



Fibro dT 0.4 mL, 4-pack

Instructions for Use



1 Introduction

Fibro dT 0.4 mL comes as a 4-pack and is a ready-to-use unit for capturing mRNA and polyadenylated oligonucleotide chains. The single-batch, disposable format eliminates the need for sanitization. However, the alkali-tolerant, monomeric oligonucleotide chain ligand allows for the use of 0.1 to 0.5 M NaOH for cleaning-in-place (CIP) in case of challenging mRNA materials and extended cycling.

In the remainder of this document, we refer to fibro dT 0.4 mL as the fibro dT unit or the unit.

The matrix consists of derivatized electrospun cellulose fibers and the ligand is made up of a single-stranded sequence of deoxythymidine (dT). The unit has high binding capacity at short residence times. This allows for cycle times shorter than 15 minutes. Short cycle times enable high-throughput screening of binding capacities and running conditions.

High capacity, low ligand leakage, and a rigid matrix make the unit ideal for purifying mRNA and polyadenylated oligonucleotide chains. The ready-to-use format is well suited for preparative purifications requiring short cycle times, high productivity, and flexibility.

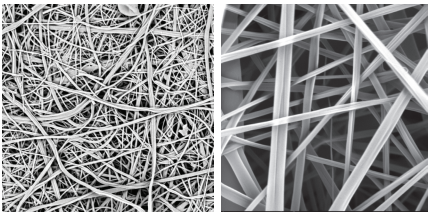
The unit can be used for up to 20 cycles before disposal, depending on the application. A full lifetime study can be performed in under 24 hours.

2 Product description

2.1 Fibro technology

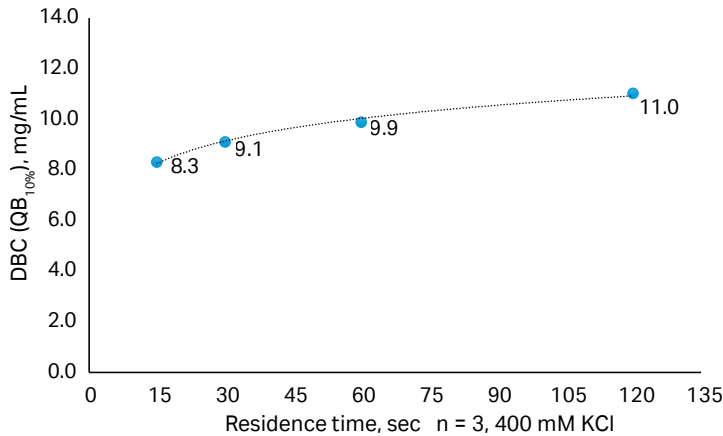
The technology used in the fibro dT unit offers faster mRNA capture compared to resin-based chromatography, with residence times measured in seconds rather than minutes.

The technology is based on electrospun cellulose fibers. The porous matrix of the unit has an open and a well-defined structure without dead-end pores, as shown in the following magnification images (4000× and 15 000×). The pore size of the matrix is optimized, the surface of the cellulose fibers is modified, and a suitable ligand is coupled.



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

Mass transfer in the matrix is driven by convective flow, unlike diffusion in chromatography beads. The immediate mass transfer allows for high binding capacity at short residence times, as shown in the following diagram illustrating DBC at different residence times. The diagram is based on a 2000-nucleotide mRNA transcript. Other sizes or constructs might show slightly different values.



2.2 Fibro unit

A fibro unit is a ready-to-use unit for research and early process development. The unit can be operated with a peristaltic pump, a chromatography system, and a syringe.

The unit can be operated using bidirectional flow.

The unit is delivered with stoppers at the inlet and outlet. The following table lists the characteristics of the unit.

Parameter	Fibro unit
Matrix material	Derivatized, electrospun cellulose fibers
Housing material	Biocompatible polypropylene ¹
Plastic cover (red part)	Polypropylene
Matrix volume (MV)	0.4 mL
Housing pressure limit	1.0 MPa (10 bar)
Recommended systems	ÄKTA pure™/ÄKTA avant™ 25 and 150

¹ Does not interact with biomolecules.

2.3 Fibro dT unit

The fibro dT unit is a ready-to-use unit for capturing mRNA and polyadenylated oligonucleotide chains. The following table lists the characteristics of the unit.

Parameter	Fibro dT unit
Ligand	Single-stranded sequence of deoxythymidine (dT)
Coupling chemistry	Single-point attachment
Dynamic binding capacity (DBC), QB _{10%} ¹	> 6 mg/mL matrix
DBC, QB _{10%} ¹ per unit (2000 nT)	> 2.4 mg/fibro dT unit
Cycle time	~ 10 min
Recommended flow rate range ²	0.4–10 mL/min (1–25 MV/min)
Maximum operating pressure	1 MPa (10 bar)
Chemical stability	Compatible with commonly used aqueous buffers for mRNA chromatography
Chemical compatibility	Compatible with chemical compounds specified in Chemical compatibility on page 7
pH stability ³ Operational ⁴ CIP ⁵	pH 3 to 12 pH 2 to 14
Temperature stability Shipping Operational Storage	Ambient ⁶ 4°C to 25°C 2°C to 8°C
Delivery conditions	20% (v/v) ethanol in water
Storage solution	20% (v/v) ethanol in water

¹ Determined at 10% breakthrough by frontal analysis in the recommended binding buffer with 400 mM KCl, 10 mM Tris-HCl, 1 mM EDTA.

² It is recommended to perform the sample application and elution steps at 0.4–1.6 mL/min. The remaining steps can be performed at up to 10 mL/min.

³ The pressure increases at pH ≤ 3 and low conductivity.

⁴ pH range where the fibro dT unit can be operated without significant change in function.

⁵ pH range where the fibro dT unit can be subjected to CIP without significant change in performance.

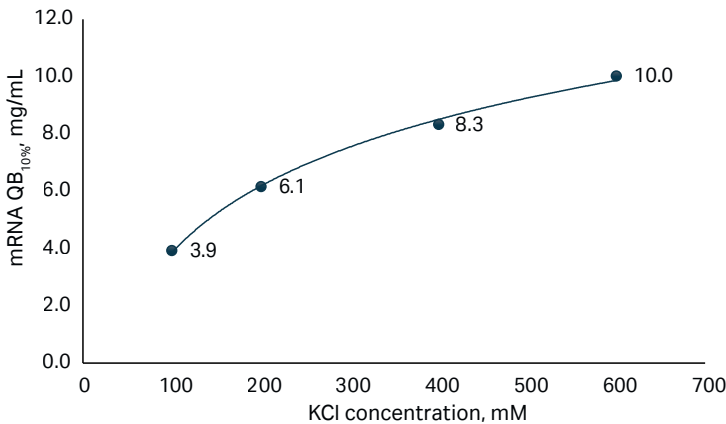
⁶ Ambient refers to short periods of time outside the temperature range of 4°C to 25°C.

Oligo dT ligand

The matrix is coupled with the oligo dT ligand. The monomeric oligonucleotide chain ligand is produced by synthesis using the phosphoramidite method (instead of in vitro transcription). Subsequent purification is performed in the absence of animal products.

Applications

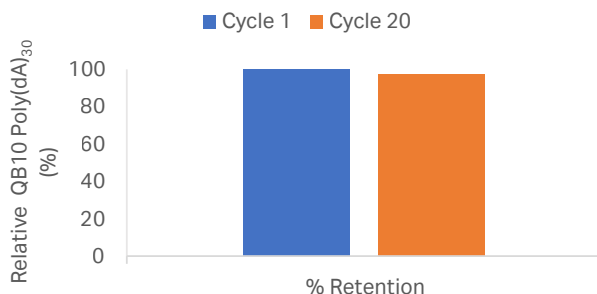
The fibro dT unit has a binding capacity of approximately 6 mg/mL for a 2000 nucleotide mRNA construct at residence times of 15 to 30 seconds. The binding capacity depends on construct size and composition, residence time, and buffer composition. The diagram below illustrates mRNA QB_{10%} for different potassium chloride concentrations.



High capacity, low ligand leakage, and a rigid matrix make the unit well suited for purifying mRNA. The ready-to-use format is well-suited for preparative purifications when short cycle times, high productivity, and flexibility are important. The single-batch, disposable format eliminates the need for sanitization.

Stability

The alkali-tolerant single-stranded sequence of deoxythymidine (dT) ligand allows for the use of 0.1 to 0.5 M NaOH for CIP in each purification cycle. The following diagram shows the relative DBC for the unit after 20 CIP cycles with NaOH concentrations of 0.1 M. The DBC was determined with Poly(dA)₃₀, 1 min contact time per cycle.



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

Binding buffer: 200 mM KCl, 10 mM Tris-HCl, 1 mM EDTA

Elution buffer: 10 mM Tris-HCl, 1 mM EDTA

Each cycle consisted of:

- 12 mL binding buffer, flow rate 10 mL/min
- 8 mL elution buffer, flow rate 2 mL/min
- 12 mL NaOH (0.1 M), flow rate 10 mL/min

DBC at 10% breakthrough by frontal analysis, QB_{10%}, for Poly(dA)₃₀, was measured before and after 20 cycles during the study.

Chemical compatibility

The fibro dT unit has verified compatibility with the compounds in the following table. More than 75% of the DBC (Poly(dA)₃₀) remained after 24 hours of storage at room temperature in the compounds listed.

Compound	Concentration
NaOH	0.5 M
NaOH	0.1 M
EtOH	70%
EtOH	20%
Acetic acid	1 M
Acetic acid	0.1 M
Isopropyl alcohol	30%
Sodium acetate + EtOH	0.2 M + 20%
Thioglycerol + Tris pH 8.5	100 mM + 25 mM
Guanidine hydrochloride (GuHCl)	6 M

3 Operation

3.1 Preparations

Preparing the chromatography system

- Disconnect the mixer chamber on the system by connecting the tube from the pump valve directly to the injection valve. A separate mixer valve can be bypassed in the method.
- Bypass the flow restrictor in the method to minimize the system back pressure.

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

Preparing the buffers

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

For examples of suitable buffers, see [Buffers, on page 12](#).

Prepare the sample for DBC

Pre-purified mRNA with the recommended concentration of 0.1 to 0.2 mg/mL is preferably used for determination of DBC by frontal analysis.

Prepare the sample as follows:

Step	Action
1	Dilute the sample in binding buffer with additional salt.
2	Determine the delay volume of the fibro dT unit and the system in the frontal analysis for determination of DBC. For more information, see Delay volume, on page 15 .
3	Adjust the sample to the composition of the start buffer by dilution using binding buffer with additional salt.

Connecting the unit to the system

Materials required:

- fingertight PEEK connector 1/16" male

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

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

A drop-to-drop connection or venting is not needed. Any air in the unit is removed when flushing with buffer during the first blank run.

The fibro dT unit does not require a holder.

Instrument configuration

To configure the UNICORN™ method for the fibro dT unit, follow the steps below. For the steps, the following is assumed:

A1 line – binding buffer

B1 line – elution buffer

A2 – CIP solution

Step 1: Method settings

- 1.1 Set **Pre column pressure limit** to 1.4 MPa.
- 1.2 Set **Delta column pressure limit** to 1 MPa.
- 1.3 Set **Flow rate** to 2 mL/min.
- 1.4 Select the column valve position on the ÄKTA system that the unit is attached to.
- 1.5 Set **UV1** to 280 nm and **UV2** to 260 nm.

Step 2: Equilibration 1

- 2.1 Select the **Reset UV monitor** checkbox.
- 2.2 Clear the **Use the same flow rate as in Method Settings** checkbox.
- 2.3 Set **Flow rate** to 10 mL/min.
- 2.4 Set **Volume** to 20 CV.
- 2.5 Leave the **Inlet A1** position blank.
- 2.6 Set the **Inlet B1** position to 0%.

Step 3: Sample application using a sample pump (option 1)

- 3.1 Select the **Use the same flow rate as in Method Settings** checkbox.
- 3.2 Set **Flow rate** to 1.6 mL/min.
- 3.3 Set **Inject sample using** to **Pump injection**.
- 3.4 Set the **Sample inlet** position to **S1**.
- 3.5 Clear the **Inject all sample using air sensor** checkbox.
- 3.6 Clear the **Interrupt sample application at UV** checkbox.
- 3.7 Set **Inject fixed sample volume** to 30 mL.
- 3.8 Set **Fractionate** to **using fraction collector**.
- 3.9 Set **Fractionation Type** to **Fixed volume fractionation**.
- 3.10 Set **Fractionation destination** to **50 mL tube**.
- 3.11 Set **Fixed Fractionation Volume** to **30 mL**.

Text editing

- 3.12 Change **Watch UV1** to **Watch UV2** (260 nm).
- 3.13 Delete the **Finalize sample injection** block.

Step 4: Sample application using Superloop™ (option 2)

- 4.1 Clear the **Use the same flow rate as in Method Settings** checkbox.
- 4.2 Set **Flow rate** to 1.6 mL/min.
- 4.3 Set **Inject sample using** to **Loop injection**.
- 4.4 Set **Fill the loop using** to **Manual load**.
- 4.5 Set **Loop type** to **Superloop** and select **50 mL**.
- 4.6 Clear the **Interrupt sample application at UV** checkbox.
- 4.7 Select the **Use the same flow rate as in Method Settings** checkbox.
- 4.8 Set the **Inlet B** position to 0%.
- 4.9 Set **Fixed sample volume** to 30 mL.
- 4.10 Set **Fractionate** to **using fraction collector**.
- 4.11 Set **Fractionation Type** to **Fixed volume fractionation**.
- 4.12 Set **Fractionation destination** to **50 mL tube**.
- 4.13 Set **Fixed Fractionation Volume** to **30 mL**.

Step 5: Column wash

- 5.1 Clear the **Use the same flow rate as in Method Settings** checkbox.
- 5.2 Set **Flow rate** to 10 mL/min.
- 5.3 Leave the **Inlet A1** position blank.
- 5.4 Set the **Inlet B** position to 0%.
- 5.5 Set **Wash until** to 20 CV.

Step 6: Isocratic elution 1

- 6.1 Select the **Use the same flow rate as in Method Settings** checkbox.
- 6.2 For **Isocratic elution**, set **Volume/Time** to 20 CV.
- 6.3 Set the **Inlet B1** position to 100%.
- 6.4 Select the **Fill the system with the selected buffer** checkbox.
- 6.5 Set **Fractionate** to **using fraction collector**.
- 6.6 Set **Fractionation Type** to **Fixed volume fractionation**.
- 6.7 Set **Fractionation destination** to **15 mL tubes**.
- 6.8 Set **Fixed Fractionation Volume** to **10 mL**.

Text editing

- 6.9 Type `2.50 Block Start frac (Elution).`
- 6.10 Type `17.50 Block Stop frac (Elution).`

Step 7: Column wash

- 7.1 Clear the **Use the same flow rate as in Method Settings** checkbox.
- 7.2 Set **Flow rate** to 10 mL/min.
- 7.3 Clear the **Fill the system with the selected buffer** checkbox.
- 7.4 Set **Wash until** to 30 CV.
- 7.5 Set **Fractionate** to **in waste**.
- 7.6 Leave the **Inlet B1** position blank.
- 7.7 Set the **Inlet B** position to 0%.

Step 8: Equilibration

- 8.1 Clear the **Use the same flow rate as in Method Settings** checkbox.
- 8.2 Set **Flow rate** to 10 mL/min.
- 8.3 Set **Volume** to 40 CV.
- 8.4 Set the **Inlet B1** position to 100%.

Step 9: Equilibration ethanol fill step (optional)¹

- 9.1 Set **Flow rate** to 10 mL/min.
- 9.2 Set **Volume** to 20 CV.

¹ This step can be used to fill the fibro dT unit with 20% ethanol (storage solution) before disconnecting the unit from ÄKTA.

3.2 Recommended starting conditions

General recommendations

To minimize buffer consumption and time, some steps can be omitted from the method. The following three steps can be replaced by switching to the next buffer and clearing out the previous buffer:

1. System wash
2. Pump wash
3. Priming

Buffers

The following table lists suitable buffers.

Buffer	Composition
Binding buffer	10 mM Tris-HCl, 400 mM KCl, 1 mM EDTA
Elution buffer	10 mM Tris, 1 mM EDTA
Post-load wash buffer 1	Same buffer system as the binding buffer
Post-load wash buffer 2 (optional)	Same buffer system as the elution buffer, except the KCl concentration is reduced to 200 mM

Operating flow rate and volume

The following table lists the recommended operating flow rates and volumes for the fibro dT unit on the recommended systems.

Phase	Fibro dT unit			
	ÄKTA pure/avant 25		ÄKTA pure/avant 150	
	Volume (mL)	Flow rate (mL/min)	Volume (mL)	Flow rate (mL/min)
Equilibration	10	10	10	10
Wash	10	10	10	10
Elution	6	2	8	2
CIP	10	10	10	10
Re-equilibration	10	10	22	10

Perform a blank run

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

Step	Action
1	Prime inlets and system with buffers.
2	Equilibrate the unit with loading buffer at 10 mL/min to remove the storage solution (20% ethanol). The storage solution can also be washed out with distilled water before equilibration.
3	Perform a blank run including all buffers and CIP solution in the method except for sample load. This removes any loosely (non-covalently) bound ligand.

The unit is ready for use.

Run the recommended method

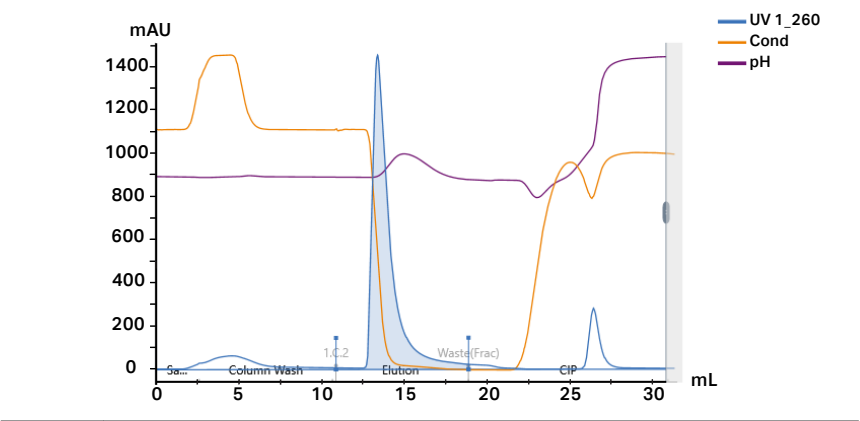
Before running a method, it is possible to manually equilibrate the fibro dT unit with binding buffer until the UV baseline, pH, and conductivity are stable. In that case, step 1 in the method below can be shortened.

In this method for the ÄKTA pure or ÄKTA avant 25 system, the A-pump is used in all steps except elution and sample load. Elution is performed using the B-pump, and sample load is performed using the sample pump.

For the recommended flow rate, see [Recommended flow rate, on page 15](#).

Step	Action
1	Equilibrate with 6 mL of binding buffer. Make sure that the UV baseline, the pH, and the conductivity are stable.
2	Load the sample.
3	Wash with 10 mL of binding buffer. Make sure that the UV returns to near base line.
4	Elute by step elution with 6 mL of elution buffer.
5	Perform CIP with 8 mL at 8 mL/min for a contact time of 1 min, or at 16 mL/min for a contact time of 0.5 min.
6	Wash with 6 mL of binding buffer at the same flow rate as in step 5.
7	Re-equilibrate with 10 mL of binding buffer at 10 mL/min. Make sure that the pH and the conductivity are stable.

The following diagram shows a typical chromatogram for the fibro dT unit on an ÄKTA pure 25 system.



4 Optimization

4.1 Introduction

This chapter contains procedures for optimizing processes such as determination of DBC, and optimizing wash and elution conditions.

The fibro dT unit is intended for research and early process development. Use the unit for high-throughput purification, screening of buffer conditions, and lifetime studies.

4.2 Determining DBC

DBC for the fibro dT unit, typically at 10% breakthrough ($QB_{10\%}$), is determined by frontal analysis in the same way as for resin-based chromatography. The prepurified target mRNA is loaded beyond the breakthrough to be evaluated, and the amount of bound/loaded mRNA is calculated, see [Evaluating DBC on page 17](#). Evaluation is possible using the UNICORN extension **DBC calculations**. For more information, refer to [How to determine dynamic binding capacity \(DBC\) of chromatography resins](#), available on cytiva.com.

The following sections outline how to determine the input parameters that are required for evaluating DBC.

DBC extension

The [DBC calculation](#) extension, available on cytiva.com, provides DBC calculations in an extension for UNICORN. It can be used in UNICORN with version 6.4 or later releases. For help on installing the **DBC calculation** extension, contact your local ÄKTA system specialist.

Recommended flow rate

The binding capacity is a function of the residence time. A residence time from 15 to 60 seconds is recommended. The DBC needs to be determined at flow rates that match the residence time for the intended application. For the load phase, a flow rate of 1.6 mL/min (40 MV/min) and a residence time of 15 seconds are recommended.

Sample

Pre-purified mRNA is preferably used for DBC determination by frontal analysis. High mRNA concentrations in the sample result in low volume applications for the fibro dT unit and complicate DBC determination. For the instructions for sample preparation and the recommended concentrations, see [Prepare the sample for DBC on page 8](#).

Delay volume

The delay volume for the fibro dT 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.

Diluting the mRNA sample to the specified concentration with a higher KCl concentration than the binding buffer (e.g., 450 mM KCl) results in an initial conductivity shift during sample loading.

Unbound species

Samples containing species that do not bind to the ligand show a UV 260 nm offset plateau—the **Contaminant offset**—in the UNICORN **DBC calculation** extension. The offset is determined from the UV 260 nm offset plateau that is reached before breakthrough.

Maximum absorbance

Determine the maximum 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 mRNA sample.
4	When the UV signal is stable, determine the 100% UV absorbance at 260 nm.
5	Run binding buffer through the system until the UV signal returns to zero.

Frontal analysis

Determine the DBC 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, the pH, and the conductivity are stable. Afterward, auto-zero the UV signal.
2	Load the sample to a breakthrough beyond the breakthrough to be evaluated.
3	Wash the sample with binding buffer.
4	Elute the bound mRNA with elution buffer.
5	Perform CIP with a contact time of 0.5 min.
6	Re-equilibrate the unit with binding buffer.

Step	Action
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Result:

From the frontal analysis, the following input parameters for DBC evaluation can be determined:

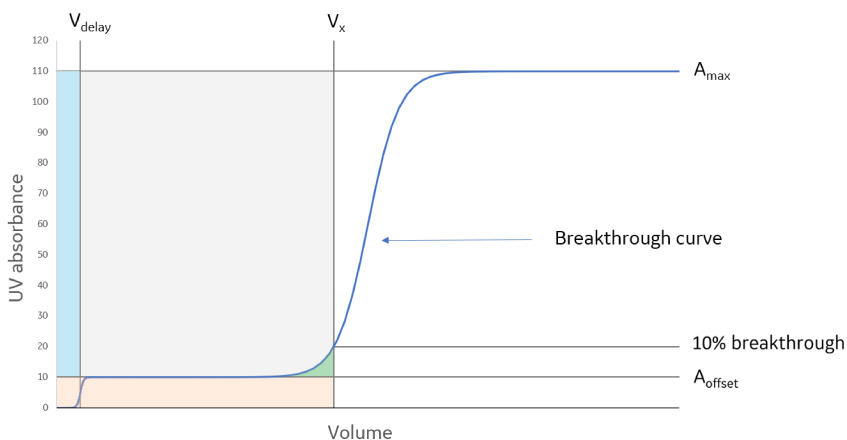
- V_x , the retention volume at selected breakthrough
- V_{delay} , delay volume of the unit and the system
- $A(V)$, absorbance at given retention volume
- A_{offset} , absorbance for the offset plateau for unbound species

Evaluating DBC

The UNICORN **DBC calculation** extension can be used for DBC evaluation at different breakthrough levels. For an outline of the calculation, refer to [How to determine dynamic binding capacity \(DBC\) of chromatography resins](#), available on [cytiva.com](#).

For the fibro dT unit, the delay volume relative to the matrix volume is greater than the delay volume relative to the column volume in resin-based chromatography. Therefore, considering the delay volume in the calculation is important.

The blue area in the following figure shows the delay volume. The pale orange area shows the amount of unbound species, that is, the UV offset. The green area shows the amount of unbound mRNA that breaks through. The gray area corresponds to the evaluated DBC using the equation on the next page.



The calculation of $QB_{10\%}$ as shown in the previous figure is expressed with 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

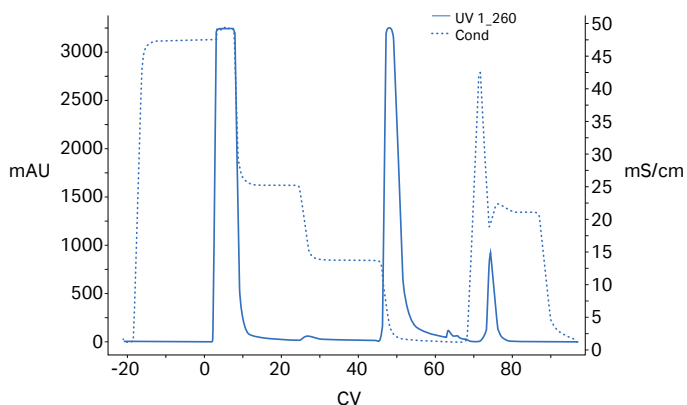
- C_0 = the concentration of the sample
- MV = the volume of the matrix
- V_x = the retention volume at selected breakthrough
- V_{delay} = the delay volume of the unit and the system
- $A(V)$ = the absorbance at given retention volume
- A_{offset} = the absorbance for the offset plateau for unbound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

4.3 Wash conditions

In the recommended methods in [Section 3.2 Recommended starting conditions, on page 12](#), the binding buffer is used for washing after sample load. For more efficient removal of tailless constructs and other process-related impurities, additional post-load washes are an option.

A typical post-load wash buffer can be a low-salt buffer (100–200 mM KCl) at, or near, neutral pH. For examples of buffers, see [Buffers, on page 12](#).

The following figure shows an example chromatogram for a fibro dT unit operated on an ÄKTA pure 25 system including post-load washes with 10 mM Tris-HCl, 200 mM KCl, 1 mM EDTA.



4.4 Cycling and lifetime studies

Since the fibro format is suitable for rapid cycling, it is relevant to perform lifetime studies. A study of 20 cycles can be completed in under 5 hours.

5 CIP

5.1 General description

CIP removes tightly bound, precipitated, or denatured substances from the fibro matrix. Accumulated contaminants can affect the chromatographic properties of the fibro dT unit, increase back pressure, decrease binding capacity, or cause carry-over between cycles. Fibro oligo dT, a highly alkali-tolerant matrix, allows for the use of 0.1 to 0.5 M NaOH for CIP. NaOH causes decreased binding capacity due to caustic ligand degradation. However, for the recommended contact times and concentrations (0.1 to 0.5 M NaOH), capacity loss is minimal. If needed, higher NaOH concentrations, other chemicals, and increased contact times can be used, see [Chemical compatibility, on page 7](#) and [Section 5.3 CIP optimization, on page 20](#).

CIP must be performed in every cycle to prevent contaminant buildup and maintain the unit's capacity, flow properties, and overall performance. CIP is included in the recommended method, see [Run the recommended method, on page 14](#).

5.2 CIP protocol

Note: *Prepare a volume in step 3 that is sufficient to match the pH and conductivity of the binding buffer.*

Step	Action
1	Perform CIP using 8 mL of NaOH (0.5 M), at a flow rate of 10 mL/min and a contact time of 0.6 min.
2	Wash immediately with 8 mL of binding buffer at a flow rate of 10 mL/min.
3	Re-equilibrate at a flow rate of 10 mL/min using the following amount of binding buffer: • 10 mL for ÄKTA pure/avant 25 • 22 mL for ÄKTA pure/avant 150 or until the effluent pH and conductivity match those for the binding buffer.

5.3 CIP optimization

Reversed flow for CIP and subsequent re-equilibration can be used for the fibro dT unit to more efficiently remove impurities from the top surface of the fiber matrix.

Higher NaOH concentrations and longer contact times increase CIP efficiency. If needed, NaOH concentrations up to 1.0 M can be used. The contact time for CIP can be increased to 3 minutes/cycle.

Other chemicals, including polar solvents, detergents, and various cleaning reagents, can also be used, see [Chemical compatibility, on page 7](#).

5.4 System CIP

To reduce the risk of RNase contamination in the flow path of the ÄKTA system, perform a system CIP using 0.5 to 1.0 M NaOH with a contact time of 30 to 60 minutes. Perform the procedure before connecting the fibro dT unit to the system. The effectiveness of NaOH molarity and contact time might need to be verified.

6 Storage

Store the fibro dT unit in 20% ethanol at 2°C to 8°C.



NOTICE

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

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

After storage, equilibrate the unit with binding buffer and perform a blank run, including CIP, prior to use.

7 Troubleshooting

The following table lists issues that might arise when using the fibro dT unit, possible causes, and corrective actions.

Issue	Possible cause	Corrective action
Leakage		
High ligand leakage during the first cycle.	Loosely (non-covalently) bound ligand that elutes with the mRNA during the first cycle.	Perform a blank run, including CIP, before the first purification cycle on a new unit.
Unit leakage when starting a flow.	Connectors are not properly screwed into the unit.	Tighten the connections between column valve and unit.
Pressure		
High pressure during the first run.	There is an inline flow restrictor.	Disconnect the flow restrictor.
	Solutions with high viscosity or low temperature are used.	Use a lower flow rate or control the flow by pressure.
	The inline filter is clogged.	Replace the inline filter.
	Damaged/clogged PEEK tubing between the column valve and the unit.	Replace the PEEK tubing between the column valve and the unit.
High pressure during elution.	High concentrations of nucleic acids combined with low conductivity leading to a pressure increase.	Decrease the flow rate during the elution to half the recommended flow rate.
		Add a low concentration of salt (≤ 5 mM NaCl) to the elution buffer.
Large increase in pressure over multiple cycles.	Fouling of fibro matrix due to inefficient CIP.	Perform CIP after each purification cycle.
		Increase the NaOH concentration or the CIP contact time if the problem persists.
		Perform CIP followed by wash/re-equilibration in reversed flow.
	Inefficient elution.	Optimize the elution conditions. Add a strip step with RNase-free water after elution.

Issue	Possible cause	Corrective action
Recovery		
Low recovery.	The delay volume of the system is incorrect.	Make sure that the delay volume is correct (System settings).
	The elution peak collection criteria are not optimal.	Change the pool collection criteria.
	The target mRNA is breaking through during sample application because the sample load is too high.	Decrease the sample load.
Decreasing recovery over multiple cycles.	The binding capacity decreases over multiple cycles due to fouling of the fibro matrix that is caused by inefficient CIP.	Perform CIP after each purification cycle.
		Increase the NaOH concentration or the CIP contact time if the problem persists.
		Perform CIP followed by wash/re-equilibration in reversed flow.
	The binding capacity decreases over cycles because of inefficient elution.	Optimize the elution conditions. Add a strip step with a lower conductivity buffer or RNase-free water after elution.
Elution volume		
Broader elution peaks than expected.	The flow path has not been minimized.	Make sure that the flow path is minimized by disconnecting or bypassing the mixer chamber.
	Low conductivity of elution buffer and high conductivity of the wash buffer prior to elution.	Include a prime in the elution step by either using the Fill system command or switching the unit out of the flow path until the buffers exchange.
		Add an extra post-load wash step with a conductivity that is in between that of the binding buffer and the elution buffer.

Issue	Possible cause	Corrective action
Peak broadening over multiple cycles.	Fouling of fibro matrix due to inefficient CIP.	Perform CIP after each purification cycle.
		Increase the NaOH concentration or the CIP contact time if the problem persists.
		Perform CIP followed by wash/re-equilibration in reversed flow.
Performance		
Reduced performance despite elution and CIP optimizations.	Product longevity, mainly dependent on the sample type and sample preparation.	Replace the unit.

8 Product recycling

This chapter provides information on product materials and recycling when the product has reached the end of its usable life.

The fibro dT 0.4 mL unit is made of the following polymers:

- 84% polypropylene (PP)
- 12% polyetheretherketone (PEEK)
- 4% cellulose fiber

Follow national and local environmental regulations for recycling.

Note: *Before recycling, rinse the unit with 1 M NaOH at a low flow rate (e.g., 0.5 mL/min) for at least 30 minutes to remove biological contamination and RNA.*

In most regions, the following applies:

- The complete unit (including stop plugs) can be recycled as mixed plastic waste.
- The unit without the stop plugs can be recycled as polypropylene (PP) waste.

The packaging is made of cardboard. Recycle the packaging according to national and local environmental regulations.

9 Ordering information

For additional information, refer to [cytiva.com](https://www.cytiva.com).

Products

Product	Pack size	Product code
Fibro dT 0.4 mL, 4-pack	4 units	17553101

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