Endotoxin Removal

Endotoxin Removal

Endotoxin Removal from Water Buffer, Neutral Sugars, and Certain Biological Solutions

Endotoxin is a complex aggregate of acidic lipopolysaccharides (LPS) and consists of an innermost core of hydrophobic fatty acid groups and a central and outermost region composed of hydrophilic polysaccharides. In aqueous solutions, endotoxin can exist in various states of aggregation up to 1 MDa. Divalent cations, such as Ca2+ and Mg2+, are found to stabilize the aggregated structure of LPS, whereas detergents help to break down the structure into smaller sub-units. When producing recombinant proteins in E. coli and other gram-negative bacteria, it is often necessary to remove LPS from the final product. This is especially important when carrying out immunological readouts and when developing manufacturing processes. Endotoxin can cause false readings in cell-based assays. There are limits to the amounts of endotoxin allowed in human products.

Cause of Endotoxin Contamination

Endotoxin is continuously shed from the outer membrane of viable gram-negative bacteria and is released when the bacterial cell dies. Although bacteria are often removed by using a 0.2 µm sterilizing grade filter, LPS itself is difficult to remove or inactivate because it is extremely heat and pH stable. The pyrogenic threshold of an endotoxin reaction is on the order of 1 EU (endotoxin unit ~0.1 ng) per kg of body weight. This amount of endotoxin can come from 105 bacterial cells.

Removal of endotoxin is one of the most difficult downstream processes during protein purification. Many commercially available products are unable to remove endotoxin satisfactorily, or require time-consuming incubation steps. In many cases, complete endotoxin removal is only achieved with massive substrate loss. Because endotoxin is negatively charged at pH above 2, a positively-charged membrane surface can remove endotoxin. The Acrodisc® unit with positively-charged, hydrophilic Mustang® E membrane is ideal for the removal of endotoxin from solution due to its highly crosslinked quaternized amine charged surface. This gives very high dynamic capacities under selected conditions for the removal of endotoxin from process feedstreams, buffers, and water. The Acrodisc syringe filter has a high performance endotoxin removal capability of approximately 500,000 EU/unit in saline. The device properties are summarized in Table 2.64.

Table 2.64
Properties of the Acrodisc® Unit with Mustang® E Membrane, 25 mm

Specification	Parameter
Materials of Construction	
Membrane	Mustang E (positively-charged quarternary amine surface)
Housing	Polypropylene
Effective Filtration Area	2.8 cm^2
Membrane Pore Size	0.2 µm
Membrane Bed Volume	0.12 mL
Mean Endotoxin Removal Capacity (EU)*	~500,000 EU/Acrodisc unit from saline**
Maximum Pressure Limit	5.5 bar (550 kPa, 80 psi)

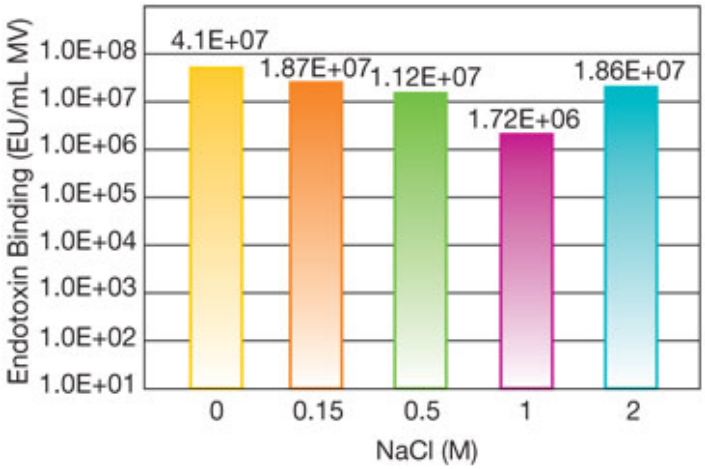
Mean Flow Rate	1-4 mL/min
Inlet/Outlet Connectors	Female luer-lok inlet, male slip luer outlet
Sterilization	Sterilized by gamma irradiation and individually packaged

**The endotoxin removal capacity may vary by flow rate, protein surface charge, type and concentration of protein, pH, salt concentration, or other components of the sample solution such as surfactants and glycols.*
***Endotoxin source: Escherichia coli strain 055:B5 (no dispersing agents) at a minimum challenge level of 10,000 EU/mL in a volume of 60 mL of 0.9% saline.*

Application Data for Endotoxin Removal from Water Buffer, Neutral Sugars, and Certain Biological Solutions

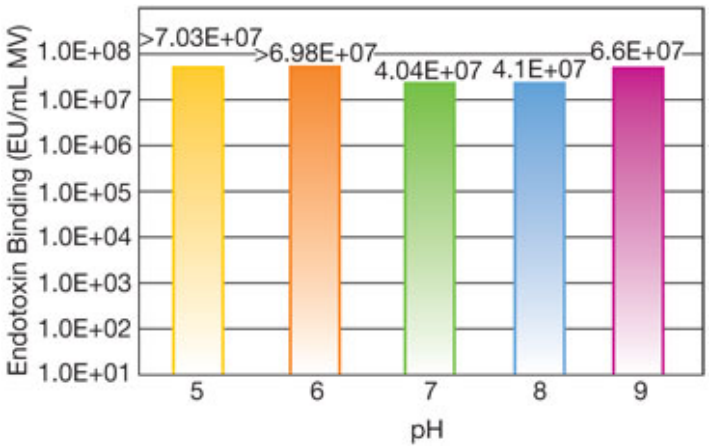
The impact of composition of the sample medium was assessed for ionic strength and pH on the dynamic binding capacity of the Acrodisc unit with Mustang E membrane. Data is summarized in Figure 2.60 for NaCl and Figure 2.61 for pH. The results show that NaCl at all concentrations reduced the dynamic binding capacity suggesting that an ion exchange mechanism is responsible for adsorption of endotoxin onto the Mustang E surface. Interestingly the dynamic binding capacity appeared to be higher at 2.0 M than at 1.0 M NaCl, suggesting that a secondary hydrophobic interaction may be present under high salt conditions.

Figure 2.60
Effect of NaCl Concentration in 25 mm Tris (pH 8) on Endotoxin Dynamic Binding Capacity to Acrodisc® Unit with Mustang® E Membrane



Endotoxin source: Escherichia coli strain 055:B5 (no dispersing agents) at a minimum challenge level of 10,000 EU/mL. A range of NaCl concentrations were tested in a volume of 60 mL. The dynamic binding capacity was calculated at 10% of the breakthrough curve.

Figure 2.61
Effect of pH on Endotoxin Dynamic Binding Capacity to Acrodisc Unit with Mustang E Membrane



Following process as described in Figure 2.60, with pH varied between 5.0 and 9.0.

Ordering Information for Endotoxin Removal from Water Buffer, Neutral Sugars, and Certain Biological Solutions

Acrodisc® Unit with Mustang® E Membrane

Part Number	Description	Pkg
MSTG25E3	0.2 µm, 25 mm, sterile, blister packs	10/pkg

References for Endotoxin Removal from Water Buffer, Neutral Sugars, and Certain Biological Solutions

1. Nelsen, L. (1977). Filtration removal of endotoxin in solution in different states of aggregation. Applied and Environmental Microbiology, (34), 382–385.

2. Olson, W.P. (1995). Separations Technology, Pharmaceutical and Biotech Appl., 57–194.

3. Zimmerman, G., et al. (1976). Drugs made in Germany-pyrogen elimination from paren. med. by means of UF. (19), 123–128.

4. Karbachsch, M., et al. (1984). Drugs made in Germany-depyrogen of soln of low Mol. Wt. Subs. by UF. 27(72), 74–76.

Top