volume of the unit and the system • A(V), absorbance at given retention volume • A_{offset}, absorbance for the offset plateau for unbound species

Evaluation of DBC

The UNICORN extension DBC calculations can be used for evaluation of DBC at different breakthrough levels. For description of the calculations, refer to *How to determine dynamic binding capacity (DBC) of chromatography resins* at cytiva.com.

For Fibro PrismA units the delay volume compared to the matrix volume is larger than the delay volume compared to the column volume for resin-based chromatography. Therefore, it is important that the delay volume is considered in the calculation. This corresponds to the blue area in the figure below.

The orange area in the figure describes the amount of unbound species, that is, UV offset.

The unbound amount of protein that breaks through is shown as the green area. Using the calculation below the DBC is evaluated as the grey area.

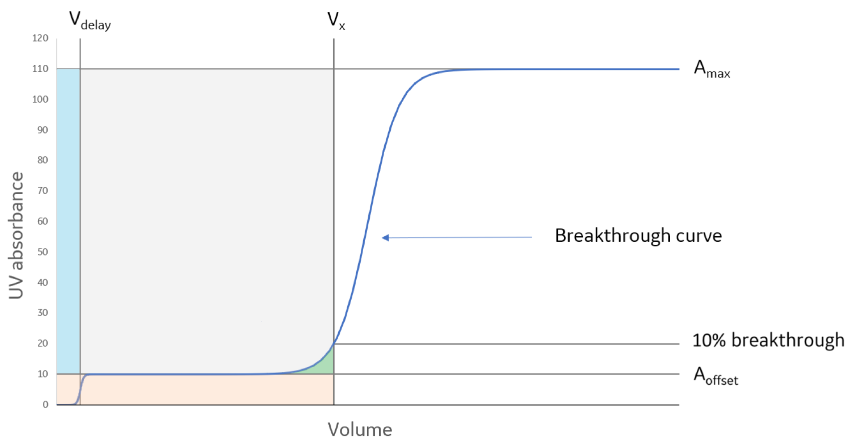


Figure 4.1: Evaluation of DBC.

Calculation of Q_{B10} as described in the figure above is described in the following equation.

$$Q_{BX\%} = \frac{C_0}{MV}\left[V_X - V_{delay} - \int_{V_{delay}}^{V_X} \frac{A(V) - A_{offset}}{A_{max} - A_{offset}}\,dv\right]$$

where

C_0	=	the concentration for the sample
MV	=	the volume of the matrix
V_x	=	the retention volume at selected breakthrough
V_{delay}	=	delay volume of the unit and the system
A(V)	=	absorbance at given retention volume
A_{offset}	=	absorbance for the offset plateau for unbound species
A_{max}	=	max absorbance for the sample solution

4.2 Wash conditions

In the recommended methods in *Section 3.2 Recommended starting conditions, on page 10* the binding buffer is used for washing after sample load. For more efficient removal of host cell proteins and other process-related impurities additional post load washes can be used.

A typical post load wash buffer can be a high salt containing buffer (0.5 to 1 M NaCl) at pH neutral or close to neutral. After the salt wash, before eluting, it is recommended to wash the unit with the same buffer system as the elution buffer, but at a higher pH. This will eliminate salt in the eluate pool and condition the unit with the same buffer system as the elution buffer. For examples of buffers, see *Buffers, on page 11*.

The figure below shows an example chromatogram for a HiTrap Fibro PrismA unit operated on an ÄKTA pure 25 with post load wash with 20 mM phosphate, 0.5 M NaCl, pH 7, followed by 50 mM acetate, pH 6.0.

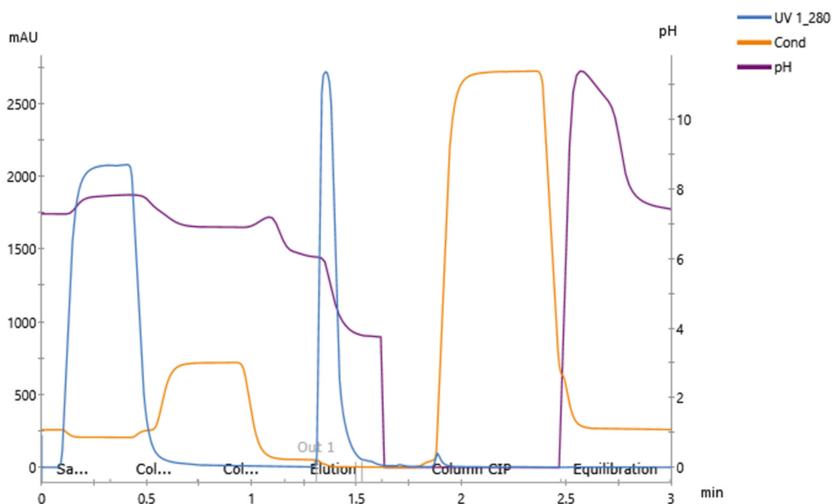


Figure 4.2: Example chromatogram including additional post load washes.

4.3 Elution conditions

Optimizing pH

Determine the highest pH that allows efficient elution of the mAb. This prevents denaturation of sensitive mAb caused by exposure to low pH.

Neutralizing fractions

Neutralize the fractions by adjusting the pH after elution, or elute into an alkaline buffer, for example 1 M Tris base.

Stepwise elution

Stepwise elution allows the target antibody to be eluted in a more concentrated form, reducing buffer consumption, and shortening cycle times.

4.4 Cycling/Lifetime studies

Since the Fibro format is suitable for rapid cycling it is relevant to perform lifetime studies. A study of 200 cycles can take less than 24 hours.

Trend the purification performance over the cycles by collecting the eluate from every 50th cycle in a separate test tube for off-line analysis. The rest of the eluates can be collected in a common outlet (bulk pool). The elution profile and pressure curves are compared by overlays of the chromatograms.

5 Removal of leached ligand from final product

The PrismA protein A ligand can be analyzed using commercially available protein A immunoassays. The ligand leakage from Fibro PrismA is generally very low. For example, the eluate from the purification run shown in *Section 4.2 Wash conditions, on page 18* contained 9 ppm (ng ligand/mg antibody) of leached ligand.

However, in some monoclonal antibody applications it is a requirement to eliminate leached ligand from the final product. There are a number of chromatographic solutions to remove leached ligand, such as ion exchange chromatography, multimodal exchange chromatography, and size exclusion chromatography. The optimal conditions for removal of leached ligand must be evaluated for each individual antibody.

6 Cleaning-In-Place (CIP)

6.1 General description

CIP removes very tightly bound, precipitated, or denatured substances from the Fibro matrix. The accumulated contaminants can affect the chromatographic properties of the unit, increase back pressure, reduce the capacity, or lead to carry-over between cycles. Fibro PrismA is a highly alkali-tolerant matrix that allows the use of 0.5 to 1.0 M NaOH for CIP. The use of NaOH will lead to a decrease in binding capacity, due to caustic degradation of the protein-based ligand. For the recommended contact times and concentrations of 0.5 to 1.0 M NaOH loss of capacity is of less concern, see *Fig. 2.3, on page 6*. When needed, higher concentration of NaOH, other chemicals, and increased contact time can be used, see *Chemical compatibility, on page 6* and *Section 6.3 CIP optimization, on page 21*.

CIP must be performed in every cycle to prevent the enrichment of the contaminants and to maintain the capacity, flow properties, and general performance of the unit. CIP is included in the recommended methods, see *Recommended method for HiTrap Fibro PrismA, on page 12* and *Recommended method for HiScreen Fibro PrismA, on page 13*.

Mixing of acidic and alkaline solutions can cause a rise in temperature. In the recommended methods, CIP is performed using the A-pump and elution is performed using the B-pump. Since system washes are not used, the CIP solution will chase the previous buffer in the A-line. This will prevent direct contact between low pH and high pH solutions in the unit, and the risk for rise in temperature is avoided.

6.2 CIP protocol

Make sure the volume in step 3 is sufficient to reach the same pH and conductivity as for the binding buffer.

HiTrap Fibro PrismA

1. CIP with 8 mL NaOH (0.5 to 1.0 M), flow rate 16 mL/min for a contact time of 0.5 min, or 8 mL/min for a contact time of 1 min.
2. Wash immediately with 6 mL binding buffer at the same flow rate as in step 1.
3. Re-equilibrate with binding buffer, 16 mL/min, for
   - 10 mL for ÄKTA pure/avant 25
   - 22 mL for ÄKTA pure/avant 150

or until the effluent pH and conductivity reach the values for the binding buffer.

HiScreen Fibro PrismA

1. CIP with 4 MV of NaOH (0.5 to 1.0 M), flow rate 30 mL/min (8 MV/min) or 15 mL/min (4 MV/min) for a contact time of 0.5 or 1 min.
2. Wash immediately with 2 MV binding buffer at the same flow rate as in step 1.
3. Re-equilibrate with binding buffer, 30 mL/min, for 10 MV, or until the effluent pH and conductivity reach the values for the binding buffer.

6.3 CIP optimization

Reversed flow for CIP and subsequent re-equilibration can be used for both units for more efficient removal of impurities on the top surface of the fiber matrix.

Higher concentrations of NaOH and/or longer contact time increases the CIP efficiency. If needed, NaOH concentrations up to 2.0 M can be used. The CIP contact time can also be increased to up to 3 minutes/cycle. However, these conditions might also lead to a decrease in the DBC, see *Fig. 2.3, on page 6*

Other chemicals such as polar solvents, detergents, and other cleaning reagents can also be used, see *Chemical compatibility, on page 6*.

7 Storage

Store HiTrap and HiScreen Fibro PrismA in 20% ethanol at 2°C to 8°C.

Before use, equilibrate with binding buffer and perform a blank run, including CIP.

NOTICE

The unit must be filled with 20% ethanol before storage.

Note: *Decrease the flow rate when 20% ethanol is used.*

8 Troubleshooting

This chapter describes different problems that may arise when using the Fibro units, and how to solve the problems.

Table 8.1: Troubleshooting

Problem	Possible cause	Corrective action
Leakage		
Leakage from unit when starting a flow.	Connectors not properly screwed into the unit.	Tighten connections from column valve to unit.
Pressure		
High pressure in the first run.	Flow restrictor in line.	Disconnect the flow restrictor.
	Solutions with high viscosity or low temperature.	Use a lower flow rate or control the flow by pressure.
	Online filter is clogged.	Change online filter.
	Damaged/clogged PEEK tubing connecting the unit to the column valve.	Change the PEEK tubing connecting the unit to the column valve.
High pressure during elution/ acidic strip.	Low pH combined with low conductivity can lead to pressure increase.	• Decrease the flow rate on the elution or strip step to half the recommended flow rate. • Add low molarity of salt (≤5 mM NaCl) to the elution or strip buffer.

Problem	Possible cause	Corrective action
Large increase in pressure over multiple cycling.	Fouling of Fibro matrix due to inefficient CIP.	• Run CIP after each purification cycle. • Increase the NaOH concentration and/or CIP contact time if problem persists. • Run the CIP and wash/re-equilibration after CIP in reversed flow.
	Inefficient elution.	• Optimize elution condition. • Add a strip step, with lower pH and higher molarity than elution buffer, after elution.
	Challenging feed stream (mAb harvest) material.	Consider pre-treatment of the feed stream by filtering through depth filter/charged depth filter.
Recovery		
Low recovery.	Delay volume of the system is not correct.	Make sure that the delay volume is correct (System settings)
	Elution peak collection criteria not optimal.	Change the pool collection criteria. Recommended is to collect the elution peak from 20 to 100 mAU for the HiTrap Fibro PrismA unit and from 20 to 150 mAU for the HiScreen Fibro PrismA unit.
	Target protein is breaking through during sample application because of too high sample load.	Decrease the sample load.

Problem	Possible cause	Corrective action
Decreasing recovery over multiple cycles.	Decrease in binding capacity over multiple cycles because of fouling of the Fibro matrix due to inefficient CIP.	• Run CIP after each purification cycle. • Increase the NaOH concentration and/or CIP contact time if problem persists. • Run the CIP and wash/re-equilibration after CIP in reversed flow.
	Decrease in binding capacity over cycles because of inefficient elution.	• Optimize elution condition. • Add a strip step, with lower pH and higher molarity than elution buffer, after elution.
Elution volume		
Broader elution peaks than expected.	Flow path has not been minimized.	Ensure that the flow path is minimized by disconnecting or bypassing the mixer chamber.
	Low molarity of elution buffer and high molarity of the wash buffer prior to elution.	Include a prime in the elution step either by using the fill system command or by switching the unit out of the flow path until the buffers exchange.
Peak broadening over multiple cycles.	Fouling of Fibro matrix due to inefficient CIP.	• Run CIP after each purification cycle. • Increase the NaOH concentration and/or CIP contact time if problem persists. • Run the CIP and wash/re-equilibration after CIP in reversed flow.

Problem	Possible cause	Corrective action
Ligand leakage		
High ligand leakage in the first cycle.	Loosely (non-covalently) bound ligand that elutes with the mAb in the first cycle.	Perform a blank run, including CIP, before the first purification cycle on a new unit
Performance		
Reduced performance despite optimized elution and CIP	Unit longevity, which depends mainly on the sample type and sample preparation	Discard the unit and change to a new.

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