PALL Life Sciences

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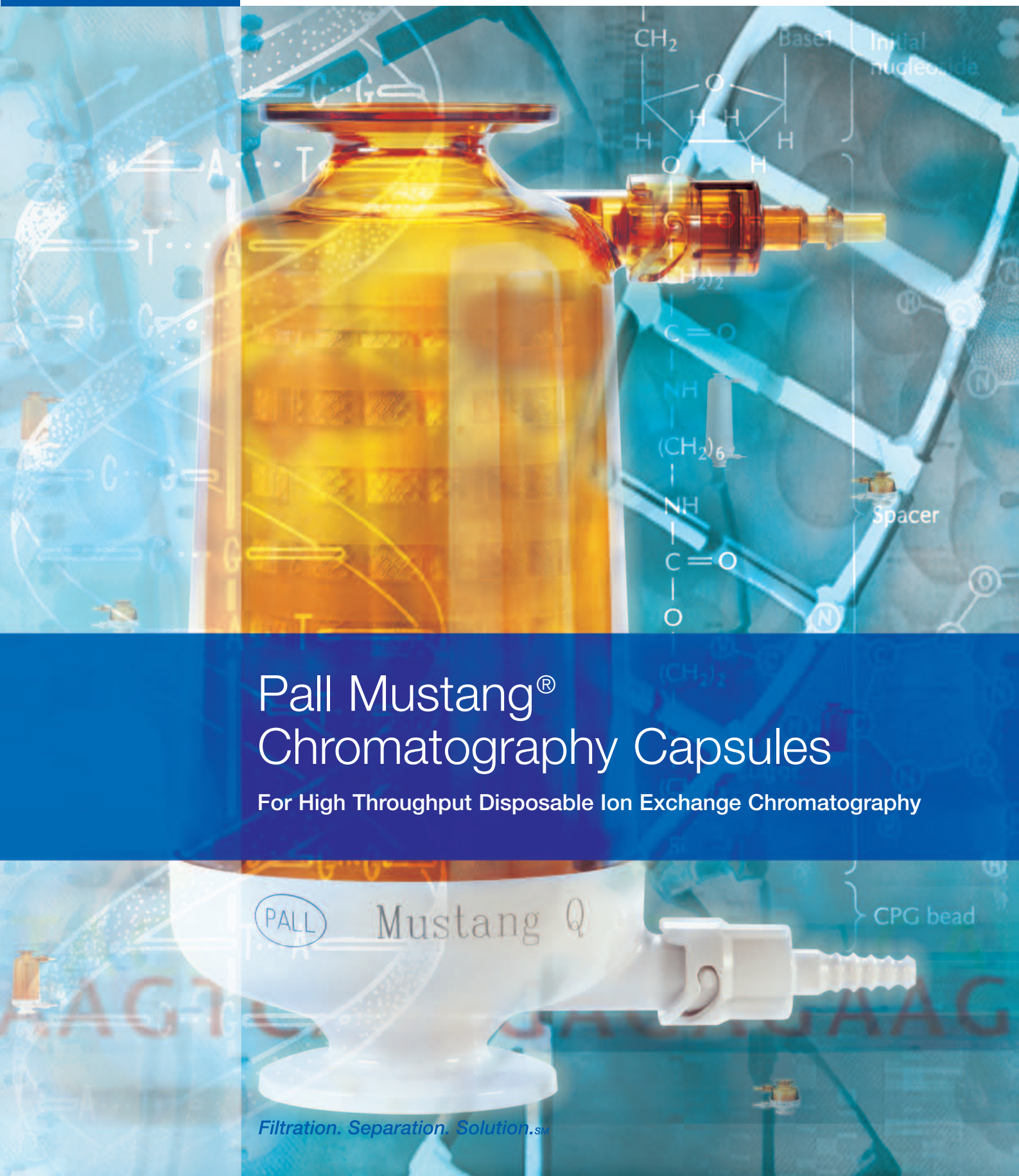


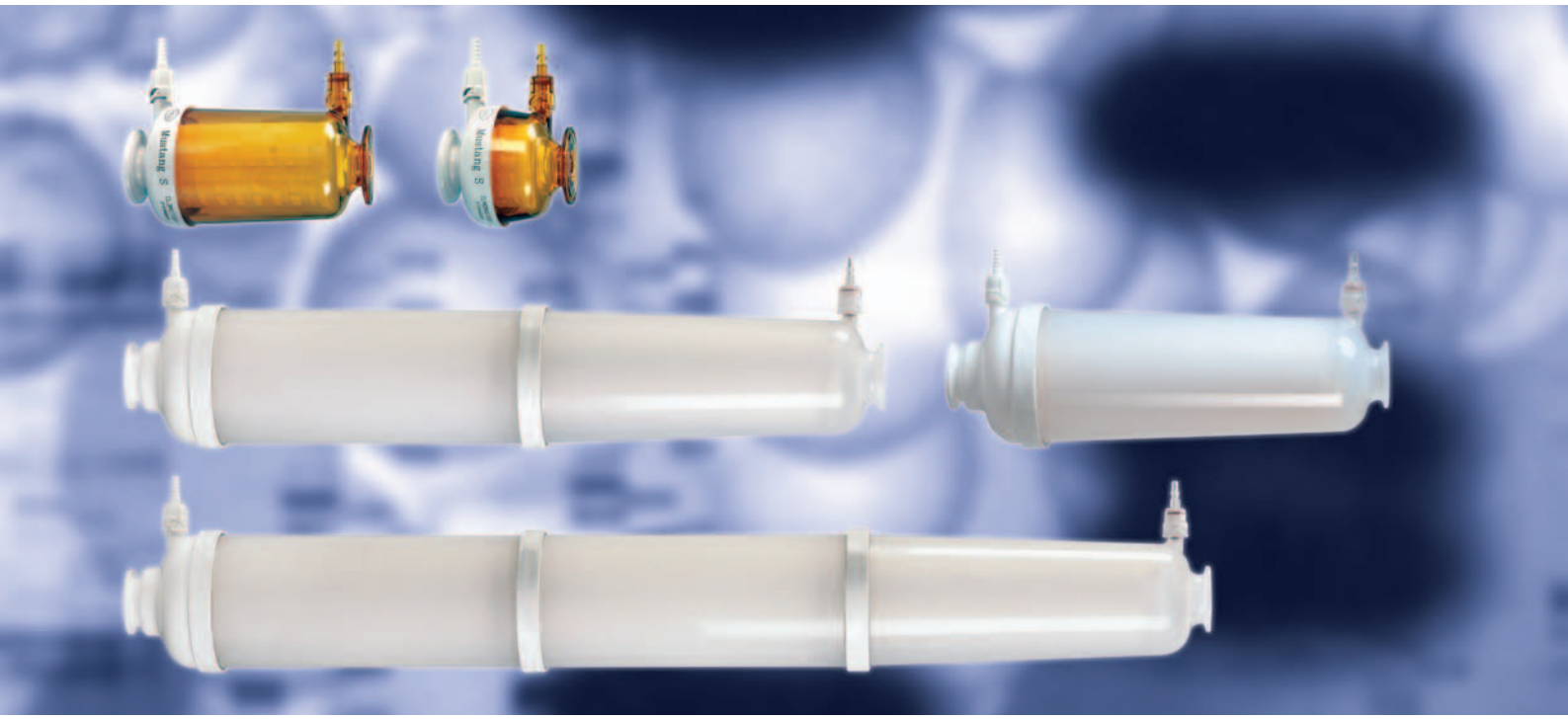
Pall Mustang® Chromatography Capsules

For High Throughput Disposable Ion Exchange Chromatography

*Filtration. Separation. Solution.*SM

Mustang Chromatography Capsules

High Throughput Disposable Membrane Chromatography Products



Ion exchange column chromatography is a well established purification method in bioprocesses, and ion exchange resin columns are effective and reliable. However, column packing and packing validation are time consuming, and are critical for the success of the purification. Due to the high equipment cost of process columns and associated equipment, cleaning and storage validation are also critical for the economics of the processes.

Mustang ion exchange chromatography capsules are prepacked single use capsules designed to provide high throughputs. Therefore, no column packing or cleaning are required resulting in significantly shorter process times. These capsules are ideal scaleable purification products for cGMP productions and cleaning and storage validation are not required, since the capsules are disposable.

Mustang capsules are particularly well suited for purifying target products in the initial capture steps or for removing DNA, virus, endotoxins and other contaminants in the final polishing steps. These capsules combine the innovative **Mustang** ion exchange membrane with a unique capsule design.

Mustang Chromatography Capsules

Unique Ion Exchange Membranes

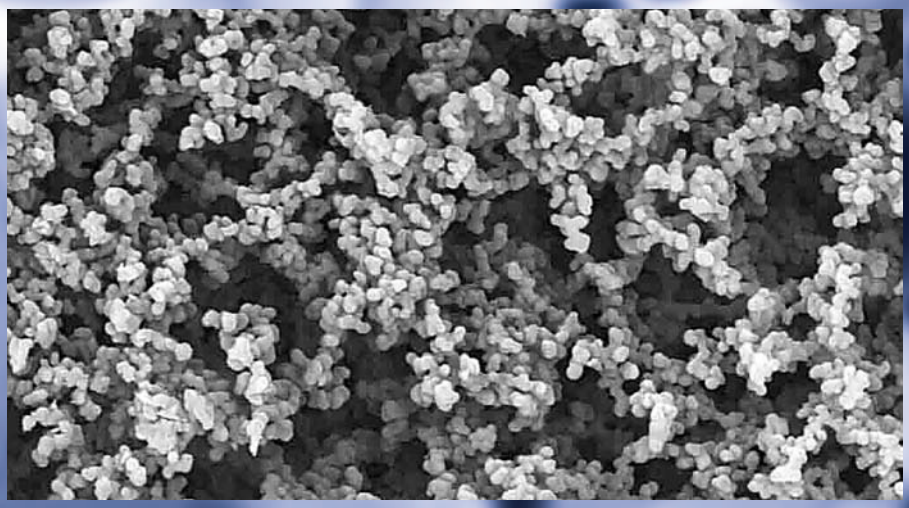


Figure 1

Mustang capsules are innovative products containing surface modified (PES) membranes with a polymeric coating, which contains charged functional groups (either sulfonic acid for **Mustang** S capsules or quaternary amine for **Mustang** Q capsules). The PES membranes have nominal 0.8 micron pores. These pores are large enough to allow very large biomolecules such as plasmids, genomic DNA and viruses to access all the binding sites by direct fluid convection.

Figure1: Electron microscopy of **Mustang** membrane shows the large surface area responsible for effective binding properties.

This results in higher dynamic capacity for large molecules in comparison to traditional beads, which are limited by diffusive pores. High dynamic binding capacity can be achieved with **Mustang** membranes even at very high flow rates such as 10 to 40 membrane volumes / min. These are ideal for both positive (capture) as well as negative (flow through) chromatography. **Mustang** E capsules have been designed to remove endotoxins from complex solutions, and especially from high salt buffers. Its unique chemistry using a surface modified PES membrane allows very efficient removal of endotoxins up to 10^5 EU/mL of media.

Features and benefits of the Mustang membrane

- Uniform flow characteristics throughout the membrane
- Uniform density of ion exchange functional groups
- High dynamic binding capacity
- High throughput
- Chemically stable under typical CIP (cleaning in place) conditions

Mustang Chromatography Capsules

High Capacity for Large Biomolecules

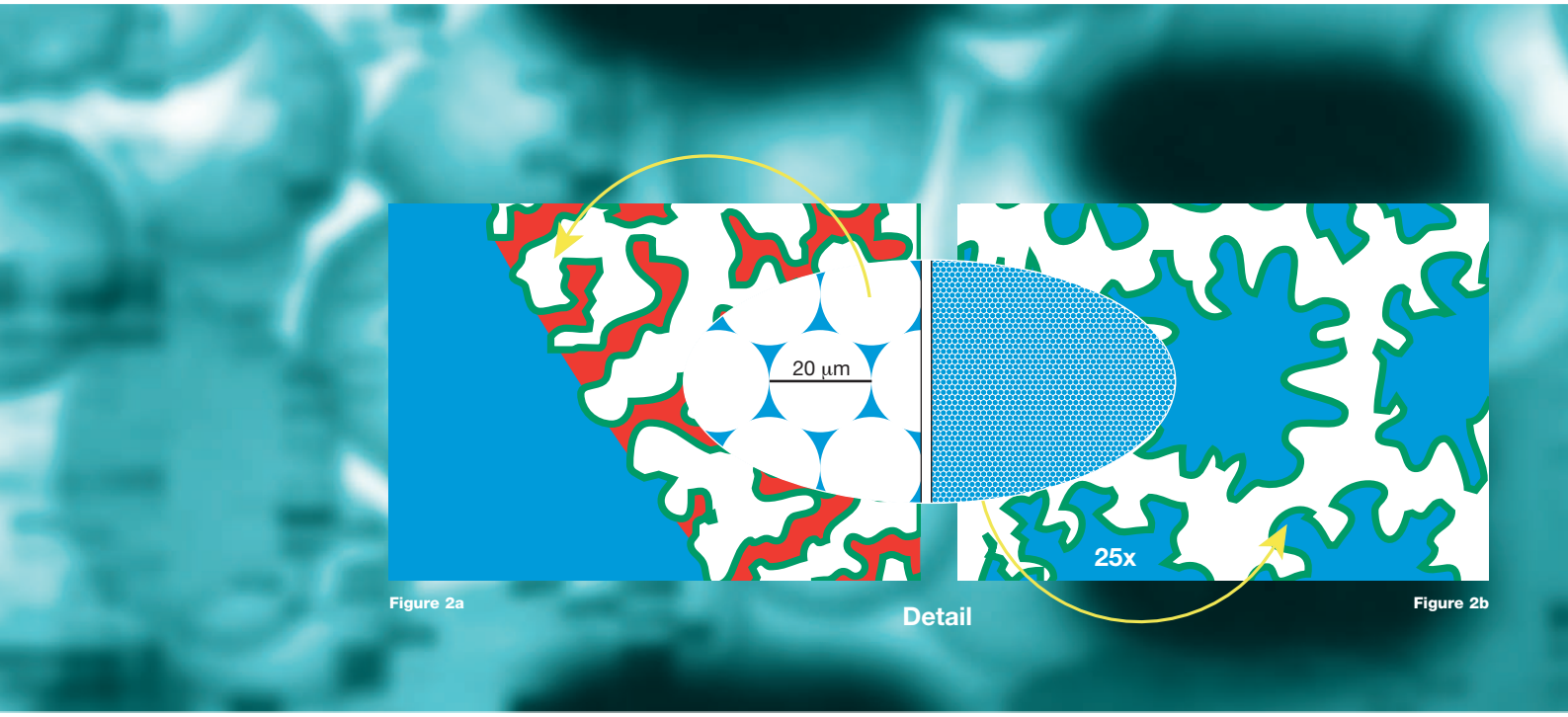


Figure 2a

Figure 2b

Conventional ion exchange chromatography media rely on internal diffusive pores to create large surface areas to achieve high binding capacity. However, the binding capacity of traditional resin columns is flow sensitive due to the diffusive nature of the pores. It is also difficult for large biomolecules such as DNA or viruses to access the internal binding sites of the pores; resulting in low capacity at high flow rates for traditional resins. In contrast, **Mustang** membranes possess large convective pores, and their dynamic binding capacity is relatively insensitive to the effect of high throughput and of large molecules such as plasmids.

Comparison of Mustang membranes with conventional chromatography beads

Figure 2a: This shows a magnified view of typical ion exchange chromatography media.

Figure 2b: Mustang membranes have convective pores for high flow rates and fast binding.

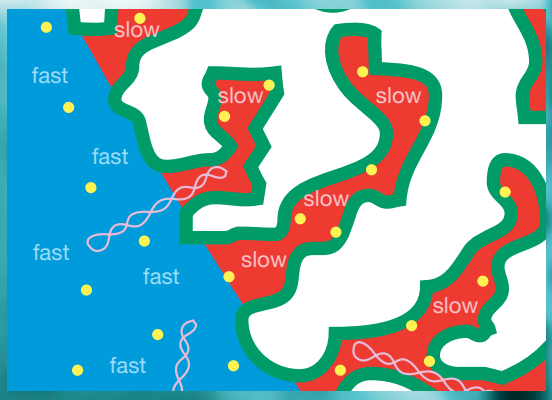


Figure 3a

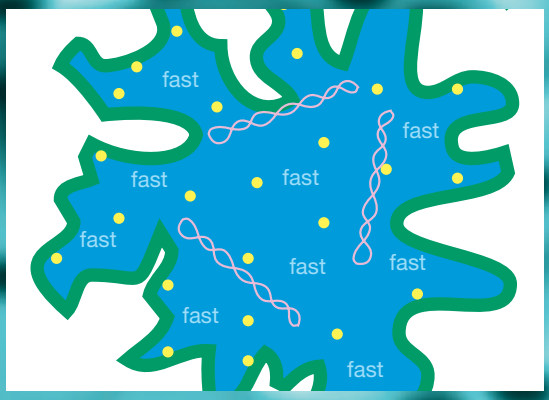
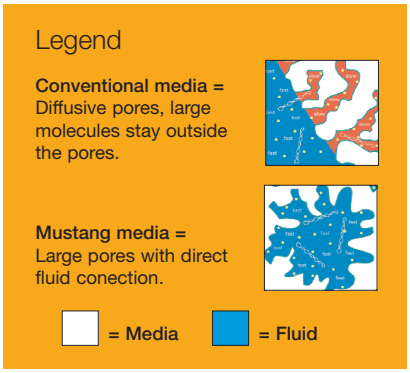


Figure 3b

Figure 3a: A long diffusion path and the internal pores diameters limit large molecule access to the internal surface area of the beads.

Figure 3b: This magnified view of Figure 2 shows the open pores of the **Mustang** membrane and the easily accessible binding sites.



Mustang Chromatography Capsules

Unique Capsule Design for Ease of Use

Figure 4

Sixteen layers of **Mustang** Q or S membrane are pleated in a unique open pleat configuration, which is then assembled in a single use capsule for ease of use.

Figure 4: Cut cartridge with dye

In the above photo, a solution with a red cationic dye was passed through a **Mustang** capsule. Flow was stopped before breakthrough, and the capsule was then sectioned to examine the distribution of dye. The uniform penetration of the red dye throughout the capsule cross section is clearly visible. It indicates uniform binding within the pleating structure.

Features and Benefits

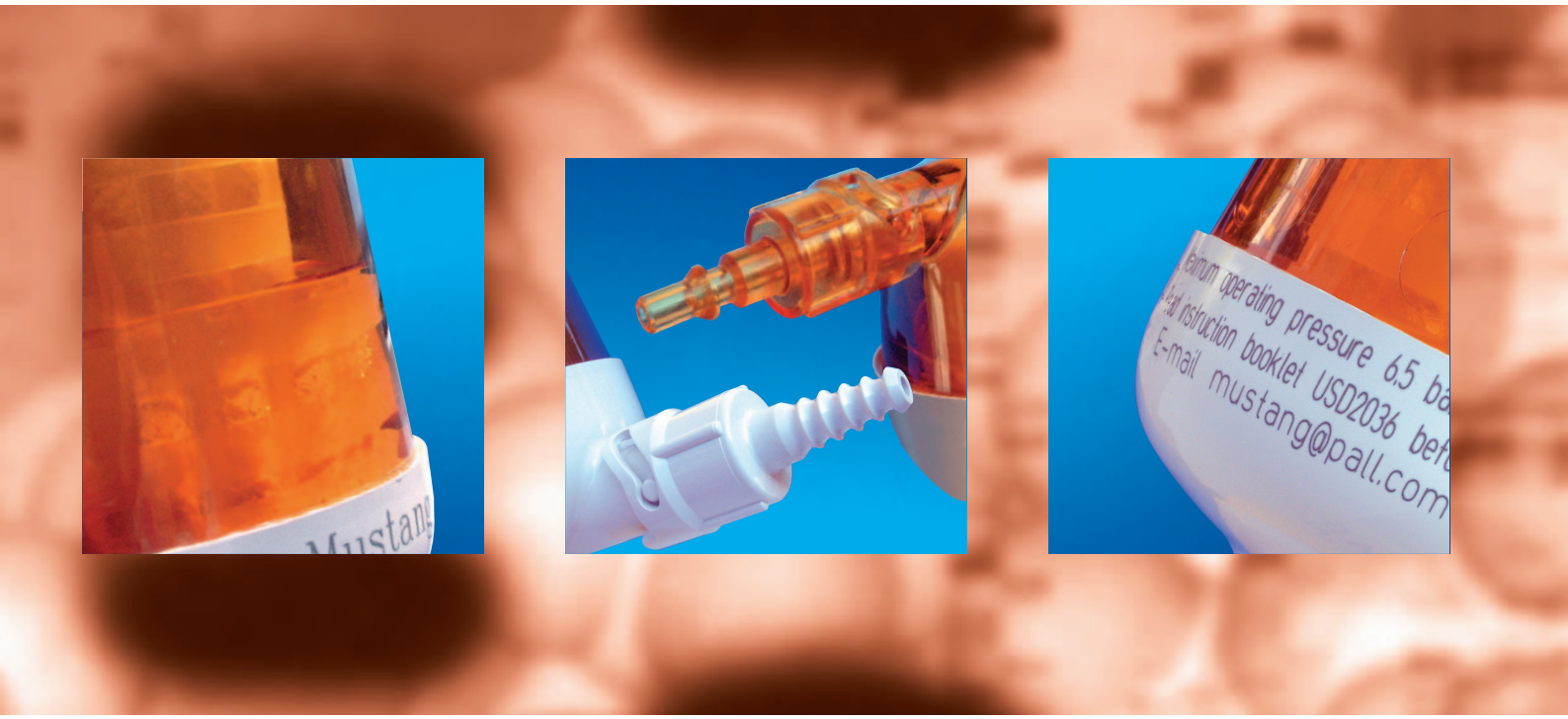
- 1½" sanitary flanges - Easy inlet and outlet connections
- Transparent shell - Liquid level always visible
- Purposed designed vent and drain valves - Ease of use
- Laser etched part number, serial number and safety information - Easy to read

Ease of use

Mustang capsules are supplied endotoxin free. They are ready to use so no packing is required. They can be chemically sanitized (1M NaOH for up to 30 minutes) or autoclaved (121°C for 30 minutes, dry cycle). **Mustang** capsules are convenient and easy to use after a quick preconditioning step. These are single use products, therefore no cleaning and no cleaning validation is needed.

Mustang Chromatography Capsules

High Dynamic Binding Capacity



High capacity and high flow rates

Mustang capsules have high dynamic binding capacities, and their capacities for large molecules are greater than those of traditional resins. Furthermore, these high capacities can be achieved at high flow rates of 10 to 40 membrane volumes / minute (MV/min).

Table1: Typical Dynamic Binding Capacity of **Mustang** Membrane for Standard Biomolecules.

Molecule	MW or Size	Chemistry	Mustang Membrane Capacity
Lysozyme	14.3 kD	S	45-50 mg/mL
Human IgG	160 kD	S	~60 mg/mL
BSA	65 kD	Q	50-60 mg/mL
Thyroglobulin	650 kD	Q	~30 mg/mL
Genomic DNA	500-30,000 bp	Q	20-25 mg/mL
Plasmid DNA	12 kb plasmid	Q	~15 mg/mL
Adenovirus		Q	5 x 10^{12} particles/mL
Endotoxin		E	4 x 10^{6} EU/mL

Mustang Chromatography Capsules

High Throughput Capture Step

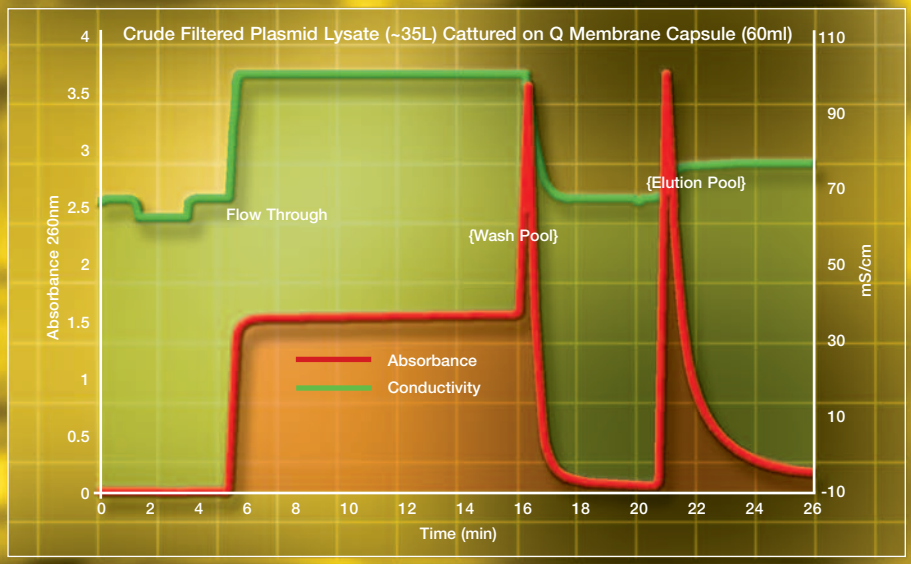


Figure 5

Usually the design of the purification scheme for a biomolecule will involve more than one chromatography step. The high capacity has made ion exchange chromatography a commonly used method for initial product capture in many purification processes.

The ability to achieve high capacity at high flow rates makes **Mustang** capsules very attractive for fast processing of large sample volumes. The use of **Mustang** capsules at this stage has proved to be very efficient as large volumes can be processed quickly especially with dilute feedstocks. Batch processing times are reduced and hence limiting protease activity.

We have applied this technique for the purification of a plasmid coming from *E.coli* fermentation, and we have demonstrated that gram quantities can be processed in less than 2 hours.

Figure 5: Separation of 4.5 kB pCAT plasmid on 60 mL **Mustang** Q capsule.

Thirty-five liters of *E.coli* cell lysate (after prefiltration and conductivity adjustment) were loaded on to a 60mL **Mustang** Q capsule at 600mL/min. After a washing step with a 0.5M NaCl in 25mM Tris-HCl, pH 8.0, plasmid was eluted with a buffer containing 1.0M NaCl and 25mM Tris, pH8.0. Approximatively 1.0 g of pCAT plasmid was obtained using this protocol. Good purity and recovery were achieved.

Mustang Chromatography Capsules

High Throughput Contaminant Removal

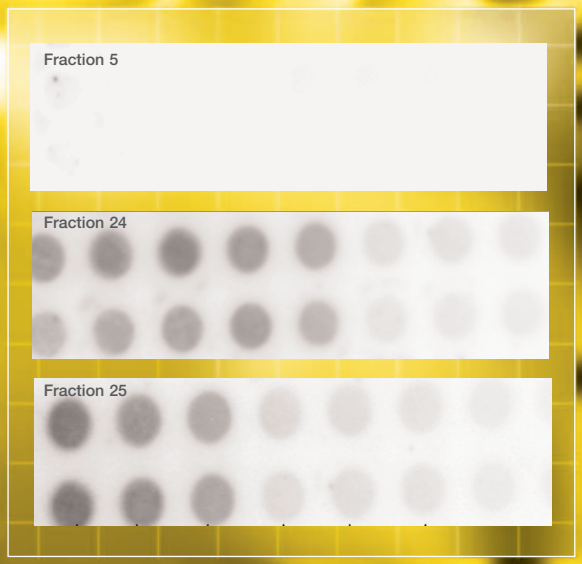


Figure 6a

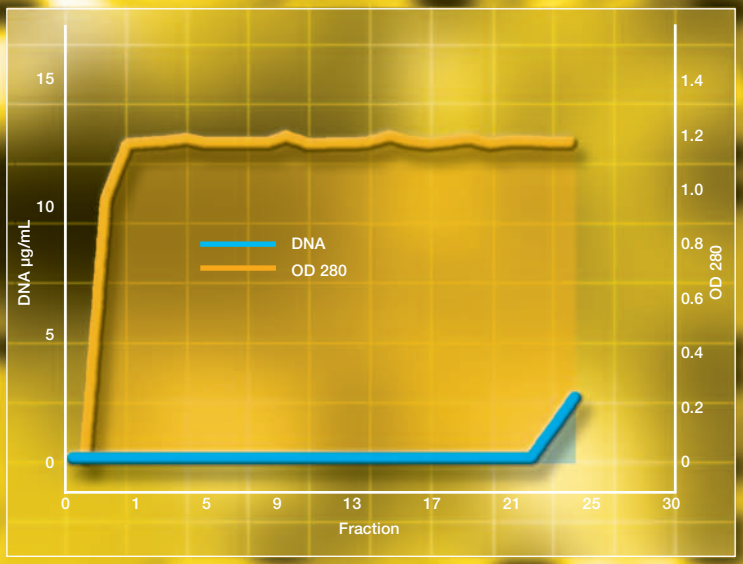


Figure 6b

The demand for therapeutic biologics has grown exponentially, and this growth has created a need for quick and efficient methods to remove DNA , viruses, HCP and other undesirable contaminants from these biologicals. Although traditional column chromatography has been used for decades, it usually employs an over-sized column in order to accommodate the throughput requirement. Since **Mustang** capsules can offer both high capacity and high throughput, a small capsule is then capable of processing large volumes of sample with short processing time. Furthermore, since **Mustang** capsule are a single use product, no cleaning or cleaning validation is needed, this can dramatically simplify the process development and validation.

Mustang capsules are the clear choice for contaminant removal from biopharmaceuticals.

We have demonstrated that **Mustang** Q capsules can typically reduce DNA from 10µg/mL to less than 10 pg/mL, resulting in a 6 logs of clearance in a single pass at 10 to 40 membrane volume / minute as shown by dot blots.

Ion exchange chromatography has also been widely used for virus removal, and it is often considered as a contributive step because of the validation complexity. **Mustang** Q capsules can efficiently remove viruses from protein solutions. Typically 4-7 logs titer reduction has been achieved for several model viruses.

Figure 6: Results of DNA challenge test

A bovine Ig G solution (4mg/mL) was spiked with Calf Thymus DNA (20 µg/mL) and loaded on a **Mustang** Q coin. Absorbance at 280 nm and DNA content using a p32 labelled probe were continuously monitored. No DNA was found before fraction 24, thus the DNA clearance factor was >6.8 log; whereas protein recovery was greater then 95% in the chosen conditions.

a. Scan of autoradads
Fraction 5, 24 and 25.

b. Chromatogram

Mustang Chromatography Capsules

Cost effective Disposable Scaleable Ion Exchange Chromatography

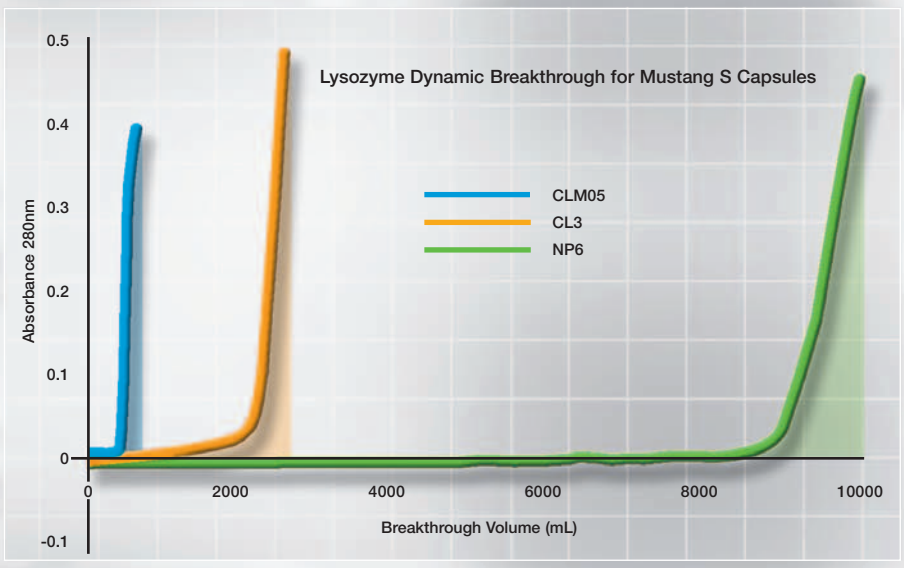


Figure 7

The use of **Mustang** capsules eliminates packing, cleaning and associated validation. This can greatly shorten the process development cycle, thereby the time to market. The use of **Mustang** capsules can also greatly reduce the consumption of WFI water and buffers, column storage, and other expenses associated with the reuse of a column. Therefore, the **Mustang** capsules can help to achieve a lower overall operating cost.

Low Capital Investment Cost

Mustang capsules have much smaller footprints than the traditional columns since less bed volume is needed due their higher throughput and capacity. It requires no investment in column hardware and complex automated chromatography skids, and reduces the space and number of tanks required for buffer make-up and storage.

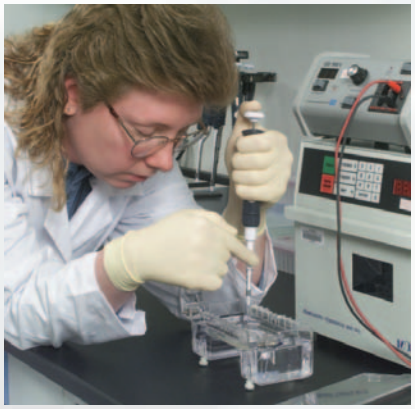
Scaleability and Flexibility

Current **Mustang** capsules are available in three chemistries (Q, S and E) and six different sizes, from 0.18 mL to 780 mL per single unit of Q and S chemistries and from 0.12 mL to 480 mL per single unit of E chemistry.

The breakthrough curves shown above demonstrate (Figure 7) the linear scaleability and the high breakthrough capacity of **Mustang** S capsules. After preconditioning and equilibration with 10mM MES pH 5.5, a solution of 1.6 mg/mL, 2 mg/mL and 1.4 mg/mL of lysozyme in 10mM MES ph 5.5 was loaded respectively on to a 10 mL, 60 mL and 260 mL capsule at a flow rate of 10-20 MV/min. The absorbance at 280 nm was continuously monitored downstream of the capsule.

Mustang Chromatography Capsules

Customer Support and Validation



Extensively Validated

Mustang capsules go through an extensive validation process to ensure consistent and reliable performances in pharmaceutical applications.

Extractables from **Mustang** capsules are low to ensure product safety. **Mustang** capsules are manufactured for use in conformance with cGMP productions as all components have been tested according to the current USP Class VI at 50°C and ISO 10993 for bio-compatibility.

Mustang capsules are supplied with a certificate of analysis, which certifies the performance of each membrane lot for protein binding capacity (Q and S) and typical endotoxin binding capacity (E).

Validation guide and application notes are available upon request.

Customer Support

One of the key elements in Pall's customer support operations is the Scientific and Laboratory Services (SLS) Group. Pall has the capability to support our customers in development of separation and purification processes. Therefore in response to the increasing demand of biotechnology processes and products, specialized biologics laboratories which can handle equipment for optimization of downstream processing chromatography including membrane chromatography have been developed.

Validation Services

Pall Validation Services Group plays a major role in helping filter users meet the current process validation requirements of regulatory authorities. It provides a comprehensive service to Pall customers, ranging from initial filter selection through to final documentation. Our validation specialists ensure that test protocols are consistent with latest regulatory requirements and that your validation is completed on time and to budget.

PALL Life Sciences

2200 Northern Boulevard
East Hills, New York 11548-1289

+1 800.717.7255 toll free
+1 516.484.5400 phone
+1 516.801.9548 fax
pharmafilter@pall.com e-mail

Europa House, Havant Street
Portsmouth PO1 3PD, United Kingdom

+44 (0)23 9230 3303 phone
+44 (0)23 9230 2506 fax
BioPharmUK@pall.com e-mail

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