Mustang™ Q XT

CHROMATOGRAPHY CAPSULES

Reusable ion exchange membrane chromatography

Meeting process demands for scalability and economy

The use of ion exchange chromatography for purification in downstream processing is well established. Many regulatory-approved processes make use of large process columns packed with ion exchange sorbent for effective and reliable performance. As demand intensifies for improved throughput and reduced processing costs, Mustang™ ion exchange membrane technology keeps pace, enabling process developers to harness:

- Reduced process times through use of high volumetric flow rates.
- Improved process economics through increased throughput and reduced buffer consumption.
- Enhanced process flexibility through use of a smaller operating footprint.

Membrane capsule design and chromatography competencies have been combined to develop a range of high performance, scalable Mustang Q XT membrane chromatography capsules. These capsules are robust and easy to use, and enable users to optimize their process by reducing buffer consumption, increasing throughput and decreasing capital expenditure.

Processes benefit from:

- High binding efficiency Mustang Q XT capsules exhibit high capacities and high flow at low pressure drops, allowing binding of large charged biomolecules such as plasmids and lentiviruses in a single pass.
- Speed Mustang Q XT capsules are designed for highthroughput membrane chromatography, with operating flow rates of up to 10 MV/min (up to 50 L/min for XT5000), enabling the processing of large volumes in less than a single working shift. Importantly, membrane binding capacity remains largely unaffected by flow rate, making this technology significantly faster than conventional chromatography sorbents.

While high flow rates are ideal for rapid processing, operating at lower flow rates presents an opportunity to fine-tune performance for specific process needs. Users are encouraged to evaluate both higher and lower than typical flow rates to optimize recovery, purity, and overall process efficiency.



Fig 1. Mustang Q XT chromatography capsule range.

This flexibility allows Mustang Q XT capsules to be tailored to a wide range of bioprocessing applications.

- Scalability and flexibility Our range of capsule sizes accommodates various volumes and capacities required in biopharmaceutical processing, from process development to full-scale manufacturing.
 - Capsules can be deployed in single-use applications or can be regenerated and reused.
- Reproducibility Capsules are manufactured using the statistical process control (SPC) system to enable consistent process reproduction.
 - This facilitates the conformance to specifications and delivers consistent process results.
- Convenience Easy-to-use capsules eliminate the need for packing protocols. In single-use applications, there are no cleaning, cleaning validation or cross-contamination issues.
- **Lower cost** Membrane capsules offer lower operating and capital investment costs than conventional columns that require validated packing and cleaning protocols.
- Reduced buffer consumption The small footprint requires less buffer, yet maintains efficient contaminant removal performance.

Mustang Q capsules XT5 to XT5000 are suitable for screening and optimization studies, as they are made with the same construction materials and consistent void volume-to-membrane ratio for scalable chromatographic performance. The XT5 and XT40 capsules are suited to scale-down optimization studies.



Applications

Mustang Q XT capsules are used in a wide range of approved processes and clinical trials for applications, such as:

- · Capture of lentiviruses
- · Plasmid purification
- · Adeno-associated virus (AAV) and adenoviruses purification
- Purification of large proteins
- · Monoclonal antibody polishing

In capture applications where the target molecule is relatively large, Mustang membrane exhibits improved capacities and recoveries and higher flow rates compared to packed bed sorbents. Each Mustang Q XT capsule is engineered with a uniform flow path and very low total volume-to-membrane-volume ratio, enabling good resolution with high yields and low elution volumes.



Fig 2. Mustang Q XT140 capsule and Mustang Q XT5 capsule.



Fig 2. Fig 3. Mustang Q XT5000 capsule on stand base unit (lower section). (Not shown in image: top [upper] section[s] can be added to operate two to three XT5000s capsules in series.)

Application examples

Capture and recovery of plasmid DNA (7 kb) from *E. coli* lysate

Table 1. Efficient recovery of plasmid DNA from clarified lysate with Mustang Q XT140 capsule

Mustang XT140 fraction (7 kb)		pDNA (g/L)	Endotoxin (EU/mg)	Total pDNA (g)	Yield (%)
Load	36.80	0.10	1	3.68	-
Mustang Q XT pDNA eluate	3.33	0.89	204	2.96	80

Loading conductivity 89.6 mS/cm following alkaline lysis loaded in two cycles at 10 MV/min flow rate.

Not measured, but refer to typical endotoxin levels from E. coli lysate 2.1 × 106

(S. Zhang, A. Krivosheyeva and S. Nochumson. Biotechnol. Appl. Biochem. [2003] 37, 245-249.)

Data courtesy of A. Carnes, Nature Technology Corporation, Wilbio Conference, November 2008.

Viral vector purification

Mustang Q XT membrane provides efficient reduction of empty capsids and enrichment of full capsids in a single bind and two-step elution method. The eluate of the first elution step consists of predominantly empty capsids. The eluate of the second elution step consists of enriched full capsids.

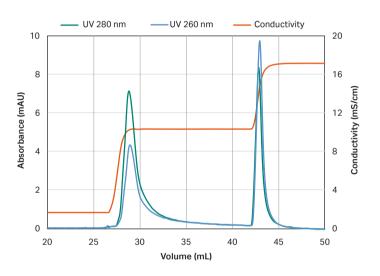


Fig 3. Mustang Q XT Acrodisc unit (0.86 mL) two-step elution method with AA5 capsids; (left peak) predominantly empty capsids, (right peak) enriched full capsid peak.

High standards of quality

- Manufactured to high quality assurance standards in accordance with ISO 9001.
- Membrane lots tested for dynamic protein and DNA binding capacity.
- Identified by lot number together with a unique serial number for traceability of manufacturing history, satisfying QC/QA requirements.
- Supplied with certificate of analysis to confirm quality standards.
- Meets USP biological reactivity tests in vivo in accordance with requirements for USP Class VI-50°C.

Technical specifications

Table 2. Specifications of Mustang Q membrane in XT Acrodisc™ unit

Product code	MSTGXT25Q16
Membrane bed volume	0.86 mL
Housing	Polypropylene (PP)
Media	16-layer Mustang Q 0.8 µm membrane
Connections	Female luer lock inlet and outlet
Maximum operating pressure	4 bar (58 psi)
Recommended connectors for chromatography workstation	Luer to ¼–28 female adapter (Bio-Rad, product code 732-0113)



Fig 4. XT Acrodisc units with Mustang Q membrane (0.86 mL).

Table 3. Specifications of Mustang Q XT capsules

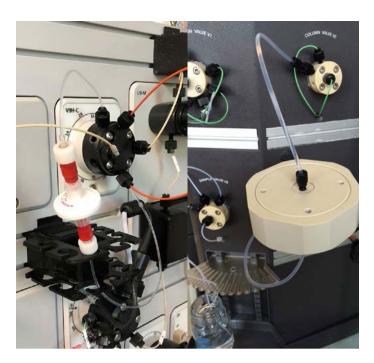


Fig 5. Preliminary evaluation of membrane performances executed with Acrodisc units (0.86 mL – left) and scaled up with Mustang XT5 capsule (5 mL – right).

Type of Mustang Q XT capsule	5 mL scale down (XT5MSTGQPM6)	40 mL scale up (XT40MSTGQP05)	140 mL pilot scale (XT140MSTGQP05)	450 mL process (XT450MSTGQP05)	5 L process (XT5000MSTGQP1)
Materials of co	nstruction				
Membrane	, , ,	, ,	mbrane with cross-linked q 53–84 mg/mL at 80% breal	,	
Membrane support and drainage	PP	РР	PP	PP	PP
Core	PP	PP	PP	PP	PP
Cage	PP	PP	PP	PP	PP
End caps	PP	PP	PP	PP	PP
Housing	PP	PP	PP	PP	PP
O-rings	Silicone	Silicone	Silicone	Silicone	Not applicable
Valve	Not applicable	PP	PP	PP	PP
Nominal dimer	nsions				
Capsule length without protective caps	39 mm (1.5 in.)	132 mm (5.20 in.)	190 mm (7.48 in.)	339 mm (13.35 in.)	605 mm (23.8 in.)
Maximum diameter of capsule body	97 mm (3.8 in.)	107 mm (4.21 in.)	107 mm (4.21 in.)	107 mm (4.21 in.)	270 mm (10.6 in.)
Weight dry	187 g (6.6 oz.)	555 g (1.22 lb.)	768 g (1.5 lb.)	1436 g (3.16 lb.)	10.3 kg (22.7 lb.)
Weight in use (filled)	195 g (6.88 oz.)	653 g (1.44 lb.)	1009 g (2.0 lb.)	2207 g (4.86 lb.)	19.6 kg (43.2 lb.)
Connectors	Female M6 threads	½ in. sanitary flange	½ in. sanitary flange	½ in. sanitary flange	⅓ in. sanitary flange

Operating characteristics

Table 4. Operating characteristics of Mustang Q XT capsules²

Type of Mustang Q XT capsule	5 mL scale down (XT5MSTGQPM6)	40 mL scale up (XT40MSTGQP05)	140 mL pilot scale (XT140MSTGQP05)	450 mL process (XT450MSTGQP05)	5 L process (XT5000MSTGQP1)
Maximum operating temperature	38°C	38°C	38°C	38°C	38°C
Maximum operating pressure	5 bar (72.5 psi) at 38°C	3 bar (43.5 psi) at 38°C	3 bar (43.5 psi) at 38°C	3 bar (43.5 psi) at 38°C	3 bar (43.5 psi) at 38°C
Storage condition	0.1 M NaOH + 1 M NaCl	0.1 M NaOH + 1 M NaCl	0.1 M NaOH + 1 M NaCl	0.1 M NaOH + 1 M NaCl	0.1 M NaOH + 1 M NaCl

²With fully compatible fluids that do not soften, swell or adversely affect the capsule or its materials of construction.

Advanced Mustang membrane technology

Mustang membrane is a PES-based based membrane with a $0.8~\mu m$ nominal pore size and a surface coating of an irreversibly cross-linked polymer containing quaternary amine groups. Mustang membrane chromatography allows rapid and direct access to the membrane binding sites for large biomolecules (nucleic acids and proteins) and virus particles by direct fluid convection.



Fig 6. All sizes use 16 layers of Mustang Q membrane in Ultipleat structure.

Capsule design

Mustang Q XT capsules are available in multiple scalable sizes from 5 mL to 5 L volumes, and are constructed with 16 layers of Mustang Q membrane for consistent performance. Set-up is simple, and each capsule is integrity tested before shipment for additional quality assurance. If required, process capacities can be increased by linking units in parallel or series.

The durable polypropylene housings have been designed to reduce upstream and downstream hold-up volume, enhancing chromatographic performance (Table 5). The housings provide the chemical stability needed for cleaning, reuse and long-term storage, allowing sanitization and storage in 0.1 M NaOH/1 M NaCl for up to 12 months.

Seamless scalability

Scalability is enabled via using 16 layers of membrane across all product sizes. By maintaining a constant membrane bed height, chromatographic performance is maintained across the product range. Scalable Mustang Q XT products meet processing requirements in a range of volumes:

- Screening and pre-clinical: Mustang Q membrane in XT Acrodisc unit (0.86 mL) and Mustang Q XT5 capsule (5 mL)
- Scale process development work and small-scale clinical manufacturing runs: Mustang XT40 (40 mL) and XT140 (140 mL) capsules
- Process scale clinical manufacturing: Mustang Q XT450 (450 mL) and XT5000 (5 L) process scale capsules

Table 5. Hold-up volume comparison across sizes

Bed volume (mL) (A)	Void volume ³ (mL) (B)	Ratio A/B	EFSA ⁴ (cm ²)
5	8	1.60	22
40	98	2.45	236
140	260	1.86	660
450	770	1.71	2121
5000	9200	1.84	22 000
	(mL) (A) 5 40 140 450	(mL) (A) (mL) (B) 5 8 40 98 140 260 450 770	(mL) (A) (mL) (B) 5 8 1.60 40 98 2.45 140 260 1.86 450 770 1.71

³ Total liquid capsule fill volume including pores (mL).

Table 6. Typical BSA dynamic binding capacity at 10 MV/min, demonstrating linear scalability (samples selected at random)

Mustang Q capsule	Membrane volume (mL)	Breakthrough (mg/mL)	Pressure drop (bar [psi]) ⁵
XT Acrodisc unit	0.86	83 ±6	0.35 (5)
XT5	5	85 ±5	0.68 (10)
XT40	40	82 ± 15	0.41 (6)
XT140	140	69 ± 10	0.48 (7)
XT450	450	87 ± 3	0.55 (8)
XT5000	5000	75 ± 3	0.62 (9)

⁵ Measured at 10 MV/min with Tris buffer.

⁴ Effective functional surface area.

Ordering information

Product	Pkg	Membrane volume	Product code
Mustang Q membrane in XT Acrodisc unit	4/pkg	0.86 mL	MSTGXT25Q16
Mustang Q XT	1/pkg	5 mL	XT5MSTGQPM6
		40 mL	XT40MSTGQP05
		140 mL	XT140MSTGQP05
		450 mL	XT450MSTGQP05
		5 L	XT5000MSTGQP1

Mustang XT5000 capsules can be connected to increase total available process volume by up to three capsules using the Mustang XT5000 stand.

Product	Pkg	Product code
Stand base unit (lower section)	1/pkg	XT5000B100
Top (upper) section	XT5000T100	
Lifting handle		XT5000H100

Please contact us for more information on the XT5000 capsules stand, and other cartridges and configurations.



Fig 7. Mustang Q XT capsules 5 mL to 5 L units.

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