



Life Sciences

Validation Guide

USTR2901

Cadence™ Single-Use TFF Modules with Omega™ Membrane

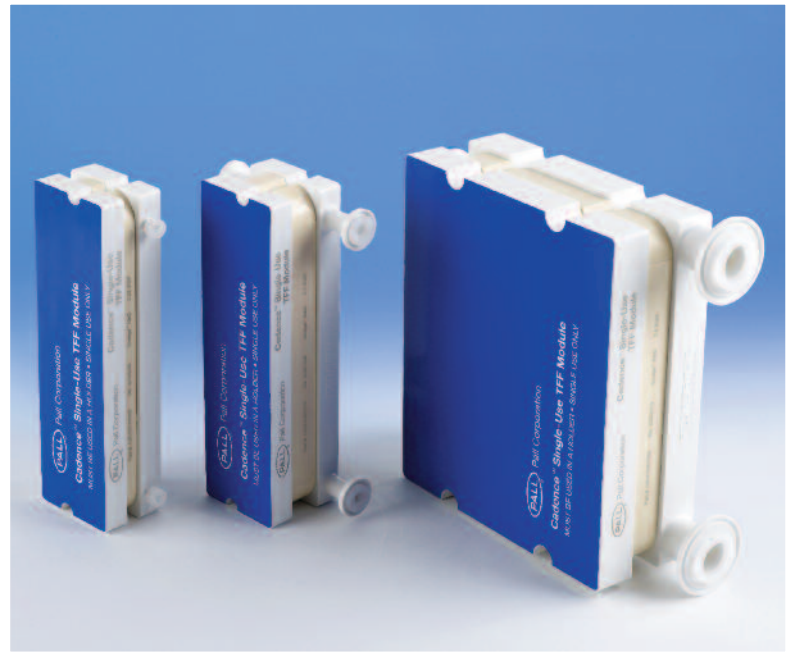


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1. Validation Overview

1.1 Introduction

The purpose of this validation guide is to document the testing that has been performed to demonstrate the suitability of the Cadence single-use TFF modules for use in Biopharmaceutical applications.

Pall Cadence single-use TFF modules with Omega polyethersulfone (PES) membrane are pre-assembled, gamma irradiated modules. The TFF filter element is assembled between two manifold plates, which allow for easy installation and disposal. The modules are also pre-flushed with water and stored in water to reduce flush volume requirements for the end user. Additionally, cleaning and cleaning validation requirements are eliminated because the modules are intended for single use only.

The modules are available in three Nominal Molecular Weight Cutoffs (NMWC): 10, 30, and 100 kD, and five membrane areas:

- Centramate™ cassette format:
 - 93 cm² part number CSUMxxxT001⁽¹⁾
 - 186 cm² part number CSUMxxxT002
 - 0.1 m² part number CSUMxxxT010
- Centrasette™ cassette format:
 - 0.5 m² part number CSUMxxxT050
 - 2.5 m² part number CSUMxxxT250

⁽¹⁾: xxx corresponding to the NMWC code: 010, 030 and 100 for 10, 30, and 100 kD respectively

All modules are intended to be torqued and operated in Pall Cadence holders for single-use TFF for the corresponding formats.

This validation guide summarizes the tests that were performed to qualify the performance of Cadence single-use TFF modules. It includes data on functional and mechanical properties, biological safety, extractables and shelf life.

Cadence single-use TFF modules with Omega membrane are supplied after receiving a gamma irradiation dose equal to or greater than 25 kGy. All the validation work in this guide has been performed on modules receiving a gamma irradiation dose of 50-60 kGy, which represent a worst-case scenario. This assures that Cadence single-use TFF modules with Omega membrane meet all the criteria specified in this validation guide post exposure to gamma irradiation at a dosage up to and including 50 kGy.

Additionally, all modules were torqued in their respective Cadence holders for single-use TFF at the recommended nominal torque settings.

Please contact your Pall representative for any question on this validation guide.

1.2 Summary of Conclusions

1.2.1. Mechanical and Functional Tests

1.2.1.1 *Stability of the Membrane to Gamma Irradiation*

Omega membrane samples exposed to >50 kGy gamma irradiation were tested for water permeability and solute retention and the results were compared to non gamma exposed Omega membrane results. No significant change in performance was shown between Omega membranes exposed to >50 kGy gamma irradiation compared to non-gamma exposed Omega membrane.

1.2.1.2 *Maximum Operating Pressure at Minimum Temperature*

The Cadence single-use TFF modules were tested with deionized water at 4 barg (58 psig) and 4 °C for 2 cycles of 8 hours each. After completion of the testing, the modules met the acceptance criteria for integrity and the differential pressure (Delta P) values or Feed Channel Air Flow values, confirming that the modules can be used at a maximum pressure of 4 barg (58 psig) and a minimum temperature of 4 °C for 8 hours.

1.2.1.3 *Maximum Operating Pressure at Maximum Temperature*

The Cadence single-use TFF modules were tested with deionized water at 4 barg (58 psig) and 40 °C for 2 cycles of 8 hours each. After completion of the testing, the modules met the acceptance criteria for integrity and the Delta P values, confirming that the modules can be used at a maximum pressure of 4 barg (58 psig) and a minimum temperature of 40 °C for 8 hours.

1.2.1.4 *Life Time (Operating Time)*

Tests confirmed that the Cadence single-use TFF modules could maintain integrity after being processed with deionized water at 4 barg (58 psig) and either minimum (4 °C) or maximum temperature (40 °C) for 2 cycles of 8 hours each. Since these temperatures represent extreme operating conditions, the modules can therefore be processed in an 8-hour operating window, at temperatures between 4 and 40 °C.

1.2.1.5 *Clamping Values*

Cadence single-use TFF modules in Centramate cassette format were torqued to between 9 and 11 Nm (80-100 in-lb) in the 4-bolt Cadence CM holder for single-use TFF designed for modules with a Centramate cassette format. The integrity and the Delta P values met the acceptance criteria. A torque range of 9-11 Nm (80-100 in-lb) was recommended for the modules in Centramate cassette format.

Cadence single-use TFF modules in Centrasette cassette format were torqued to between 80 and 125 Nm (700-1100 in-lb) in the 2-bolt Cadence CS holder for single-use TFF designed for modules with a Centrasette cassette format. The integrity and the Delta P values met acceptance criteria. A torque range of 80-125 Nm (700-1100 in-lb) was recommended for the modules in Centrasette cassette format.

1.2.1.6 *Pre-Use Flush Studies*

The Cadence single-use TFF modules were flushed with de-ionized water, with 75% of the feed flow going through the permeate and 25% through the retentate.

Typical total flush-out volumes (feed volumes) to decrease the TOC value to 1 ppm in both the retentate and permeate lines were:

- 20 L/m² (2 L/ft²) de-ionized water for the 10 and 30 kD NMWC modules.
- 30 L/m² (3 L/ft²) de-ionized water for the 100 kD NMWC modules.

In general, 1 ppm TOC levels were reached for the feed-retentate channel within 10 L/m² flush-out volumes.

1.2.1.7 *Compatibility with Pre-Use Sanitization Solutions- High and Low pH*

The Cadence single-use TFF modules were subjected to two 30 minute cycles with either 400 ppm peracetic acid (pH of 2), or 0.5 N NaOH (pH of 14). Both tests were performed at 25 °C. After completion of the testing, the modules met the acceptance criteria for integrity and the differential pressure (Delta P) values or Feed Channel Air Flow values, confirming that the modules can be pre-use sanitized for 30 minutes at 25 °C using either a 400 ppm peracetic acid solution or a 0.5 N NaOH solution.

However, depending upon end-user requirements and operating conditions, a pre-sanitization step may not be required.

1.2.1.8 *Post-Use Decontamination*

The Cadence single-use TFF modules were tested for post-use decontamination with 1 N NaOH exposure for 1 hour at ambient temperature. The modules were visually inspected after exposure and no external damage was noted. The modules installed in their holder also passed a pressure hold test at 30 psig and are thus compatible with 1 N NaOH exposure for 1 hour for decontamination purposes.

It should, however, be noted that post-use decontamination is for safety issues only, as the modules are only qualified for single-use.

1.2.2 **Shelf Life Information**

An accelerated aging study has been completed for Cadence single-use TFF modules stored at 45 °C for 79, 159 and 238 days, which are equivalent to storage for 1, 2 and 3 years, respectively, at ambient conditions. The test modules met the acceptance criteria for integrity and the differential pressure (Delta P) values. A real-time ambient shelf life study is also planned. Results will be available as developed. Please contact Pall for the latest information.

1.2.3 **Extractables**

Four Cadence single-use TFF modules were flushed at 18 to 25 °C with 20 L/m² (2 L/ft²) of DI water to waste prior to the extraction. Two extraction cycles of 8 hours each were run in recirculation with de-ionized water at 40 °C +/-2.5 °C. The total extractables based on the Non-Volatile Residue were less than or equal to 18 mg for modules with 0.5 m² membrane area and less than or equal to 13 mg for modules with 0.1 m² membrane area.

For further information regarding extractable quantitative and qualitative characterization, please contact your Pall representative.

1.2.4 **Biological Safety**

Tests were performed as specified in reference to USP chapters on the fluid path material of construction of the Cadence single-use TFF modules.

The fluid path of Cadence single-use TFF modules with Omega membrane met the requirements for:

Biological Reactivity Tests (*in vivo*) for Class VI-70 °C Plastics, USP <88>

Biological Reactivity Tests (*in vitro*) for Cytotoxicity, USP <87>

1.2.5 Endotoxins

The Cadence single-use TFF modules were flushed with 20 L/m² (2 L/ft²) LAL reagent water, per standard flush procedures (5-10 L/min/m² [0.5-1.0 L/min/ft²] feed flux, with 75% of the feed flow through the permeate and 25% through the retentate). The endotoxin level of the permeate and retentate pools after flushing was ≤ 0.005 EU/mL.

2. Mechanical and Functional Tests

2.1 Stability of the Membrane to Gamma Irradiation

2.1.1 Introduction

Omega membranes are routinely used for re-use tangential flow filtration (TFF) biopharm applications in T-series cassettes. In order to implement the existing membrane into the Cadence single-use TFF module product line, the membrane required verification work to prove gamma stability for single-use applications.

The objective of this work was to verify that irradiated Omega membrane has no significant change in performance compared to non-gamma irradiated Omega membrane. Membrane performance tests consisted of water permeability and solute retention.

2.1.2 Test Method

Representative membrane samples were evaluated after flushing and exposure to gamma irradiation (>50 kGy) and compared to controls. Membrane samples were tested using stirred cell format for characterization of water permeability and solute retention.

2.1.3 Results

Table 1

| Membrane | Stirred Cell Test | Comments | Pass/Fail |
|--------------|--|--|-----------|
| Omega 10 kD | Water Flux 0.2% BSA Dextrans R90 | No statistical differences were detected for pre- versus post-gamma irradiation with regards to testing in stirred cells | Pass |
| Omega 30 kD | Water Flux 0.2% BSA Dextrans R90 | | Pass |
| Omega 100 kD | Water Flux 0.1% IgG 0.2% BSA | | Pass |

2.1.4 Conclusion

Omega membranes exposed to >50 kGy gamma irradiation have no significant change in performance compared with non-gamma exposed Omega membrane.

2.2 Maximum Operating Pressure at Minimum Temperature

2.2.1 Introduction

The purpose of this testing was to demonstrate product robustness and compatibility at the extreme operating conditions of high pressure (4 barg/58 psig) and low temperature (4 °C). Cadence single-use modules considered as representative of the whole product range were tested.

2.2.2 Test Methods

Three CSUM010T002 (186 cm², 10 kD NMWC) and two CSUM010T050 (0.5 m², 10 kD NMWC) single-use modules were gamma irradiated and then flushed with 20 L/m² (2 L/ft²) deionized water at a feed pressure of 2 barg (30 psig), with 95% of the flow through the permeate and 5% through the retentate. The modules were subsequently characterized for Delta P, Feed Channel Air Flow (FCAF) and integrity.

The Delta P (DP), also called differential pressure, is corresponding to the difference between the feed pressure and the retentate pressure.

Deionized water was then recirculated for 8 hours at 4 barg (58 psig) and 4 °C at a minimum retentate Cross Flow Flux (CFF) of 5 L/min/m² (0.5 L/min/ft²). The modules were subjected to two 8 hour cycles to represent a safety factor of two times compared to the recommended 8-hour operating time for the Cadence single-use TFF modules and were characterized after completion of each cycle.

Module acceptance criteria were:

- for integrity: <1500 mL/min/m² (<150 mL/min/ft²) permeate air flow at 2 barg (30 psig)
- for Delta P: 0.3-1 bar (5-15 psid), at 5 L/min/m² (0.5 L/min/ft²) average Cross Flow Flux
- for Feed Channel Air Flow (FCAF): 2.5 to 6.5 L/min/m² (0.25 to 0.65 L/min/ft²) at 0.7 barg (10 psig)

2.2.3 Results

All modules tested met acceptance criteria for integrity after completion of testing. Meanwhile, the Delta P values or FCAF values remained within the acceptance criteria.

Table 2

Characterization Data After Cycle Testing at 4 barg (58 psig) and 4 °C

| Part Number | Serial Number | Time (hr) | FCAF⁽¹⁾ at 0.7 barg (10 psig) | DP⁽¹⁾ at 0.5 L/min/ft² CFF | IT⁽¹⁾ at 2 barg (30 psig) |
|--------------------|----------------------|------------------|---|---|---|
| CSUM010T002 | 32247203 | 0 | Pass | n/a ⁽²⁾ | Pass |
| | | 8 | Pass | n/a | Pass |
| | | 16 | Pass | n/a | Pass |
| CSUM010T002 | 32252202 | 0 | Pass | n/a | Pass |
| | | 8 | Pass | n/a | Pass |
| | | 16 | Pass | n/a | Pass |
| CSUM010T002 | 32252203 | 0 | Pass | n/a | Pass |
| | | 8 | Pass | n/a | Pass |
| | | 16 | Pass | n/a | Pass |
| CSUM010T050 | 32040202 | 0 | n/a | Pass | Pass |
| | | 8 | n/a | Pass | Pass |
| | | 16 | n/a | Pass | Pass |
| CSUM010T050 | 32040203 | 0 | n/a | Pass | Pass |
| | | 8 | n/a | Pass | Pass |
| | | 16 | n/a | Pass | Pass |

⁽¹⁾ FCAF: Feed Channel Air Flow; DP: Delta P; IT: Integrity Test

⁽²⁾ n/a : not applicable to the size of the tested Cadence single-use TFF modules

2.2.4 Conclusions

The Cadence single-use TFF modules can be processed for one 8-hour cycle at 4 °C and 4 barg (58 psig) while maintaining product robustness.

2.3 Maximum Operating Pressure at Maximum Temperature

2.3.1 Introduction

The purpose of this testing was to demonstrate product robustness and compatibility at extreme operating conditions of high pressure (4 barg/58 psig) and high temperature (40 °C). Cadence single-use modules considered as representative of the whole product range were tested.

2.3.2 Test Methods

Two CSUM010T010 (0.1 m², 10 kD NMWC) and two CSUM010T050 (0.5 m², 10 kD NMWC) Cadence single-use modules were gamma irradiated and then flushed with 20 L/m² (2 L/ft²) DI water, with 95% of the feed flow through the permeate and 5% through the retentate. They were then characterized for Delta P and integrity. Deionized Water was recirculated at 4 barg (58 psig) and 40 °C at a minimum retentate CFF of 5 L/min/m² (0.5 L/min/ft²) for an 8-hour cycle and the modules were characterized after completion of each cycle. The modules were processed for two cycles to represent a safety factor of two times compared to the recommended 8-hour operating time for the Cadence single-use TFF modules.

Module acceptance criteria were:

- for integrity: <1500 mL/min/m² (<150 mL/min/ft²) permeate air flow at 2 barg (30 psig)
- for Delta P: 0.3-1 bar (5-15 psid), at 5 L/min/m² (0.5 L/min/ft²) average Cross Flow Flux

2.3.3 Results

All modules met acceptance criteria for integrity after completion of testing. Meanwhile, the Delta P values remained within the acceptance criteria.

Table 3

Characterization Data After Cycle Testing at 4 barg (58 psig) and 40 °C

| Part Number | Serial Number | Time (hr) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|-----------|--|---------------------------------------|
| CSUM010T010 | 32251206 | 0 | Pass | Pass |
| | | 8 | Pass | Pass |
| | | 16 | Pass | Pass |
| CSUM010T010 | 32035205 | 0 | Pass | Pass |
| | | 8 | Pass | Pass |
| | | 16 | Pass | Pass |
| CSUM010T050 | 32035205 | 0 | Pass | Pass |
| | | 8 | Pass | Pass |
| | | 16 | Pass | Pass |
| CSUM010T050 | 32035206 | 0 | Pass | Pass |
| | | 8 | Pass | Pass |
| | | 16 | Pass | Pass |

⁽¹⁾ DP: Delta P; IT: Integrity Test

2.3.4 Conclusions

The Cadence single-use TFF modules can be processed at 40 °C and 4 barg (58 psig) for an 8 hour cycle while maintaining product robustness.

2.4. Life Time (Operating Time)

2.4.1 Introduction

The purpose of this testing was to verify that the modules could maintain integrity after being processed in a typical 8-hour operating time.

2.4.2 Test Methods

As noted in previous sections 2.2 and 2.3, three CSUM010T002, two CSUM010T010, and four CSUM010T050 modules were tested at either 4 °C or 40 °C and 4 barg (58 psig) for two cycles to represent a safety factor of two times compared to the recommended 8-hour operating time for the Cadence single-use TFF modules.

2.4.3 Results

As previously confirmed, the modules maintained integrity after being processed at 4 barg (58 psig) and either minimum (4 °C) or maximum temperature (40 °C) for two 8 hour cycles.

2.4.4 Conclusions

Since these temperatures represent extreme operating conditions, the Cadence single-use TFF modules can therefore be processed in an 8-hour operating window (at temperatures between 4 and 40 °C) at a maximum pressure of 4 barg (58 psig).

2.5 Clamping Values

2.5.1 Introduction

Cadence single-use TFF modules need to be installed and torqued into a holder prior to operation. Studies were conducted to determine an acceptable torque range for Cadence single-use modules. Modules considered as representative of the whole product range were tested.

2.5.2 Test Methods

All modules were gamma irradiated prior to testing.

Three CSUM010T010 (0.1 m², 10 kD NMWC) Cadence single-use modules were tested in the 4-bolt Cadence CM holder for single-use TFF designed for the modules with a Centramate cassette format. These modules were representative of modules with 93 cm², 186 cm², and 0.1 m² membrane area. The modules were torqued to between between 9 and 11 Nm (80-100 in-lb) in the holder, incorporating 4 X 0.375 inch diameter steel bolts and bronze nuts. The modules were tested for Delta P and integrity at each 1.1 Nm (10 in-lb) increment. Between each torque setting, the product was released and re-clamped.

Three CSUM100T050 (0.5 m², 100 kD NMWC) Cadence single-use modules were tested in the 2-bolt Cadence CS holder for single-use TFF designed for the modules with a Centrasette cassette format. These modules were representative of modules with 0.5 and 2.5 m² membrane area. The modules were clamped to between 80 and 125 Nm (700-1100 in-lb) in the holder, incorporating 2 X 0.75 inch diameter steel bolts and bronze nuts. The modules were tested for Delta P and integrity at each 11 Nm (100 in-lb) increment. Between each torque setting, the modules were released and re-clamped.

For all modules, the acceptance criteria were:

- for integrity: <1500 mL/min/m² (<150 mL/min/ft²) permeate air flow at 2 barg (30 psig)
- for Delta P: 0.3-1 bar (5-15 psid), at 5 L/min/m² (0.5 L/min/ft²) average Cross Flow Flux

2.5.3 Results

For all tests, all modules met acceptance criteria for integrity and the Delta P values remained within the acceptance criteria.

Table 4

Characterization Data for CSUMxxxT001, CSUMxxxT002 and CSUMxxxT010 Modules Clamped to 9-11 Nm (80-100 in-lb)

| Part Number | Serial Number | Torque (in-lb) | Torque (Nm) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|----------------|-------------|--|---------------------------------------|
| CSUM010T010 | 32033224 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |
| CSUM010T010 | 32033220 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |
| CSUM010T010 | 32033218 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |
| CSUM010T002 | 32250203 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |
| CSUM010T002 | 32250204 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |
| CSUM010T002 | 32250205 | 80 | 9 | Pass | Pass |
| | | 90 | 10 | Pass | Pass |
| | | 100 | 11 | Pass | Pass |

⁽¹⁾ DP: Delta P; IT: Integrity Test

Table 5

Characterization Data For CSUMxxxT050 and CSUMxxxT250 Modules Clamped to 80-125 Nm (700-1100 in-lb)

| Part Number | Serial Number | Torque (in-lb) | Torque (Nm) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|----------------|-------------|--|---------------------------------------|
| CSUM100T050 | 32250217 | 700 | 80 | Pass | Pass |
| | | 800 | 90 | Pass | Pass |
| | | 900 | 100 | Pass | Pass |
| | | 1000 | 115 | Pass | Pass |
| | | 1100 | 125 | Pass | Pass |
| CSUM100T050 | 32250218 | 700 | 80 | Pass | Pass |
| | | 800 | 90 | Pass | Pass |
| | | 900 | 100 | Pass | Pass |
| | | 1000 | 115 | Pass | Pass |
| | | 1100 | 125 | Pass | Pass |
| CSUM100T050 | 32250219 | 700 | 80 | Pass | Pass |
| | | 800 | 90 | Pass | Pass |
| | | 900 | 100 | Pass | Pass |
| | | 1000 | 115 | Pass | Pass |
| | | 1100 | 125 | Pass | Pass |
| CSUM100T050 | 32250220 | 700 | 80 | Pass | Pass |
| | | 800 | 90 | Pass | Pass |
| | | 900 | 100 | Pass | Pass |
| | | 1000 | 115 | Pass | Pass |
| | | 1100 | 125 | Pass | Pass |

⁽¹⁾ DP: Delta P; IT: Integrity Test

2.5.4 Conclusions

The following recommended ranges were selected:

- 9-11 Nm (80-100 in-lb) for Cadence single-use TFF modules in CSMUxxxT001, CSUMxxxT002 and CSUMxxxT010 formats in the 4-bolt Cadence CM holder for single-use TFF
- 80-125 Nm (700-1100 in-lb) for Cadence single-use TFF modules in CSUMxxxT050 and CSUMxxxT250 formats in the 2-bolt Cadence CS holder for single-use TFF

2.6 Pre-Use Flush Studies

2.6.1 Introduction

The Cadence single-use modules are stored in water. Prior to the processing of the product stream, the modules are pre-flushed with pharmaceutical grade water or buffer to remove water storage solution. The purpose of these studies was to evaluate the deionized water volume requirements for flushing to reach 1 ppm TOC in the effluent.

Cadence single-use modules considered as representative of the whole product range were tested.

2.6.2 Test Methods

All modules were gamma irradiated prior to testing.

The TFF systems were first flushed, sanitized, and re-flushed, without the Cadence single-use modules.

Eleven Cadence single-use modules were then installed and flushed with deionized water at a feed flux of 5 L/min/m² (0.5-1.0 L/min/ft²), with 75% of the flow going through the permeate and 25% through the retentate, corresponding to the recommended pre-use flush procedure for the modules. The feed pressure was not to exceed 2 barg (30 psig). Permeate and retentate samples were taken at 0, 5, 10, 15, 20, 25, 30, 35 and 40 L/m² and analyzed for TOC.

The list of the tested modules is the following:

- Three CSUM010T010: 0.1 m², 10 kD NMWC
- Three CSUM030T010: 0.1 m², 30 kD NMWC
- Three CSUM100T010: 0.1 m², 100 kD NMWC
- One CSUM010T050: 0.5 m², 10 kD NMWC
- One CSUM030T050: 0.5 m², 30 kD NMWC

Their respective Serial Numbers (SN) are shown on the flush curves.

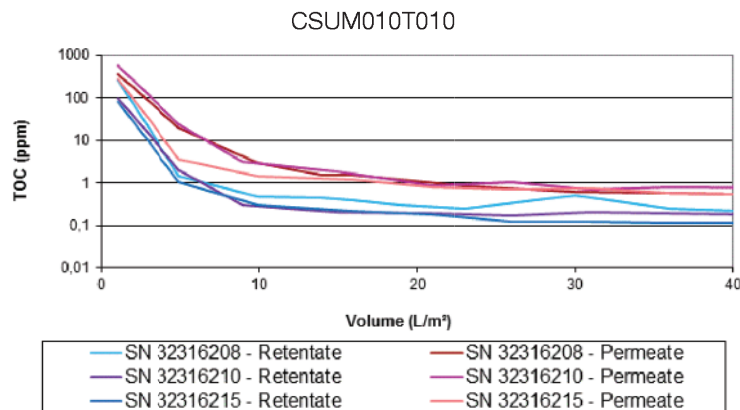
2.6.3 Results

As can be depicted from the following total flush-out curves, all Cadence single-use modules with 0.1 m² and 0.5 m² membrane area and Omega 10 and 30 kD NMWC membranes decreased to 1 ppm TOC levels on both retentate and permeate streams after processing with a total flush-out volume of 20 L/m² de-ionized water. Similarly, for the Cadence single-use modules with Omega 100 kD NMWC membrane, 1 ppm TOC levels were reached after processing with total flush-out volume of 30 L/m² de-ionized water.

In general, 1 ppm TOC levels were reached for the feed-retentate channel within 10 L/m² flush-out volumes.

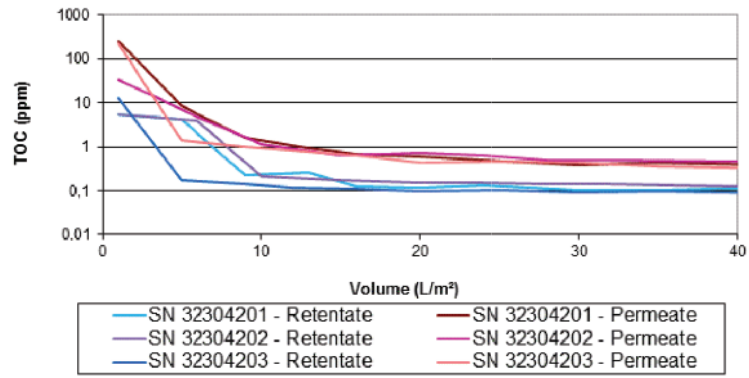
Overall, the recommended flush-out protocol conducted at a feed flux of 5 L/min/m² (0.5-1.0 L/min/ft²) and feed pressure of ≤ 2 barg (30 psig), with diverted flow of 75% and 25% through permeate and retentate, respectively, was effective to remove the storage solution and the subsequent TOC in the gamma irradiated Cadence single-use modules.

Flush curves for 3x CSUM010T010 modules



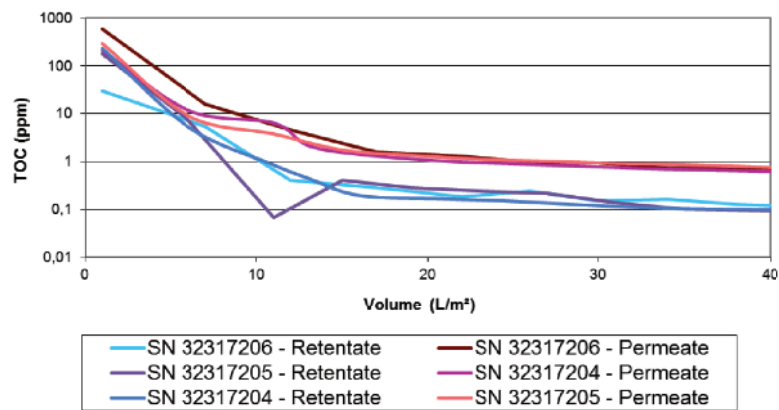
Flush curves for 3x CSUM030T010 modules

CSUM030T010



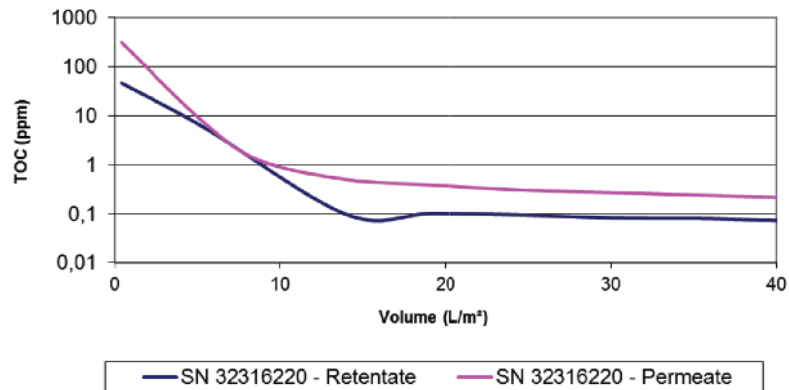
Flush curves for 3x CSUM100T010 modules

CSUM100T010

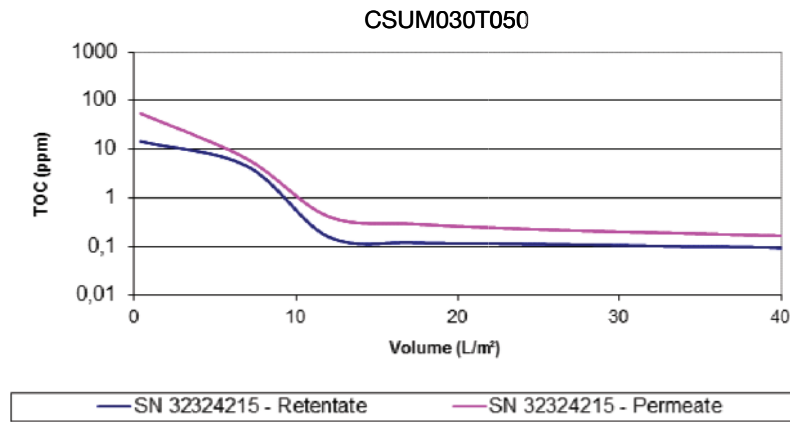


Flush curves for 1x CSUM010T050 module

CSUM010T050



Flush curves for 1x CSUM030T050 module



2.6.4 Conclusions

In conclusion, the recommended flush-out protocol conducted at a feed flux of 5 L/min/m² (0.5-1.0 L/min/ft²), with diverted flow of 75% and 25% through permeate and retentate, respectively, was effective to reduce TOC down to 1 ppm.

Typical total flush-out volumes to reach 1 ppm TOC on both the retentate and permeate lines were:

- 20 L/m² (2 L/ft²) for the 10 and 30 kD NMWC Cadence single-use TFF modules.
- 30 L/m² (3 L/ft²) for the 100 kD NMWC Cadence single-use TFF modules.

In general, 1 ppm TOC levels were reached for the feed-retentate channel within 10 L/m² flush-out volumes.

Differences in the total flush volumes can be due to different membrane morphologies between different cutoffs and module formats.

2.7 Compatibility with Pre-Use Sanitization Solutions – High and Low pH

2.7.1 Introduction

Cadence single-use TFF modules are stored in water and supplied after receiving a gamma irradiation dose equal or greater than 25 kGy. In most of the applications, no pre-use sanitization should be required prior to operate these modules on the drug product.

However, depending upon specific application requirements, Cadence single-use TFF modules may need to be sanitized pre-use with 0.5 N NaOH (pH of 14) or 400 ppm peracetic acid (pH of 2) at ambient temperature for 30 minutes.

Cadence single-use modules considered as representative as the whole product range were tested.

2.7.2 Test Methods

All modules were gamma irradiated prior to testing.

Five Cadence single-use TFF modules:

- Three CSUM010T002, 186 cm², 10 kD NMWC
- One CSUM100T050, 0.5 m², 100 kD NMWC
- One CSUM010T050, 0.5 m², 10 kD NMWC

were challenged with two 30 minute cycles of 400 ppm peracetic acid (pH of 2) at 25 °C. Two cycles representing a safety factor of two times, as only one cycle is recommended.

Another five Cadence single-use TFF modules:

- Three CSUM010T002, 186 cm², 10 kD NMWC
- Two CSUM100T050, 0.5 m², 100 kD NMWC

were challenged with two 30 minute cycles of 0.5 N NaOH at 25 °C. Two cycles representing a safety factor of two times, as only one cycle is recommended.

The systems were first cleaned with 0.5 N NaOH without the modules. The modules were then torqued in their respective holders at 10 Nm (90 in-lb) for the CSUM010T002 modules and 125 Nm (1100 in-lb) for the CSUMxxxT050 modules. Then, the pre-sanitization solution of 0.5 N NaOH or 400 ppm peracetic acid was recirculated at a feed flux of 5-10 L/min/m² (0.5-1.0 L/min/ft²), with 75% of the flow going through the permeate and 25% through the retentate. The run was stopped after 30 minutes.

The modules were flushed with 10-20 L/m² (1-2 L/ft²) deionized water in between each cycle at a feed flow rate of 5-10 L/min/m² (0.5-1 L/min/ft²), with at least 50% of the flow going through the retentate. They were then tested for integrity, Delta P or Feed Channel Air Flow (FCAF). A total of 2 cycles were completed for each module.

Module acceptance criteria were:

- for integrity: <1500 mL/min/m² (<150 mL/min/ft²) permeate air flow at 2 barg (30 psig)
- for Delta P: 0.3-1 bar (5-15 psid), at 5 L/min/m² (0.5 L/min/ft²) average Cross Flow Flux.
- for Feed Channel Air Flow: 2.5 to 6.5 L/min/m² (0.25 to 0.65 L/min/ft²) at 0.7 barg (10 psig)

2.7.3 Results

All modules tested met acceptance criteria for integrity after completion of each cycle. Meanwhile, the Delta P values or FCAF values remained within the acceptance criteria after each cycle.

Table 7

Module characterization data after challenging with 400 ppm peracetic acid at 25 °C

| Part Number | Serial Number | Number of 30 Minute Cycles | FCAF ⁽¹⁾ at 0.7 barg (10 psig) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|----------------------------|---|--|---------------------------------------|
| CSUM010T002 | 32250209 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM010T002 | 32250210 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM010T002 | 32250211 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM100T050 | 22104205 | 0 | n/a | Pass | Pass |
| | | 1 | n/a | Pass | Pass |
| | | 2 | n/a | Pass | Pass |
| CSUM010T050 | 22101206 | 0 | n/a | Pass | Pass |
| | | 1 | n/a | Pass | Pass |
| | | 2 | n/a | Pass | Pass |

⁽¹⁾ FCAF: Feed Channel Air Flow; DP: Delta P; IT: Integrity Test

Table 8

Module characterization data after challenging with 0.5 N NaOH at 25 °C

| Part Number | Serial Number | Number of 30 Minute Cycles | FCAF ⁽¹⁾ at 0.7 barg (10 psig) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|----------------------------|---|--|---------------------------------------|
| CSUM010T002 | 32250206 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM010T002 | 32250207 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM010T002 | 32250208 | 0 | Pass | n/a | Pass |
| | | 1 | Pass | n/a | Pass |
| | | 2 | Pass | n/a | Pass |
| CSUM100T050 | 22104203 | 0 | n/a | Pass | Pass |
| | | 1 | n/a | Pass | Pass |
| | | 2 | n/a | Pass | Pass |
| CSUM100T050 | 22104204 | 0 | n/a | Pass | Pass |
| | | 1 | n/a | Pass | Pass |
| | | 2 | n/a | Pass | Pass |

⁽¹⁾ FCAF: Feed Channel Air Flow; DP: Delta P; IT: Integrity Test

2.7.4 Conclusions

Cadence single-use TFF modules can be sanitized at 25 °C for a typical 30 minute sanitization time with 0.5 N NaOH solution (pH of 14) or 400 ppm peracetic acid solution (pH of 2).

2.8 Post-Use Decontamination

2.8.1 Introduction

In some applications, a post-use decontamination or deactivation of the drug product may be required post-use and prior to the disposal of Cadence single-use modules. The purpose of this study was to check that Cadence single-use TFF modules retain structural integrity when submitted to a post-use decontamination with a 1 N NaOH solution.

2.8.2 Test Methods

Two CSUM010T010 Cadence single-use modules (0.1 m², 10 kD NMWC) were gamma irradiated, installed and torqued into their holders and then characterized. They were then exposed to two 1-hour cycles of 1 N NaOH at 25 ± 3 °C, which was recirculated at 1-2 barg (15-30 psig) feed pressure with 5 psig of retentate backpressure. After each cycle, the modules were flushed with deionized water for 10 minutes, and evaluated for integrity with a pressure hold test at 2 barg (30 psig). During this test, each module remained installed and torqued into its holder. The modules were also visually inspected for leaks after each test outside of their holder.

Acceptance criteria for the pressure hold test at 2 barg (30 psig) was an air flow rate of 0 mL/min.

For the visual test, no visible leak was the acceptance criteria.

2.8.3 Results

The modules passed the pressure hold test before and after the one hour decontamination cycle with 1 N NaOH at $25 \pm 3^\circ\text{C}$. Additionally, no external damage or leaks were observed after the module was taken out of the holder following the pressure hold test.

Table 9

Module properties after 1 N NaOH post-use decontamination cycle at $25 \pm 3^\circ\text{C}$

| Part Number | Serial Number | Condition | Pressure Hold Test at 2 barg (30 psig)* |
|-------------|---------------|---------------|---|
| CSUM010T010 | 31299202 | As Received | Pass |
| | | Post 1 N NaOH | Pass |
| CSUM010T010 | 31299204 | As Received | Pass |
| | | Post 1 N NaOH | Pass |

*Module torqued in the holder during pressure hold test

2.8.4 Conclusions

Cadence single-use TFF modules are compatible with exposure to 1 N NaOH for 1 hour at ambient temperature ($25 \pm 3^\circ\text{C}$) for purposes of post-use decontamination before disposal. Please note that the post-use decontamination is performed for safety issues (operator safety) only. As the modules are single-use, they should be disposed of after this decontamination cycle.

3. Shelf Life

3.1 Introduction

Shelf life studies are conducted in order to establish up to a 3 year claim for Cadence single-use TFF modules when stored at ambient conditions ($25 \pm 2^\circ\text{C}$) in their original packaging. At the time of this document completion, a three-year accelerated aging study is completed, while a real time shelf life study is planned.

3.2 Test Method

All modules were gamma irradiated prior to the study.

For the accelerated aging studies, three CSUM010T002 (186 cm², 10 kD NMWC) and three CSUM010T010 (0.1 m², 10 kD NMWC) modules were placed in an oven at 45°C . These modules are considered as representative of the entire Cadence single-use TFF module product line. One module of each format size was removed from the oven after 79, 159 and 238 days, which correspond to real-time equivalent of 1, 2 and 3 years respectively, per ASTM F1980 standards.

The modules were tested for integrity and either Feed Channel Air Flow or Delta P (DP) at each time increment.

Module acceptance criteria were:

- for integrity: $<1500\text{ mL/min/m}^2$ ($<150\text{ mL/min/ft}^2$) permeate air flow at 2 barg (30 psig)
- for Delta P: 0.3-1 bar (5-15 psid), at 5 L/min/m^2 (0.5 L/min/ft^2) average Cross Flow Flux.
- for Feed Channel Air Flow: 2.5 to 6.5 L/min/m² (0.25 to 0.65 L/min/ft²) at 0.7 barg (10 psig)

3.3 Results

All modules met integrity acceptance criteria and either feed channel air flow or Delta P acceptance criteria after the 1, 2 and 3 year accelerated shelf life studies.

Table 10*Characterization data during accelerated aging study*

| Part Number | Serial Number | Equivalent Months at 23 °C | Actual Storage Days at 45 °C | FCAF ⁽¹⁾ at 0.7 barg (10 psig) | DP ⁽¹⁾ at 0.5 L/min/ft ² CFF | IT ⁽¹⁾ at 2 barg (30 psig) |
|-------------|---------------|----------------------------|------------------------------|---|--|---------------------------------------|
| CSUM010T010 | 32319201 | 12 | 79 | n/a | Pass | Pass |
| CSUM010T002 | 32316201 | 12 | 79 | Pass | n/a | Pass |
| CSUM010T010 | 32319202 | 24 | 159 | n/a | Pass | Pass |
| CSUM010T002 | 32316202 | 24 | 159 | Pass | n/a | Pass |
| CSUM010T010 | 32316203 | 36 | 238 | n/a | Pass | Pass |
| CSUM010T002 | 32316206 | 36 | 238 | Pass | n/a | Pass |

⁽¹⁾ FCAF: Feed Channel Air Flow; DP: Delta P; IT: Integrity Test

3.4 Conclusions

Based on accelerated shelf life data, the Cadence single-use TFF modules have a 3-year shelf life at ambient conditions (23 °C).

A real-time ambient shelf life study is also planned. Results will be available as developed. Please contact Pall for the latest information.

4. Extractables

4.1 Introduction

The purpose of the extractables study was to quantify the materials extracted from the Cadence Single-Use TFF modules when exposed to deionized water at 40 °C.

4.2 Test Methods

All modules were gamma irradiated prior to testing.

The TFF system was sanitized and flushed prior to integrating the module. The Cadence single-use module was then installed into the holder. Prior to the extraction, the module was flushed at 18 to 25 °C with 20 L/m² (2 L/ft²) of deionized water to waste, corresponding to the recommended pre-use flush conditions prior to use on the drug product. The feed flux was 5-10 L/min/m² (0.5-1 L/min/ft²), and the flow ratio was 25% through the retentate, 75% through the permeate lines.

The deionized water extractions were carried out on four modules:

- two CSUM010T010: 0.1 m², 10 kD NMWC
- two CSUM010T050: 0.5 m², 10 kD NMWC

These modules were considered as representative of the whole Cadence single-use modules product range.

The extraction was run in recirculation with deionized water at 40 °C +/-2.5 °C. The feed flux was 10 L/min/m² (1 L/min/ft²), and the flow ratio was 25% through the retentate, 75% through the permeate lines. Two successive 8 hour extraction cycles were performed.

After each cycle, the retentate and permeate streams were pooled, providing a worst-case condition concentrating the extracted compounds.

The extracts were then submitted to a Non Volatile Residues (NVR) determination to quantify the total mass of extractables after evaporation of the test fluid.

4.3 Results

The total NVR from the two successive 8-hour extractions in deionized water were no more than 18 mg for CSUM010T050 modules (0.5 m²) and no more than 13 mg or for CSUM010T010 modules (0.1 m²).

4.4 Conclusions

Cadence single-use TFF modules were tested for extractables in deionized water at 40 °C +/- 2.5 °C for two successive 8-hour cycles. The total extractables based on the Non-Volatile Residue were less than or equal to 18 mg for CSUM010T050 modules with 0.5 m² membrane area and less than or equal to 13 mg for CSUM010T010 modules with 0.1 m² membrane area.

For further information regarding extractable quantitative and qualitative characterization, please contact your Pall representative.

5. Biological Safety

5.1 Introduction

The purpose of these tests was to evaluate the biological safety of the Cadence single-use module fluid path materials of construction.

The fluid path materials of construction as described in the following table were gamma irradiated prior to the tests.

Tests performed included Biological Reactivity Tests, *in vivo*, for Class VI - 70 °C Plastics as described in USP <88> and, *in vitro*, under USP <87> (Elution Test).

Table 11

Fluid path materials of construction of the Cadence single-use TFF modules

| Cadence Single-use TFF Module Component | Flowpath Material of Construction |
|--|--|
| Membrane | Polyethersulfone |
| Support | Polyolefin |
| Screens | Polypropylene |
| Encapsulant | Polyurethane with white pigment (TiO ₂) |
| Seals | Platinum cured silicone and thermoplastic elastomer |
| Manifold plates | Glass bead reinforced polypropylene with white pigment (TiO ₂) |
| Port caps for modules with ≥0.1 m ² membrane area | Polyethylene for sanitary fittings |

The Luer Lock port caps for modules with 93 or 186 cm² membrane area are made of polypropylene. They are certified USP Class VI by the supplier, as non gamma irradiated items.

5.2 Test Methods

USP <88>

The testing procedures described under USP <88> include injection of extracts of plastic materials, as well as implantation of the material itself into animal tissue. Four extracting media are listed which simulate parenteral solutions and body fluids. These include: (1) sodium chloride injection, (2) 1-in-20 solution of alcohol in sodium chloride injection, (3) polyethylene glycol 400, and (4) vegetable oil (sesame or cottonseed oil). Extracts are prepared at one of three standard conditions: 50 °C for 72 hours, 70 °C for 24 hours, or 121 °C for one hour. Since Cadence single-use module with Omega membrane have a recommended operating temperature limit of 40 °C, module components were extracted at 70 °C to provide for the most stringent test condition that would not result in physical changes in the plastic itself.

An acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium chloride injection and 1-in-20 solution of alcohol in sodium chloride injection extracts were injected intravenously. Vegetable oil extract and polyethylene glycol 400 extract were injected intraperitoneally. An Intracutaneous Test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. The four specified extracts were used. Implantation Testing was also performed in order to subject the materials of construction to the most stringent conditions included in the United States Pharmacopoeia. Each of the components of the module was implanted separately.

USP <87>

Under USP <87>, several procedures are defined. An Elution Test was carried out to determine the response of mammalian cell cultures (L929 cells) following incubation with extracts of the materials of construction, prepared using serum supplemented Minimal Essential Medium (MEM). The Elution test determines the lysis of cells and the inhibition of cell growth caused by extracts of the test materials.

5.3 Results

All components of the fluid path of the Cadence single-use TFF modules with Omega membrane passed the tests for biological reactivity, *in vivo*, under USP <88> for Class VI - 70 °C plastics and *in vitro*, under USP <87> (Elution Test).

Copies of test certificates are available upon request. Please contact your Pall representative for details.

5.4 Conclusions

The fluid path of Cadence single-use TFF modules with Omega membrane meets the requirements for:

Biological Reactivity Tests (*in vivo*) for Class VI-70 °C Plastics, USP <88>

Biological Reactivity Tests (*in vitro*) for Cytotoxicity, USP <87>

6. Endotoxins

6.1 Introduction

The goal of this study was to quantify the endotoxin levels found in Cadence single-use modules incorporating Omega membrane.

6.2 Test Methods

Six CSUM010T010 (0.1 m², 10 kD NMWC) single-use modules, considered as representative of the whole product range were gamma irradiated to 50-60 kGy.

The system was sanitized and flushed without the module. Limulus Amebocyte Lysate (LAL) Reagent water was then recirculated through the system and a sample from the retentate and permeate lines was collected (sample called “system control LAL water”).

A Cadence single-use module was then installed into the system and torqued in its holder. 20 L/m² (2 L/ft²) of LAL reagent water was flushed through the module with 75% of the flow through the permeate and 25% through the retentate, corresponding to the recommended pre-use flush procedure for the modules. At the end of the flush, a pooled retentate and permeate sample was collected for endotoxin analysis (sample called “Pooled retentate and permeate”).

6.3 Results

The detection limit of this test is 0.005 EU/mL

The system control LAL water endotoxin levels were all < 0.005 EU/mL, while the endotoxin levels after flushing the modules were ≤ 0.005 EU/mL.

Table 12

Endotoxin results

| Part Number | Serial Number | Sample Identification | EU/mL |
|-------------|---------------|-------------------------------|--------|
| CSUM010T010 | 32316209 | Pooled Permeate and Retentate | <0.005 |
| CSUM010T010 | 32316213 | Pooled Permeate and Retentate | 0.005 |
| CSUM010T010 | 32316214 | Pooled Permeate and Retentate | 0.005 |
| CSUM010T010 | 32319213 | Pooled Permeate and Retentate | <0.005 |
| CSUM010T010 | 32298201 | Pooled Permeate and Retentate | <0.005 |
| CSUM010T010 | 32298202 | Pooled Permeate and Retentate | <0.005 |

6.4 Conclusions

The tested Cadence single-use TFF modules demonstrated an endotoxin level below or equal 0.005 EU/mL after a flush volume of 20 L/m² (2 L/ft²). They show endotoxin level below or at the detection limit of the test.



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