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Depyrogenation of Vivaspin® Turbo 15 in comparison to Ultrafiltration devices with a regenerated cellulose membrane

Application Note

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Introduction

Endotoxins (or Lipopolysaccharides) are a component of gram-negative bacteria cell wall, an often unwanted impurity in laboratory based research due to their inflammatory and pyrogenic effect on mammalian immune systems.

Here, the background levels of endotoxin from manufacturing are quantified in both Vivaspin® Turbo 15 devices and 15 ml ultrafiltration devices from another supplier (Supplier A). Additionally, both types of devices were subjected to treatment of 1N NaOH, which is commonly used in laboratories as a basic chemical for depyrogenation.
A protocol describes the depyrogenation of Vivaspin® Turbo 15 for applications where the absence, thus removal of endotoxin is of critical importance.

Method

A) Analysis of typical baseline endotoxin level

1. 2 × Vivaspin® Turbo 15 (10 kDa PES membrane) and 2 × 15 ml UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 ml HyPure water and left to stand at 20°C for 30 min.
3. Each device was centrifuged at 3000 × g for 10 min till approximately 0.5 ml of concentrate remained (approx. 30-fold) in the concentrate reservoir.
4. Samples were retrieved from the filtrate reservoir and loaded onto an Endosafe-PTS cartridge for EU/ml quantification.

B) Effect of NaOH treatment on flux and recovery

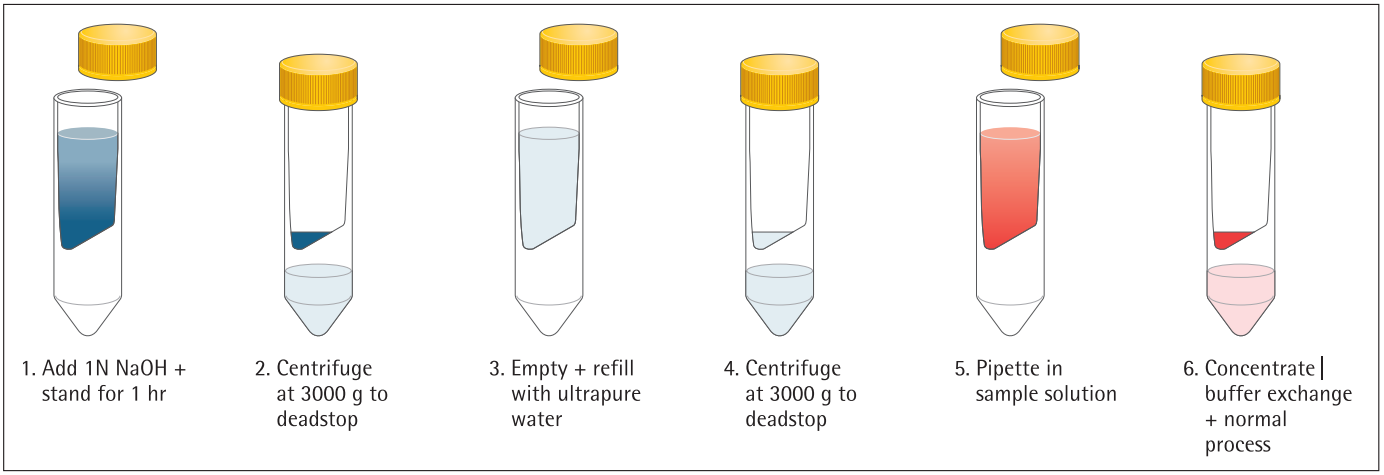
1. 4 × Vivaspin® Turbo 15 (10 kDa PES membrane) and 4 × 15 ml UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 ml 1N NaOH and left to stand at 20 °C for 1 hr.
3. Each device was then centrifuged at 3000 × g till the device deadstop was reached.
4. The devices were emptied, then re-filled with 15 ml HyPure water for the 1st wash cycle.
5. The devices were then centrifuged at 3000 × g till the deadstop was reached.
6. A 2nd wash cycle was repeated as above.
7. The same devices were then emptied and filled with 15 ml 1.0 mg/ml BSA in saline.
8. All devices were centrifuged at 3000 × g till the final concentrate volume was < 0.5 ml.
9. A recovery measurement was then performed on a spectrophotometer.

Results and discussion

The typical endotoxin levels were an order of magnitude below the guideline maximal threshold of 0.1 EU/ml for intravenous work with a 20 g mouse, showing the inherent cleanliness of the devices in both the Vivaspin® Turbo 15 and the 15 ml UF device, Supplier A, even when untreated (table 1).

Upon treatment with 1N NaOH, the flow rate and protein retention and recovery value in Vivaspin Turbo 15 remained unaffected (table 3).
In contrast, the 15 ml UF device with a regenerated cellulose membrane from Supplier A showed a significant reduction in the filtration rate following the use of high pH 1N NaOH, despite decreasing pH after each wash cycle (table 2),

The total process time following the depyrogenation protocol described above in method B) was over twice as fast when using the Vivaspin® Turbo 15 compared to the 15 ml UF device, Supplier A (table 3).



Schematic depyrogenation process, followed by sample concentration.

Equipment and test samples

- Vivaspin® Turbo 15 10 kDa PES (Sartorius, VS15T01)
- 15 ml UF device, Supplier A
- NaOH (Sigma, S0899)
- NaCl (Sigma, S7653)
- HyPure Cell Culture Grade Water, Endotoxin Free (< 0.005 EU/ml) LAL water (HyClone, SH30529.03)
- Albumin from Bovine Serum (Sigma-Aldrich, 1001430867)
- Genova Spectrophotometer (JENWAY, 1282)
- Megafuge 1.0R Centrifuge (Heraeus instruments, 100000494)
- Standard Pipettes and tips

Tables and figures

	Vivaspin® Turbo 15		Amicon Ultra 15®	
	1	2	1	2
Final volume (ml)	0.54	0.42	0.75	0.52
EU/ml	< 0.006	< 0.005	< 0.005	< 0.009

Table 1: Both the Vivaspin® Turbo 15 and 15 ml UF device, Supplier A presented less than 0.01 EU/ml of endotoxin when untreated and tested as a water wash control.

Device type	Vivaspin® Turbo 15 10 kDa PES	15 ml UF device, Supplier A 10 kDa Regenerated Cellulose
Average time to concentrate BSA 30 × prior to NaOH treatment	15	25
Average time for NaOH treatment and 2 wash cycles (min)	90	225
Average time to concentrate protein 30 x post NaOH treatment (min)	15	45
Final concentrate volume (ml)	0.4	0.25 – 0.3
Recovery percentage (%)	97.0	84.9
Total process time (min)	105	240

Table 2: Process time taken when devices centrifuged at 3000 × g. Depyrogenated Vivaspin® Turbo 15 lead to higher recovery of protein after treatment with NaOH. Additionally, the PES membrane remained unaffected by high pH treatment, leading to a faster total processing time by 135 min compared to the time take by the 15 ml UF device, Supplier A.

	After NaOH treatment	1st wash cycle	2nd wash cycle
pH of filtrate	13.51	11.03	9.32

Table 3: The pH levels of device filtrates were assayed during each wash cycle to demonstrate that even when the pH level was lowered, the negative effect of NaOH on the flow rate of regenerated cellulose was not reversed. The low endotoxin HyPure water and the filtrate from an untreated device presented a baseline pH of 7.55.

Conclusion

For applications, in which the absence of endotoxins is essential, we describe a method for fast and reliable depyrogenration of Vivaspin® Turbo 15 devices.

Additionally, it could be shown that Vivaspin® Turbo 15 has superior performance in both flow rate and recovery compared to Supplier A with a regenerated cellulose membrane, following 1N NaOH soaking treatment for 1 hr.

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