

Fibro dT adsorber, 0.4 mL

AFFINITY CHROMATOGRAPHY FOR RNA WITH POLYADENOSINE (POLY A) TAIL

Adsorbers with an immobilized oligonucleotide deoxythymidine (dT) sequence are regularly used for the purification of RNA sequences with a 3' poly ribo adenosine (poly A) tail. A dT functionalized adsorber allows for enrichment of poly A-tailed RNA, and removal of impurities that do not have poly A stretches. Cytiva fibro dT adsorber is an adsorber of electrospun cellulose fiber, functionalized with dT oligonucleotides. The product is ideal for the purification of *in vitro* transcribed mRNA. The open structure of the fibro adsorber enables high mass transfer at high flow rates. In addition, the fibro properties allow for high binding capacity, particularly *in vitro* transcribed RNA, i.e., RNA that is longer than 1000 nucleotides. The RNA must have a poly A-tail to bind complementary to the fibro dT adsorber. The fibro dT 0.4 mL product (Figure 1) can, connected to an ÄKTA™ chromatography system, be used repeatedly to purify RNA with:

- a chromatography cycle time of less than 15 minutes
- high capacity. Over 6 mg/mL is repeatedly observed for 2000 nucleotide mRNA
- high reproducibility. Over at least 20 cycles and between different fibro dT batches

Product overview

Fibro chromatography matrix offers a large chromatography surface area for high binding capacity. The rigid open structure of the electrospun cellulose has high mechanical strength, allowing high flow rates. The flow can be up to 25 membrane volumes (MV) per minute for fibro dT 0.4 mL. This translates to a residence time of 2.4 seconds. The proprietary structure of the cellulose fibers allows for overcoming the diffusional and flow limitations of packed bed chromatography purification.



Fig 1. The fibro dT units are delivered ready for use.

Cytiva fibro dT adsorber 0.4 mL units

These ready-to-use units are designed for research and early process development, and are suitable for screening and stoppers at the inlet and outlet. A normal chromatography cycle for mRNA purification using fibro dT adsorber 0.4 mL is less than 15 minutes.

The fibro dT adsorber 0.4 mL product is developed for operation with an ÄKTA chromatography system (Figure 2). It is recommended to initially load using salt concentration of ~ 400 mM. This concentration can be lowered to optimize purification performance and lower risk for precipitation of the mRNA sample. The fibro dT adsorber 0.4 mL characteristics are listed in Table 1, and the product design is shown in Figure 3.



Fig 2. Use of fibro dT 0.4 mL unit on ÄKTA chromatography instrument.

Table 1. Main characteristics for fibro dT 0.4 mL unit

Matrix	Derivatized electrospun cellulose fibers
Ligand	dT oligonucleotide
Ligand coupling	Single point attachment in 5'-end of oligonucleotide dT
Ligand density	> 6 $\mu\text{mol/g}$ dry fibro ⁽¹⁾
Dynamic binding capacity (DBC)⁽²⁾	≥ 6 mg mRNA/mL matrix (2000 nucleotide mRNA)
Typical DBC/unit	2.4 mg mRNA/0.4 mL unit
Connector	1/16" fingertight
Membrane diameter	26.4 mm
Membrane height	1.6 mm
Hold up volume	0.85 mL
Membrane volume	0.4 mL
Cycle time⁽³⁾	< 15 min
Max flow⁽⁴⁾	10 mL/min
Flow, recommended operating	1.6 mL/min or 4 MV/min

Elution volume	up to 6 mL
Maximum operating pressure	1 MPa (10 bar)
Chemical stability	Compatible with aqueous buffers commonly used in column chromatography
pH stability, operational⁽⁵⁾	pH 5 to 9
pH stability, cleaning in place (CIP)⁽⁶⁾	pH 2 to 14
Temperature stability, operational	4°C to 30°C
Temperature stability, storage	2°C to 8°C
Temperature stability, shipping	ambient
Storage	20% ethanol at 2°C to 8°C

1. Determined by phosphate analysis
2. Determined at 10% breakthrough of a 2 kb model mRNA at 400 mM KCl
3. Purification cycle steps include: equilibration, sample loading, washing, elution, strip, CIP, and re-equilibration of the Fibro unit.
4. At room temperature, use a buffer with the same viscosity as water.
5. pH range where the fibro dT unit can be operated without a significant change in function.
6. pH range where fibro dT can be subjected to cleaning or sanitization-in-place without significant change in function

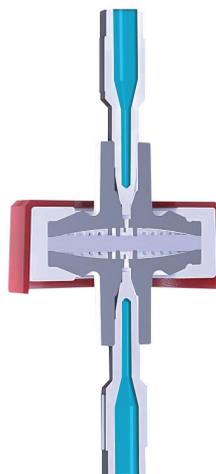


Fig 3. Cross-sectional image of fibro dT 0.4 mL unit.

Robust performance

Reliable performance of a fibro dT adsorber is key to avoiding the loss of valuable mRNA during the loading phase. Consistent mRNA capacity has been a key characteristic in the development of the fibro-dT unit. Minimal losses of mRNA during purification in combination with high binding capacity and quick chromatography cycle time make fibro dT a good fit for sustainable mRNA purification. Robust chromatography performance over many cycles is shown in Figure 4, and robust recovery over many cycles for three random batches is shown in Figure 5.

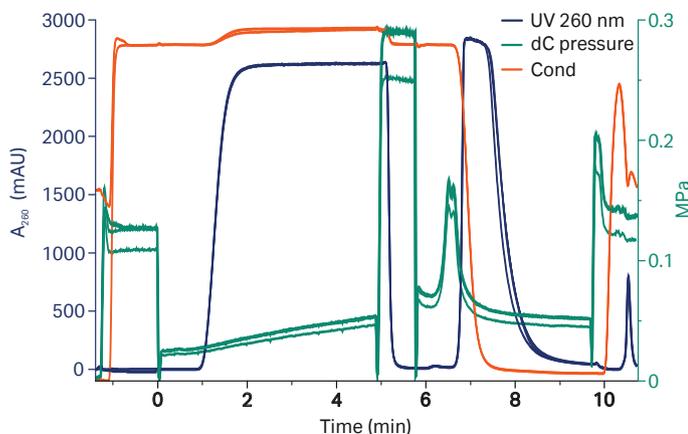


Fig 4. Overlay of mRNA purification chromatograms using a fibro dT 0.4 mL unit. Chromatography cycles 1, 5, 10, 15, and 20 are displayed. Each cycle includes equilibration, sample loading, wash, elution and cleaning. UV 260 nm — ultraviolet spectroscopy at 260 nm. dC — delta column, Cond — conductivity.

Table 2. Buffers used in typical mRNA purification cycle

Cycle step	Buffer
Equilibration	10mM Tris, 400mM KCl, 1mM EDTA
Loading	mRNA sample equilibrated to 400mM KCl
Wash	10mM Tris, 400mM KCl, 1mM EDTA
Elution	10mM Tris, 1mM EDTA or UltraPure Water
Elution	0.1M NaOH

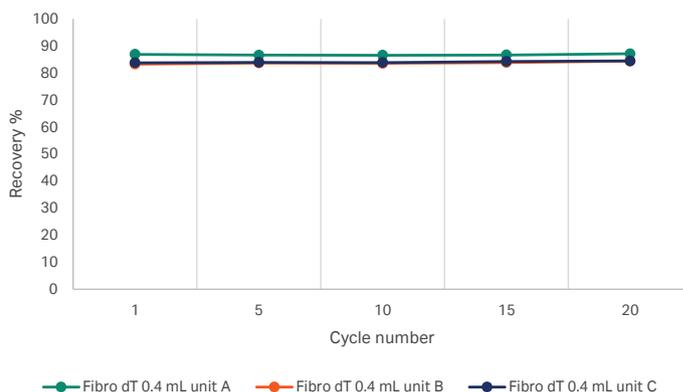


Fig 5. Recovery of mRNA over multiple cycles for three different batches of fibro dT 0.4 mL units.

Stability in cleaning

Cleaning-in-place (CIP) is a cleaning procedure to remove contaminants such as lipids, precipitates, or denatured proteins that might remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the chromatography bed and helps maintain the capacity, flow properties, and general product performance. The frequency of CIP depends on the nature and the condition of the starting material, but one CIP cycle is generally recommended every one to five separation cycles. When purifying RNA, it is important to work RNase-free to maximize recovery of integral, target RNA. Cleaning fibro dT with RNase-free water or buffers is recommended before sample application. Cleaning with NaOH is a way to further reduce the risk of RNase activity. Cytiva fibro dT unit withstands standard CIP solutions such as 0.1 M NaOH, 0.5 M NaOH, and 70% ethanol, or combinations. The fibro dT unit is stable for one exposure to 0.5 M NaOH and 30% isopropanol for 2 hours. The fibro dT product is also stable to commonly used aqueous buffers, detergents, and polar solvents. An overview is found in the Cytiva fibro dT unit Instructions for Use documentation ([cytiva.com/instructions](https://www.cytiva.com/instructions)).

Storage

The recommended storage conditions are 20% ethanol at 2°C to 8°C. Shipping at ambient. The product is supplied in a 20% ethanol storage solution.

Product recycling

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 disposal, 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.

Ordering information

17553101 Cytiva fibro dT 0.4 mL, 4-pack

Related literature

Instructions for use 29806353 available at [cytiva.com/instructions](https://www.cytiva.com/instructions). Search for product 17553101.

More information: [Fiber chromatography | Cytiva](#)

Related services

Our Fast Trak™ training and education team provides high-level, hands-on training for all key aspects of bioprocess development and manufacturing. Learn more about mRNA purification. Explore our [online courses in biotechnology and biopharma](#) or reach out to your local Cytiva representative for more information.

Related Products

Software/process specifications

Product	Column size	Product code
Sera-Mag™ Oligo (dT) coated magnetic particles	1 mL	38152103011150
Sera-Mag Oligo (dT) coated magnetic particles	5 mL	38152103010150
Sera-Mag Oligo (dT) coated magnetic particles	100 mL	38152103010350

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