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Tech Note

Sterilizing Grade Filtration Unit Operations for Plasmid DNA Processes

Recommendations

Millipore Express® SHC can be used to achieve high filtration capacity, flux and yield for Plasmid DNA (pDNA) filtration in a variety of formats including pre-sterile capsules with sizes ranging from 0.014 m^2 to 3.0 m^2. Capacity and yield of the unit operation can vary significantly, especially with larger plasmids (~10 kbp and greater), and as such, process development should be carefully considered for optimization of the step.

Overview

Attributes

Sterilizing Grade Filtration unit operations for pDNA processes should include:

- A membrane with the ability to remove bioburden from the feed stream
- A device and filtration system that can prevent the introduction of bioburden
- A membrane that can reduce particulates, provides high capacity and high flux, and allows pDNA to flow through

Parameters

A Sterilizing Grade Filtration unit operation for pDNA processing can be optimized by changing the:

- Membrane used for sterile filtration
- Filtration device and system used
- Driving force (flowrate or pressure)
- Formulation of the pDNA solution
- Purity of the pDNA solution
- Conformation of the pDNA (supercoiled, linear, open-circular, etc.)
- Endpoint of the filtration

Key Considerations

The large size of pDNA can present a challenge for sterile filtration unit operations, as the product can be retained by the filters, leading to yield loss and low filtration capacity. Additionally, large pDNA molecules can be shear sensitive and use of a sterile filtration step has the potential to cause shear-induced denaturization of the product. Viscosity must also be considered as flow rates for sterile filtration steps can be low due to viscous material. Finally, a sterile filter must be proven to retain bacteria, which can be problematic for pDNA vaccines containing adjuvants.

Attributes	Parameters	Issues
Sterility assurance	Membrane pore size	Large size of pDNA
Particulate reduction	Membrane chemistry	Shear sensitivity of pDNA
Filtration capacity and flux	Driving force	Viscosity of pDNA solution
pDNA yield	Formulation	Bacterial retention for adjuvanted pDNA solutions
	Filtration endpoint	

Table 1. Key considerations for sterile filtration of pDNA solutions.

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Technical Data

Process parameters should be optimized to achieve highest sterilizing grade filtration performance. While some plasmids present unique filtration challenges, for many smaller plasmids of less than 10 kbp, development of a robust unit operation could be as simple as confirming filter sizing using Vmax™ or Pmax™ methodology.[1]

A review of internal data for sterilizing grade filtration of pDNA feeds showed that filtration capacity, flux and yield can vary significantly, depending on the size of the plasmid, with larger plasmids presenting the greatest filtration challenge. Other researchers have also shown that filtration performance declines as plasmid size increases. The most significant filtration challenge occurs with pDNA of 20 kbp and larger – although 10–20 kbp pDNA often also cause filtration issues.[2,3] Table 2 summarizes the review of internal data and published studies.

Plasmid DNA Size (kbp)	Expected Sterilizing Grade Filtration Yield (%)	Expected Sterilizing Grade Filtration Capacity (L/m²)
<10	>90	>50
10–20	>80	Variable
>20	<80	<20

Table 2. Expected performance for sterilizing grade filtration of purified pDNA based on internal studies and literature search.

While the size of pDNA impacts sterilizing grade filtration performance, internal data and published studies both show that buffer composition can alter the plasmid conformation and subsequent radius of gyration. Specifically, salt concentrations have been shown to directly impact both the radius of gyration and diffusion coefficient of pDNA (Table 3).[4,5,6]

NaCl Concentration[a] (mM)	R_s[b] (nM)	D[c] (m^2/s)
10	6.9	4.0×10^{-12}
40	5.8	5.2×10^{-12}
100–300	4.5	5.5×10^{-12}

Table 3. Plasmid DNA properties.

[a] In TE buffer.

[b] From Hammermann et al. (1998) for 2.69 kbp plasmid.

[c] From Nguyen and Elimelech (2007) for 3.0 kbp plasmid with values adjusted to account for TE species in buffer solution (refer to text for details).

Changing the salt concentration has empirically demonstrated a greater than 2× increase in sterilizing grade filtration capacity and yield in internal studies and published studies.[3]

Using membranes for ultrafiltration, a study demonstrated a significant change in the sieving of pDNA with a change in salt concentration, providing further evidence that salt concentration heavily influences membrane filtration of pDNA.[7]

In addition to impact of pDNA size, studies have shown that supercoiled plasmid gives better filtration performance than open-circular; the purity of supercoiled pDNA can thus significantly impact unit operation outcomes of a sterilizing grade filtration step. One study cited an increase of approximately 10× in filtration capacity going from 90% to 95% supercoiled content.[2]

The filtration endpoint has been found to be significant in internal studies. Under constant pressure, plasmid concentration in the filtrate decreases at high flux decay, while constant flowrate operation has shown yield decline when pressure drop increases above a threshold. While both findings suggest that plasmid yield correlates with membrane fouling, detailed studies are needed to investigate the mechanism of action.

Both PVDF and PES membranes have shown success in filtering pDNA solutions. PES is preferred as it tends to have both higher capacity and flux versus PVDF and can be less damaging to larger plasmids.[3] Internal studies have shown higher yield for PES filters, although more detailed studies are needed to confirm this finding.

Data from internal and published studies suggest that altering the pDNA concentration can affect yield and capacity. Some published data have shown increased mass throughput with increased pDNA concentration.[2] Internal data suggest, however, this may not always be true; increased concentration may cause some self-association of pDNA molecules depending on the background buffer and purity, resulting in lower filtration capacity and yield. While concentration of pDNA is certainly a critical operating parameter, specific approaches for optimizing performance via dilution or concentration need to be better defined.

A review of sterilizing grade filtration operation conditions showed that feed flux or pressure has little to no impact on filtration capacity or yield (Table 4). It is possible, however, that high driving force could compromise plasmid integrity from shear, especially for larger plasmids.[3]

Optimization Parameter	Yield	Capacity	Product Integrity
Salt concentration	X	X	
Supercoiled pDNA content (purity)	X	X	
Filtration endpoint	X		
Membrane type – PVDF or PES	X – PES		X – PES
pDNA concentration	X	X	
Feed flux or pressure			X

Table 4. Critical parameters for optimizing plasmid DNA sterilizing grade filtration unit operations.

After a thorough review of published and internal data, critical parameters have been defined and can be applied to process development activities. Critical quality attributes of yield, capacity, and product integrity can be optimized through various parameters.

- Yield can be optimized by increasing salt concentration, increasing pDNA purity, defining the filtration endpoint to avoid extreme fouling, screening membranes, and exploring various pDNA concentrations.
- Capacity can be optimized through increasing salt concentration, increasing pDNA purity, or testing different pDNA concentrations.
- Product integrity through sterilizing grade filtration can be impacted by membrane type and feed flux or pressure.

References

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Lit. No. MK_AN6587EN Ver. 0.1
09/2020