



Life Sciences

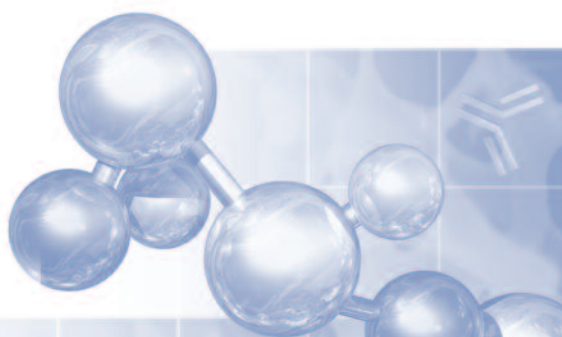
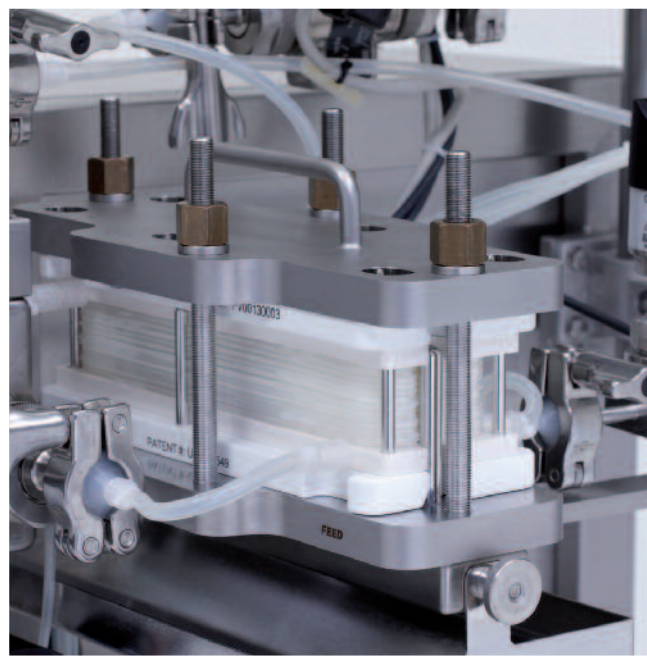
## User Guide

USTR 2705a

# Cadence™ Single-pass TFF Module with Delta 10 kD and 30 kD Regenerated Cellulose Membranes

*For Use with Cadence Single-pass TFF Systems*

*Care and Use Procedures*



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# 1. Important Information

## 1.1 Safety Notice

### Important Notice

Refer to safety instructions before use. Safety instructions in this language are available from Pall.

### Viktigt att notera

Läs säkerhetsinstruktionerna före användandet. Säkerhetsinstruktioner på svenska finns att få från Pall.

重要通知  
ご使用前に安全にお使いいただくための説明書をお読みください。  
日本語でかかれた説明書はボールより入手可能です。

### Viktig melding

Les Sikkerhetsinstruksjonen før bruk. Sikkerhetsinstruksjon på norsk vil være tilgjengelig fra Pall.

### Belangrijke informatie:

Voor gebruik veiligheidsinstructies goed doornemen. Veiligheids instructies in het Nederlands zijn bij Pall verkrijgbaar.

### Avvertenza importante

Prima dell'uso leggere le istruzioni per la sicurezza. Le istruzioni per la sicurezza in Italiano possono essere richieste a Pall.

### Aviso importante

Antes de utilizar, consultar instruções de segurança. Instruções de segurança em Português, encontram-se disponíveis na Pall.

### Aviso importante

Antes de usar, consultar Instrucciones de Seguridad. Instrucciones de Seguridad en este idioma están disponibles por Pall.

### Important

Se référer aux instructions concernant la sécurité d'utilisation avant usage. Les instructions concernant la sécurité d'utilisation sont disponibles en français chez Pall.

### Vigtigt

Læs sikkerhedsinstruktioner før ibrugtagning. Sikkerhedsinstruktioner på dansk kan fås fra Pall.

### Tärkeä tiedote

Lue turvallisuusohjeet ennen käyttöä. Pall toimittaa tarvittaessa suomenkieliset turvallisuusohjeet.

### Wichtige Anmerkung

Vor Gebrauch bitte die Sicherheitsrichtlinien lesen. Die Sicherheitsrichtlinien in dieser Sprache erhalten Sie von Pall.

Σημαντική Επισήμανση:  
Διαβάστε τις Οδηγίες Ασφάλειας πριν από τη Χρήση.  
Οι Οδηγίες Ασφάλειας στα Ελληνικά είναι διαθέσιμες από την PALL.

## 1.2 Application of the Care and Use Procedures

This guide describes the care and use of Cadence single-pass tangential flow filtration (TFF) modules with Delta 10 kD and 30 kD regenerated cellulose membranes. Do not apply these procedures to Pall's conventional tangential flow filtration cassettes. Do not apply these procedures to Cadence single-pass TFF modules having a molecular weight cutoff other than 10 kD or 30 kD.

The care and use procedures described in this document apply to the following products:

Cadence Module	Configuration	Delta 10 kD Membrane Area (m <sup>2</sup> )	Part Number
T01	4-in-series	0.065	CD 010 T010407
T01	7-in-series	0.12	CD 010 T010713
T01	8-in-series	0.14	CD 010 T010815
T01	9-in-series	0.17	CD 010 T010918
T02	4-in-series	0.13	CD 010 T020407
T02	7-in-series	0.24	CD 010 T020713
T02	8-in-series	0.28	CD 010 T020815
T02	9-in-series	0.33	CD 010 T020918
T12	4-in-series	0.7	CD 010 T120407

continued...

Cadence Module	Configuration	Delta 10 kD Membrane Area (m <sup>2</sup> )	Part Number
T12	7-in-series	1.3	CD 010 T120713
T12	8-in-series	1.5	CD 010 T120815
T12	9-in-series	1.8	CD 010 T120918
T06	4-in-series	3.5	CD 010 T060407
T06	7-in-series	6.5	CD 010 T060713
T06	8-in-series	7.5	CD 010 T060815
T06	9-in-series	9.0	CD 010 T060918

Cadence Module	Configuration	Delta 30 kD Membrane Area (m <sup>2</sup> )	Part Number
T01	4-in-series	0.065	CD 030 T010407
T01	7-in-series	0.12	CD 030 T010713
T01	8-in-series	0.14	CD 030 T010815
T01	9-in-series	0.17	CD 030 T010918
T02	4-in-series	0.13	CD 030 T020407
T02	7-in-series	0.24	CD 030 T020713
T02	8-in-series	0.28	CD 030 T020815
T02	9-in-series	0.33	CD 030 T020918
T12	4-in-series	0.7	CD 030 T120407
T12	7-in-series	1.3	CD 030 T120713
T12	8-in-series	1.5	CD 030 T120815
T12	9-in-series	1.8	CD 030 T120918
T06	4-in-series	3.5	CD 030 T060407
T06	7-in-series	6.5	CD 030 T060713
T06	8-in-series	7.5	CD 030 T060815

### 1.3 Using the Cadence Single-pass TFF Module Safely

Read all safety warnings and operating instructions before using the Cadence module. Keep this care and use guide in an accessible location and make it available to users of the Cadence modules. General safety precautions include:

- Wear protective clothing including safety glasses and gloves when working with Cadence modules, systems, and chemical solutions.
- The solutions recommended for sanitizing, cleaning, and storing components may be hazardous or corrosive. Refer to the Material Safety Data Sheets (available from the chemical suppliers) to learn how to use the recommended solutions safely.
- Do not exceed the operating limits of the Cadence module, module holder, or process components.
- Provide sufficient space around the Cadence single-pass TFF system to work ergonomically and safely when assembling and using the system.
- Disconnecting or dismantling Cadence system components without first isolating and depressurizing the system can lead to injury from the uncontrolled release of high pressure air and solutions. Depressurize the Cadence system before dismantling components.
- Some system components may be heavy. Take proper precautions when moving or lifting equipment to prevent personal injury. Hoists or other lifting equipment may be needed to assemble some Cadence system equipment.

- Do not use the Cadence module as a coded pressure vessel. Ensure the ancillary equipment and the module holder meet local safety codes.
- Wipe up spills promptly to prevent injury from contact or slipping.

#### 1.4 Locating Safety Information

This care and use guide presents safety information as follows.



##### Helpful Information

*Communicates important and helpful information about the current topic.*



##### Caution

*Identifies safety risks that could cause personal injury, or situations that may cause product damage.*



##### Warning

*Identifies safety risks that can cause serious personal injury, or situations that may cause irreversible damage to equipment.*

#### 1.5 Environmental Compliance

Users should dispose of storage, cleaning, and by-product solutions according to local, state, and federal regulations.

#### 1.6 Where to Get Help

If you do not understand the information in this guide, or need additional assistance in using Cadence modules, contact Pall Life Sciences. Pall Life Sciences provides these additional resources to help make your experience with our products trouble-free:

**Materials Safety Data Sheets** — Material Safety Data Sheets provide safety information about the chemicals used to clean and store modules. Pall supplies the Material Safety Data Sheet to users upon request.

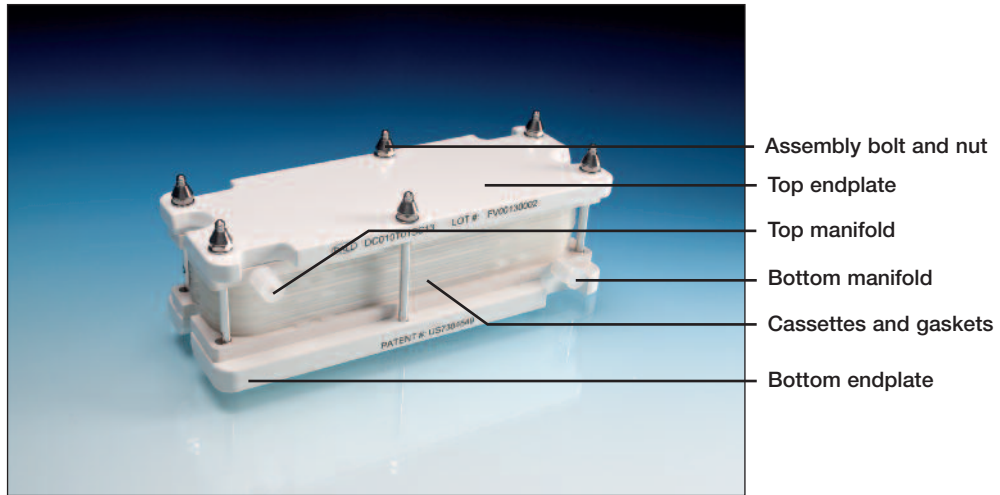
**Support Services** — The Pall Life Sciences customer support team can answer technical questions and provide replacement parts. You can reach the Pall Life Sciences customer support team, Monday through Friday, 8:00 A.M. to 5:00 P.M. Eastern Standard Time at 1-800-717-7255. Outside the USA, telephone 1-516-484-5400.

**Web Site (<http://www.pall.com/biopharm>)** — Provides contact information for Pall offices throughout the world, as well as toll-free customer support hotlines.

## 2. Learning about Cadence Single-pass TFF Module

The Cadence module is a preassembled stack of membrane cassettes. The cassettes, along with flow manifolds, are held together tightly between endplates using bolts. The flow manifolds channel fluids into and out of the stack of membrane cassettes. The manifolds include feed, retentate, and two permeate ports. Cadence modules must be used in Cadence holders and with Cadence single-pass TFF systems.

## 2.1 Cadence Single-pass TFF Modules

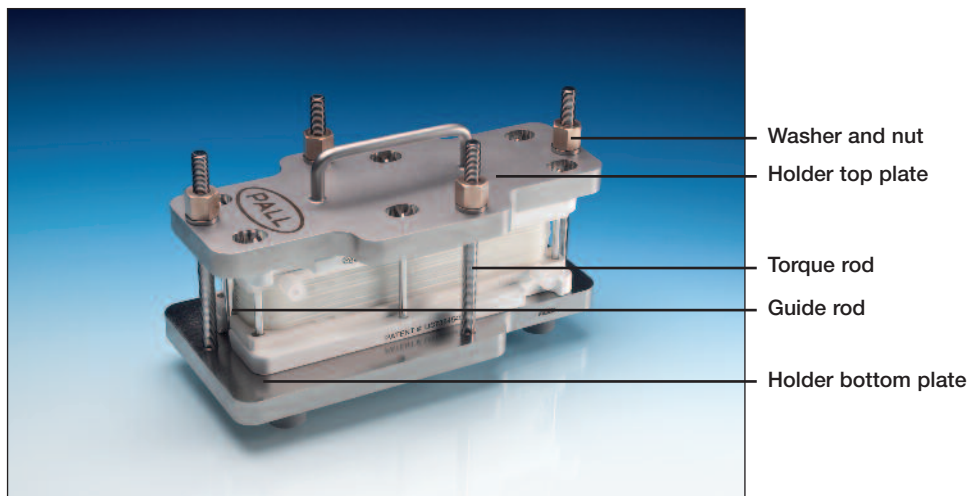


## 2.2 Cadence Single-pass TFF Module Label Information

The label on each Cadence module includes the following information:

- Company name
- Catalog number
- Serial number

## 2.3 Cadence Single-pass TFF Module Holders



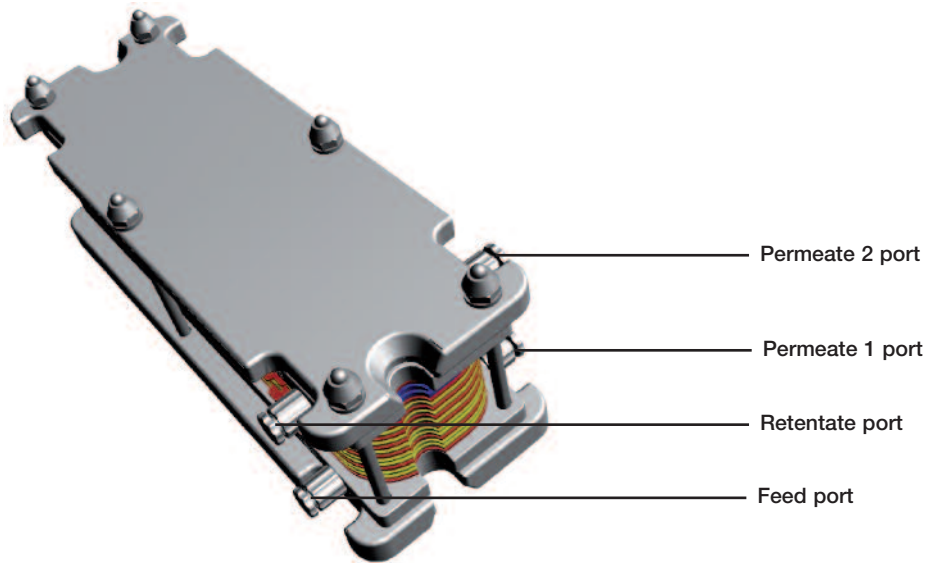
## 2.4 Cadence Single-pass TFF Module Port Locations

Cadence modules have feed, retentate, and two permeate ports. The location of the ports varies depending on the module (Figure 1). Ports on the Cadence T01 and T02 modules use luer lock connectors. Ports on the Cadence T12 modules mini-TC connectors. A label located next to each port identifies the port's function.

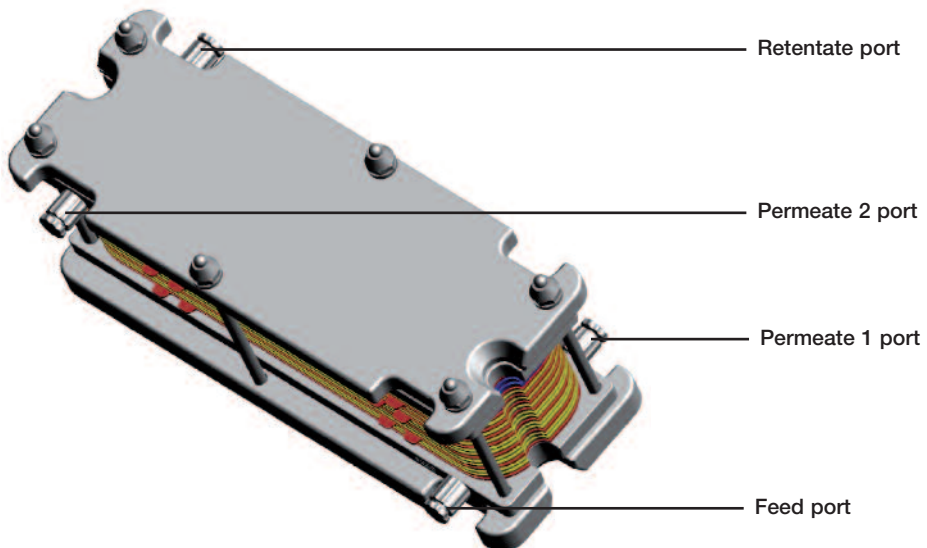
**Figure 1**

*Overhead View of Cadence T01 Modules Showing Port Locations*

*1a. View of 0.065 m<sup>2</sup> and 0.14 m<sup>2</sup> (4 and 8 in series) Cadence Module*



*1b. View of 0.12 m<sup>2</sup> and 0.17 m<sup>2</sup> (7 and 9 in series) Cadence Module*





### 3. Application of Single-pass Tangential Flow Filtration

Single-pass tangential flow filtration (TFF) is a specialized and unique technology enabling the concentration of process solutions with the simplicity of direct flow filtration:

- Concentrate product to higher levels
- In-process volume reduction
- In-process salt reduction
- Processing of fragile biomolecules

Depending upon the size of the Cadence module, volumes from several liters to thousands of liters can be processed while achieving high conversion rates. Conversion is defined by the amount of feed transferred or converted to permeate. The modules can be coupled (before or after) to other process steps (e.g., chromatography).

#### 3.1 Process Variables

Process conditions for Cadence modules are application specific. Therefore, the process conditions must be selected and adjusted based on these process variables:

- Product characteristics such as concentration, viscosity, temperature, and shear sensitivity
- Module configuration
- Target concentration factor
- Feed pressure or feed flow rate



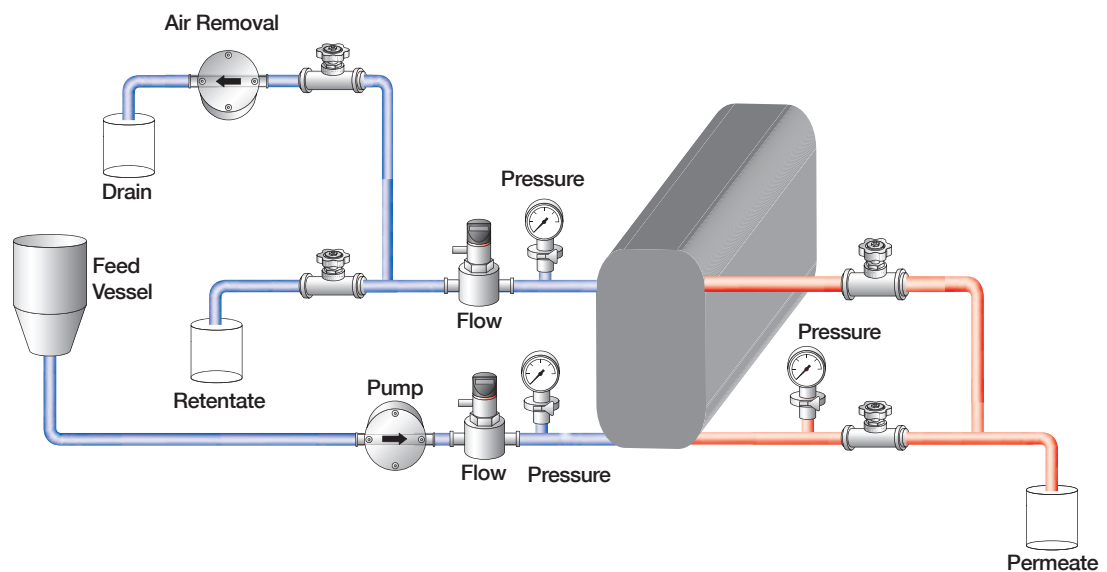
These variables influence the performance and reproducibility of single-pass TFF processing.

Typically, users establish the operating conditions for a single-pass TFF process by first performing characterization trials and analyzing results, followed by module and operating condition optimization.

Because Cadence modules can be used in repeated processing modes, proper care and use is crucial to ensure reliable and reproducible performance from run to run. Proper care also extends a Cadence module's service life.

**Figure 2**

*Example Flow Diagram of a Cadence Single-pass TFF System, Using 4 or 8 in Series Module*



### 3.2 Proper Use

The following chapters describe the procedures to properly install, prepare, clean, and store Cadence single-pass TFF modules. You may need to modify some aspects of the procedures to fit your specific objectives.

To perform these procedures, use the Cadence single-pass TFF system.

## 4. An Overview of Single-pass Tangential Flow Filtration Processing

### 4.1 The Three Parts of a Single-pass TFF Process

The single-pass TFF process can be divided into three parts:

- Part 1 — The first part involves installing the Cadence module and preparing it for use. This part (called *pre-use conditioning*) involves several sequential steps.
- Part 2 — The second part (called *processing*) involves using the Cadence module to meet the filtration objectives. Processing includes product filtration and product recovery.
- Part 3 — The third part includes the cleaning, testing, and storage of the Cadence module to enable reuse. This part is called *post-use conditioning*.

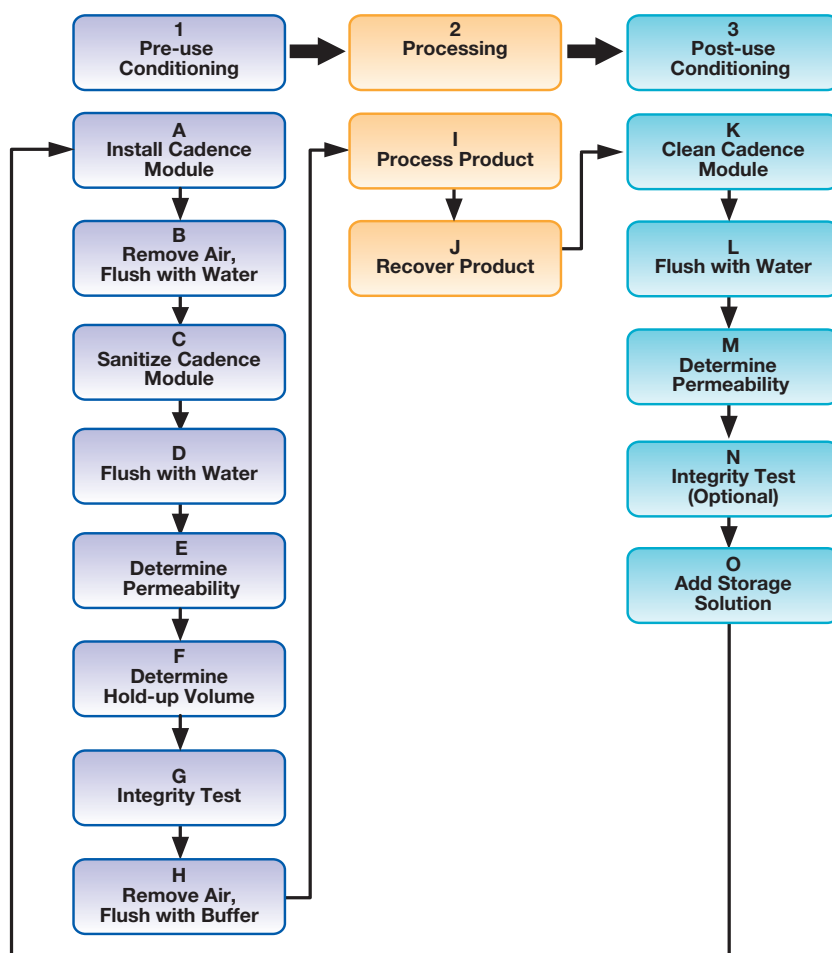
An illustration summarizes the three parts of a single-pass TFF process and provides a map for learning the procedures in each part (Figure 3).



*Helpful Information:* It may be helpful to photocopy Figure 3 and use it as a reference until you learn the procedures used in each part of the single-pass TFF process.

**Figure 3**

*Three Main Parts of a Single-pass TFF Process and the Procedures Required to Complete Each Part*



## 5. Using the Cadence Single-pass TFF Module

This chapter describes how to complete the procedures in each of the three parts of a single-pass TFF process. In addition, it also describes how to flush Cadence systems when they are new or brought back into service after long-term storage. Flushing a new system (without a Cadence module in place) removes manufacturing residue and particles that can damage a module.

This chapter is organized as follows:

- Preparing Equipment and Materials
- Flushing a New Cadence System  
Flushing assures that the storage agents have been effectively and sufficiently removed.
- Part 1 — Pre-use Conditioning  
Procedures A through G  
Pre-use conditioning establishes the fitness of the module for use or reuse.
- Part 2 — Processing  
Procedures H through I
- Part 3 — Post-Use Conditioning  
Procedures J through N  
Post-use conditioning removes contaminants and returns the module to initial, pre-use condition.

### 5.1 Preparation: Equipment and Materials

Collect the following equipment and materials:

#### Equipment

- Cadence module
- Cadence system with holder, consisting of a feed pump, retentate flow control valve, and low-cost retentate pump (for air removal)
- Torque wrench with socket
- Tubing assembly for system cleaning and flushing
- Containers to hold and collect product, water, buffer, and cleaning solutions
- Tubing and connectors — for a Cadence system with Cadence T01 or T02 modules: Luer♦ type connectors and 1/8-inch ID, high-pressure, medical-grade tubing; for a Cadence system with Cadence T12 modules: TC connectors and 1/4-inch ID, high-pressure, medical-grade tubing; for a Cadence system with Cadence T06 modules: TC connectors and 1/2-inch ID, high-pressure, medical-grade tubing
- Personnel protection equipment as required by OSHA and your facility's safety regulations
- pH meter
- Palltronic® Flowstar integrity test instrument
- Thermometer

#### Materials

- Product solution
- Buffer solution compatible with product: 20 L/m<sup>2</sup>
- Deionized, 0.2 µm filtered water: 100 L/m<sup>2</sup> (to complete all three parts of a single-pass TFF run)
- 0.1 N sodium hydroxide: 90 L/m<sup>2</sup> (to complete all three parts of a single-pass TFF run)
- Storage solution (0.1 N NaOH or 5% ethanol/1% sodium diacetate solution): 10 L/m<sup>2</sup>



*Helpful Information: Solution volumes are provided as a guide. Appropriate volumes must be determined by users for their specific applications.*

### Note on Water Quality

For the purposes of single-pass TFF, the term purified water refers to deionized, 0.2 µm filtered water. Using unfiltered water can foul the membrane in the Cadence module.

## 5.2 Flushing a New Cadence Single-pass TFF System

If a new Cadence system is placed into service (or a previously-used Cadence system is brought out of long-term storage), the Cadence system pumps and process lines must be flushed with a cleaning solution and purified water. If the Cadence system is currently in service, this procedure can be skipped (in this case, proceed to Part 1 — Pre-use Conditioning).

### Flush Cadence System Components

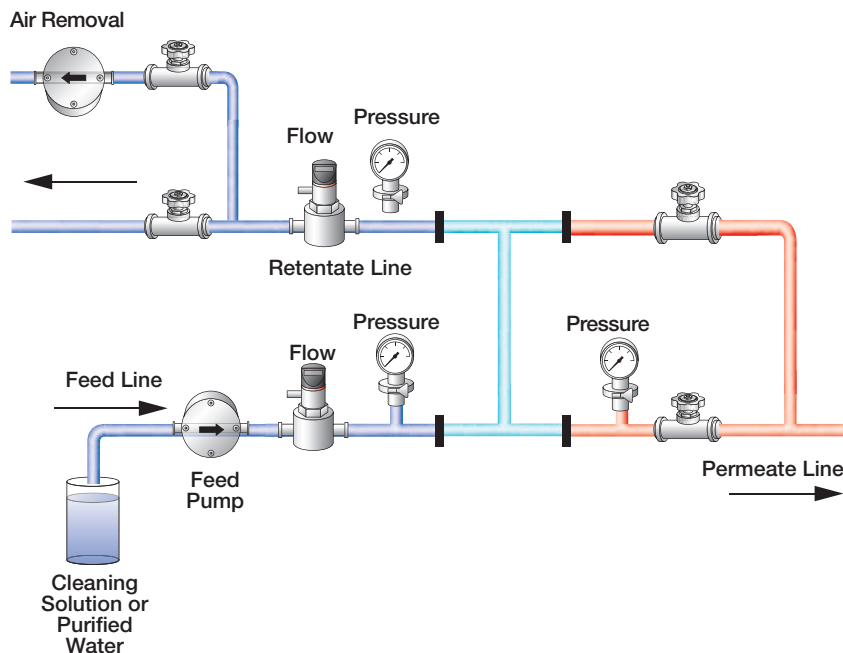
These steps describe how to sanitize and flush a Cadence system (without a Cadence module in place) with cleaning solution and then purified water:

1. Connect the supplied tubing assembly between the feed pump outlet and the retentate and permeate line hardware (Figure 4).
2. Direct permeate and both retentate lines to drain.
3. Ensure that the flow is directed to both retentate lines equipped with valve and retentate pump (Figure 4).
4. Pump 0.1 to 0.25 N NaOH through the Cadence system at a flow rate of 0.7 L/minute if using 1/8 in. ID tubing, 2.9 L/minute if using 1/4 in. ID tubing, and 11.5 L/minute if using 1/2 in. ID tubing.
5. Stop the feed pump after approximately 5 minutes. (See Cadence System Care and Use Procedures for additional information.)
6. Pump purified water through the Cadence system at a flow rate of 0.7 L/minute if using 1/8 in. ID tubing, 2.9 L/minute if using 1/4 in. ID tubing, and 11.5 L/minute if using 1/2 in. ID tubing.
7. Stop the feed pump after approximately 5 minutes or when the pH reaches  $\leq 7$ .
8. Remove and save the tubing assembly from the Cadence system.

*Helpful Information: This flushing process is provided as a guide. Users must tailor this process to meet the criteria of their specific applications.*

**Figure 4**

*Connecting the Supplied Flushing Line to the Cadence System*





*Helpful Information: The effectiveness of this sanitization procedure to reduce or eliminate bioburden will depend on the nature and level of the contamination. The user must evaluate and validate the effectiveness of the process with respect to time and temperature and the effectiveness of the sanitizing agent for their process.*

### 5.3 Part 1 – Pre-use Conditioning

#### Procedure A – Install the Cadence Module in the Cadence Holder and Connect the Tubing

A Cadence T01, T02, T06, or T12 module must be installed in a Cadence holder.

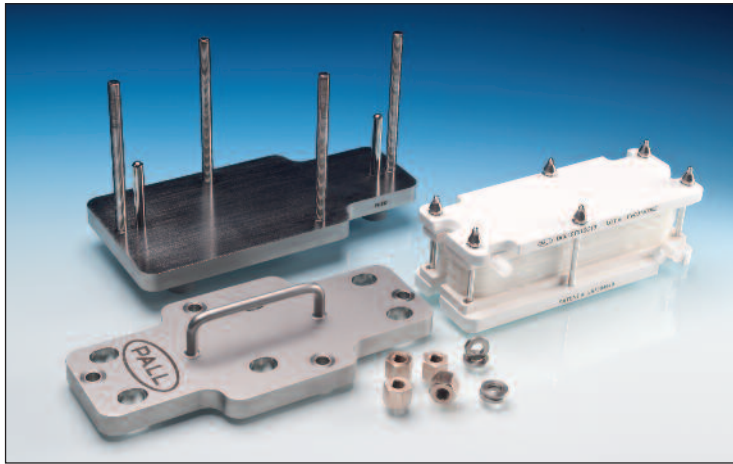
##### *Install a Cadence T01, T02, T06, or T12 Module in a Cadence Holder*

1. Place the Cadence system in the location where it will be used.
2. Remove the nuts, washers, and top plate from the Cadence holder (Figure 5).

#### Figure 5

*Installing the Cadence Module in the Cadence Holder*

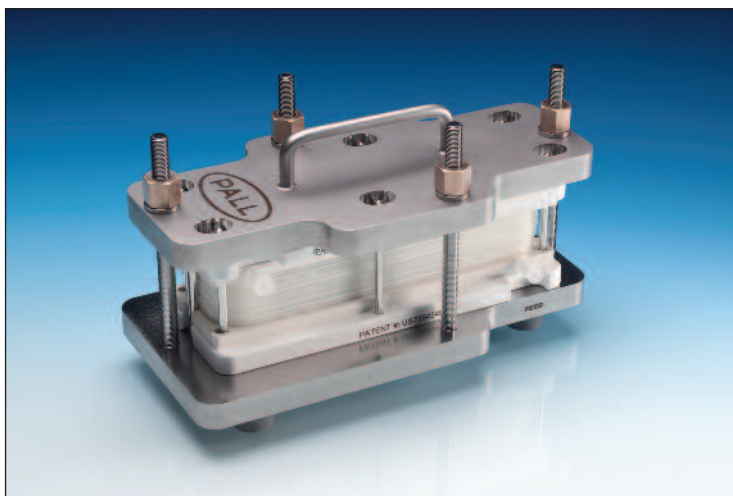
*5a. Cadence T01 Module and Holder with Top Plate Removed*



*5b. Cadence T01 Module Placed Into Holder with Top Plate Removed*



### 5c. Cadence Module Placed Into Holder with Top Plate and Torque Nuts Installed



3. Wearing protective gloves and safety glasses, open the Cadence module bag. Remove the plastic shipping plate from the top of the Cadence module.
4. Remove the caps from the ports. This must occur prior to loading the holder top plate and tightening the nuts.
5. Hold the Cadence module so that its feed port is aligned with the Feed label on the holder, then lower the Cadence module into the holder using the guides for proper placement.
6. Place the holder's top plate on top of the Cadence module, aligning the holes in the top plate with the holder's torque rods.
7. Install the washers and nuts on the holder finger tight.
8. Ensure the calibrated torque wrench is set between 80 and 90 in-lbs (8 and 9 Nm) for T01, T02, and T12 modules (in a manual holder), and between 1,800 and 2,200 psi for T06 modules (in a hydraulic holder). Using the torque wrench, tighten each nut in sequence ¼-turn at a time until nuts are tightened (Figure 6).



*Helpful Information: Use the same torque value for each process run to minimize variability in performance.*



*Helpful Information: Periodically check the torque of each nut on Cadence holders. Modules can compress after being installed for a period of time, reducing the clamping pressure. Ambient air and solution temperature changes can also change the clamping force.*



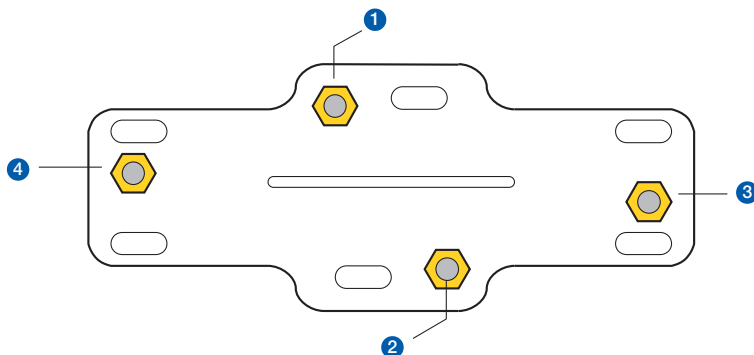
*Helpful Information: Store torque wrenches with the torque setting adjustment at zero to help maintain the wrench calibration.*



*Caution: Excessive compression of the module can cause permanent damage.*

**Figure 6**

*Sequence for Tightening Nuts when Torquing a Cadence Module in a Cadence Holder*

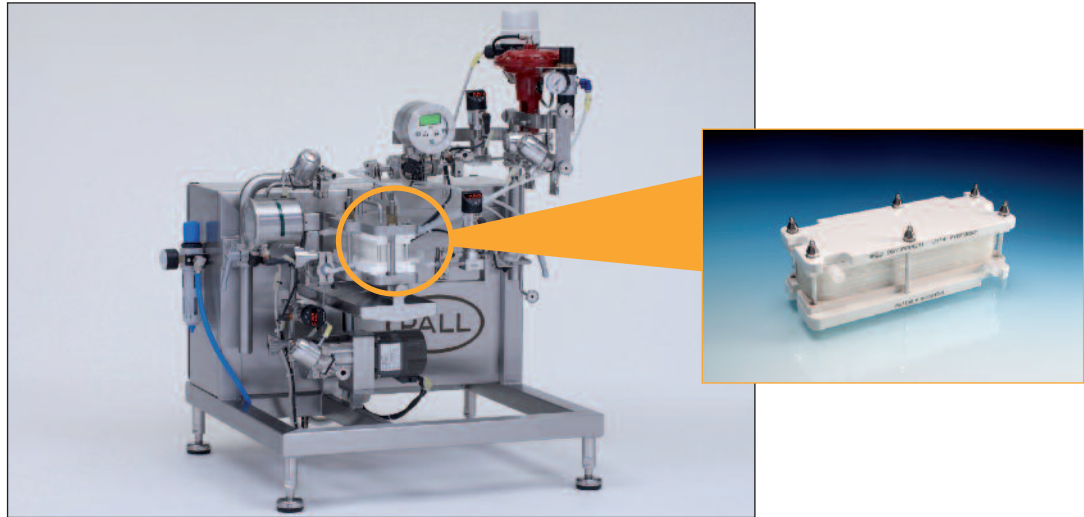


### Connect a Cadence T01, T02, T06, or T12 Module to the Cadence System

Connect the Cadence module to the Cadence system using the supplied tubing (Figure 7). This must be done while the module is in the holder to avoid damaging the ports. The module must always rest on its bottom plate and never on its side to avoid damage to the ports.

**Figure 7**

*Basic Integration of Cadence Modules into a Cadence System*



### Procedure B — Remove Air and Flush the Cadence Module with Purified Water



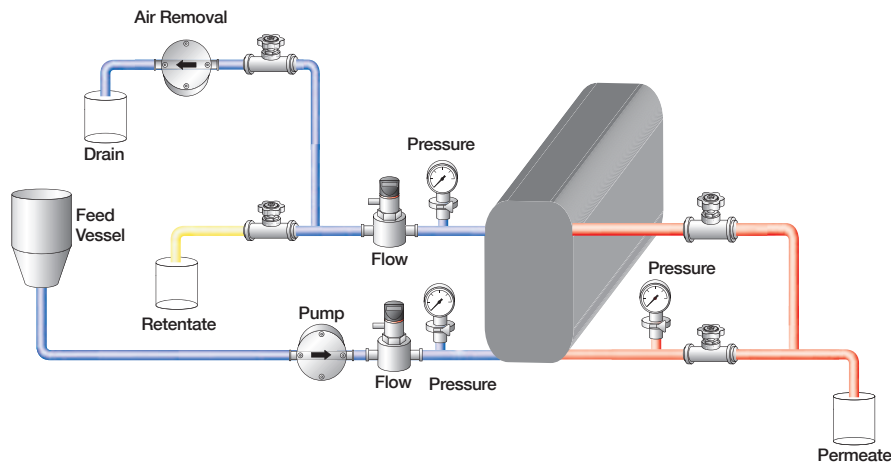
*Caution: Do not exceed a feed pressure of 6 barg (87 psig) at 23 °C during any pre-use, processing, or post-use procedures. Exceeding 6 barg (87 psig) of feed pressure can permanently damage the Cadence module's membrane. Do not exceed a feed pressure of 1.5 barg (22 psig) when the permeates are fully closed.*

New Cadence modules are stored in a solution containing 5% ethanol and 1% sodium diacetate. Previously used and stored modules can contain this storage solution or a 0.1 N NaOH solution.

The volume and flushing time can vary depending upon on the porosity of the membrane and the temperature of the flushing solution.

**Figure 8**

*Example Flow Diagram of a Cadence Single-pass TFF System, Using 4 or 8 in Series Module*



Flush the storage solution from the Cadence module following these steps:

#### ***Remove Air from Cadence Module***

1. Connect the provided peristaltic pump to the retentate line downstream of the retentate flow control valve.
2. Connect the feed line to a supply of purified water.
3. Fully open the retentate, permeate 1, and permeate 2 valves.
4. Start the feed pump and adjust pump speed to achieve a feed pressure of 2.8 barg (40 psig).
5. Close permeate 1 after one minute.
6. Open permeate 1 for 15 seconds, and then close for 10 seconds. Repeat for 4 to 5 minutes.
7. Shut down feed pump.
8. Close permeate 1 and permeate 2 valves and open the feed and retentate valves.
9. Start the feed and retentate pumps at a slow speed, and adjust to achieve a positive feed pressure of 0.35 to 1.38 barg (5 to 20 psig) and a negative retentate pressure of -0.35 to -0.69 barg (-5 to -10 psig). Do not exceed a feed pressure of 1.5 barg (22 psig) when the permeates are fully closed. Do not exceed -0.69 barg (-10 psig) on the retentate side. (That is the minimum pressure the pressure transducer will measure.)
10. Operate the Cadence system at these process conditions until air is removed from the retentate stream or after 5 L/m<sup>2</sup> of water has passed through the module.
11. Turn off the feed and retentate pumps.

#### ***Flush the Cadence Module with Water***

1. Direct permeate and retentate lines to drain.
2. Fully open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve and bypass the retentate pump.
3. Start the feed pump and slowly increase the pump speed until the feed pressure reaches 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during flushing may be set to lower than 4.1 barg (60 psig) to minimize water consumption.
4. During flushing, open the permeate 1 valve for 30 seconds. Repeat every 5 minutes.
5. Stop the feed pump once 20 L/m<sup>2</sup> of purified water has passed through the module or when the pH reaches  $\leq 7$ .



*Helpful Information: The effectiveness of this flushing protocol will depend on the composition and volume of chemicals in the module, and minimum acceptable levels in effluent at the end of the flush. The user must evaluate and validate the effectiveness of the process with respect to time, temperature and other operating conditions. Also note, for shorter path length module configurations (i.e., 4-in-series), the feed pressure used during flushing may be set to lower than 4.1 barg (60 psig) to minimize water consumption.*

#### **Procedure C — Sanitize the Cadence Module**

Sanitize the Cadence module following these steps:

1. Connect the feed line to a supply of sanitant solution (i.e., 0.1 N NaOH) at ambient temperature.
2. Direct permeate and retentate lines to drain.
3. Fully open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve. Bypass the retentate pump during the sanitization step.



4. Start the feed pump and slowly increase the pump speed until the feed pressure reaches 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during sanitization may be set to lower than 4.1 barg (60 psig).
5. Open the permeate 1 valve for 30 seconds and then close for the remainder of the sanitization step.
6. Stop the feed pump after 45 L/m<sup>2</sup> of sanitant has passed through the module.



*Helpful Information: The effectiveness of this sanitizing protocol to reduce or eliminate bioburden depends on the nature and level of the contamination. The user must evaluate and validate the effectiveness of the process with respect to time, temperature, sanitizing agent, and other operating conditions.*

#### **Procedure D – Flush the Cadence Module with Purified Water**

Flush the sanitizing solution from the Cadence module with purified water following these steps:

1. Connect the feed line to a supply of purified water.
2. Direct permeate and retentate lines to drain.
3. Fully open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve. Bypass the retentate pump during the water flush step.
4. Start the feed pump and slowly increase the pump speed until the feed pressure reaches 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during flushing may be set to lower than 4.1 barg (60 psig) to minimize water consumption.
5. During flushing, open the permeate 1 valve for 30 seconds. Repeat every 5 minutes.
6. Stop the feed pump after 25 L/m<sup>2</sup> of purified water has passed through the Cadence module, or when the pH reaches  $\leq 7$ .



*Helpful Information: The effectiveness of this flushing protocol will depend on the composition and volume of chemicals in the module, and minimum acceptable levels in effluent at the end of the flush. The user must evaluate and validate the effectiveness of the process with respect to time, temperature and other operating conditions.*

#### **Procedure E – Determine Cadence Module Normalized Water Permeability (NWP)**

If a new or previously used and stored Cadence module is used, the user will need to measure the permeability of the module before use. Later, after using and cleaning the module, the NWP of the module should be measured again. By comparing the NWP before and after use, the user can determine the effectiveness of the cleaning method and also determine when the module reaches the end of its service life.

##### ***Measure Cadence Module NWP***

Follow these steps to measure the module's NWP:

1. Connect the feed line to a supply of purified water.
2. Fully open the feed, retentate, and permeate 2 valves. Close permeate 1 valve. Bypass the retentate pump during the normalized water permeability step.
3. Direct the retentate and permeate lines to drain.
4. Start the feed pump and increase the pump speed to deliver a feed pressure of 2.1 barg (30 psig).
5. Slightly close the retentate flow control valve and adjust the feed pump speed to obtain a retentate pressure of 0.34 to 0.69 barg (5 to 10 psig) while maintaining a feed pressure of 2.1 barg (30 psig).

6. Record the feed and retentate flow rates and calculate the feed-to-retentate flow rate ratio. Use this same feed-to-retentate ratio when measuring the Cadence module water permeability at the higher feed pressures up to 60 psig and during other NWP tests. While the user keeps the ratio constant, the retentate pressure may change but should be within 0.3 to 0.4 barg (5 to 10 psig).
7. Record pressure in the feed, retentate and permeate 1 lines.
8. Record the temperature.
9. Repeat steps 4 through 7 at feed pressures of 2.8 and 4.1 barg (40 and 60 psig).
10. Stop the feed pump.

***Normalize the Permeate Flux to 20 °C***

Normalize the permeate flux to 20 °C following these steps:

1. Calculate permeate flow rates by subtracting the retentate flow from the feed flow.
2. Using the permeate flow rate measurement, calculate the permeate flux in units of L/m<sup>2</sup>/hr (LMH).
3. Using temperature correction factors (Table 1), normalize the permeate flux to 20 °C (Equation 1).

**Equation 1**

*Permeate Flux Normalized to 20 °C*

$$\text{Flux@20 °C} = \text{Permeate Flux} \times \text{TCF}_{20 \text{ °C}}$$

4. Using the feed, retentate, and permeate 1 pressures obtained while measuring Cadence module permeability, calculate the apparent transmembrane pressure (Equation 2).

**Equation 2**

*Transmembrane Pressure (TMP) Calculation*

$$\text{TMP} = \left( \frac{P_{\text{feed}} + P_{\text{retentate}}}{2} \right) - \left( \frac{P_{\text{permeate1}} + P_{\text{permeate2}}}{2} \right)$$

5. Determine the apparent NWP by dividing the permeate flux (LMH @ 20 °C) by the corresponding apparent transmembrane pressure (barg or psig) for each data point.
6. Average the three apparent NWPs and record value.

**Table 1***Temperature Correction Factors (TCF<sub>20 °C</sub>) for Cadence Module Permeability*

T °C	TCF <sub>20 °C</sub>	T °C	TCF <sub>20 °C</sub>
4	1.57	28	0.83
5	1.52	29	0.81
6	1.47	30	0.80
7	1.43	31	0.78
8	1.39	32	0.76
9	1.35	33	0.75
10	1.31	34	0.73
11	1.27	35	0.72
12	1.24	36	0.70
13	1.20	37	0.69
14	1.17	38	0.68
15	1.14	39	0.66
16	1.11	40	0.65
17	1.08	41	0.64
18	1.05	42	0.63
19	1.03	43	0.62
20	1.00	44	0.61
21	0.98	45	0.60
22	0.95	46	0.59
23	0.93	47	0.58
24	0.91	48	0.57
25	0.89	49	0.56
26	0.87	50	0.55
27	0.85		

**Procedure F – Determine the Hold-up Volume of the Cadence System and Module**

This procedure includes steps to determine the combined system and module hold-up volume for a given system setup.



*Helpful Information: This step should be performed prior to using the system for processing for the first time. For subsequent process runs, assuming that the plumbing and module size has not changed, this step is not required.*

**Determine the Hold-up Volume of the Cadence System and Module**

Follow these steps to determine the hold-up volume:

1. Open the feed and retentate valves and close both permeate valves. Bypass the retentate pump during this step.
2. Direct the retentate line to a collection vessel.
3. Disconnect the feed line from previous supply of fluid. Using the system pump to supply clean air to the feed port, pressurize the feed to 0.7 barg (10 psig). Alternatively, another source of filtered, oil-free, instrument-quality compressed air can be used to pressurize the feed to 0.7 barg (10 psig).
4. Wait until all fluid in the module is pushed out of the retentate port. Only air should be flowing from the retentate port.
5. Stop the air flow into the feed port.
6. Measure the retentate volume and record the value as the system hold-up volume.

## Procedure G — Test the Integrity of the Cadence System and Module

This procedure includes steps to test the Cadence system for external leaks and then to test the module membrane path for internal leaks or damage.



*Helpful Information: Before completing integrity testing, the user should condition the Cadence module to the temperature it will experience during processing and re-torque the holder. Users can condition the Cadence module and system to the correct temperature by completing a water flush with purified water (or the equilibration buffer) at the desired temperature. See Procedure B — Remove Air and Flush the Cadence Module with Purified Water. [If the hardware surface temperature is 20 to 25 °C and the product fluid is introduced at 4 °C, the clamping force on a module may decrease 20 to 40% and cause a leak due to contraction of the polymeric elements.]*

### *Test the Integrity of the Cadence System*

Follow these steps to test the integrity of the Cadence system. If the system hold-up volume was determined prior to this integrity test, begin with step 6. Otherwise, begin with step 1.

1. Open the feed and retentate valves and close both permeate valves. Bypass the retentate pump during the integrity test.
2. Direct the retentate line to drain.
3. Disconnect the feed line from previous supply of fluid. Using the system pump to supply clean air to the feed port, pressurize the feed to 0.7 barg (10 psig). Alternatively, another source of filtered, oil-free, instrument-quality compressed air can be used to pressurize the feed to 0.7 barg (10 psig).
4. Wait until all fluid in the module is pushed out of the retentate port. Only air should be flowing from the retentate port.
5. Stop the air flow into the feed port.
6. Close the retentate valve.
7. Open the feed port and slowly pressurize the feed line with air to 4.1 barg (60 psig).
8. Close the feed port.
9. Wait two minutes for the Cadence system to stabilize, and then observe the feed pressure decay using the feed line pressure indicator. If the system loses less than 0.07 barg (1 psig) per minute, the system is integral. If the system loses more than 0.07 barg (1 psig) per minute, check the Cadence system connections and Cadence module sealing for leaks.

### *Test the Integrity of the Cadence Module*

Follow these steps to test the integrity of the Cadence module:

1. Open the feed and permeate 2 valves and close the retentate and permeate 1 valves. Bypass the retentate pump during the integrity test.
2. Direct the permeate line to collection vessel or drain.
3. Restart the system pump or reopen the compressed air source and adjust to maintain a feed pressure of 4.1 barg (60 psig).
4. Measure the air flow out of the permeate port using the Pall Flowstar integrity tester or other integrity test instrument.
5. If the flow from the permeate port is less than 50 sccm/min/ft<sup>2</sup> (500 sccm/min/m<sup>2</sup>), the Cadence module is integral. If the flow from the permeate port is greater than 50 sccm/min/ft<sup>2</sup> (500 sccm/min/m<sup>2</sup>), the module has failed the integrity test.
6. Stop the compressed air source or pump and depressurize the module by opening the retentate valve slowly.
7. Discard modules that fail the integrity test.

## Procedure H — Remove Air and Flush with Buffer

Flushing the Cadence module with buffer removes air trapped in the module from integrity testing. It also prevents unwanted reactions between the Cadence system components and the product. For example, buffer flushing establishes the proper pH, ionic strength and temperature conditions to help prevent product precipitation or denaturation when it is introduced into the system. The buffer solution is normally the same solution used to prepare the product solution and should be at a temperature similar to the product solution temperature.



*Helpful Information: Membrane modules and hardware that experience substantial temperature changes (> 10 °C) will require a holder torque adjustment after steady state is reached.*

### **Remove Air from the Cadence Module**

1. Connect the provided peristaltic pump to the retentate line downstream of the retentate flow control valve.
2. Connect the feed line to a supply of buffer.
3. Fully open the retentate, permeate 1, and permeate 2 valves.
4. Start the feed pump and adjust pump speed to achieve a feed pressure of 2.8 barg (40 psig).
5. Close permeate 1 after one minute.
6. Open permeate 1 for 15 seconds, and then close for 10 seconds. Repeat for 4 to 5 minutes.
7. Shut down the feed pump.
8. Close permeate 1 and permeate 2 valves, and open the feed and retentate valves.
9. Start the feed and retentate pumps at a slow speed, and adjust to achieve a positive feed pressure of 0.35 to 1.38 barg (5 to 20 psig) and a negative retentate pressure of -0.35 to -0.69 barg (-5 to -10 psig). Do not exceed a feed pressure of 1.5 barg (22 psig) when the permeates are fully closed. Do not exceed -0.69 barg (-10 psig) on the retentate side. (That is the minimum pressure the pressure transducer will measure.)
10. Operate the Cadence system at these process conditions until air is removed from the retentate stream or after 5 L/m<sup>2</sup> of buffer has passed through the module.
11. Turn off the feed and retentate pumps.

### **Flush the Cadence Module with Buffer**

1. Direct the retentate and permeate lines to drain.
2. Open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve.
3. Start the feed pump and slowly increase the pump speed until the feed pressure reaches 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during sanitization may be set to lower than 4.1 barg (60 psig) to minimize buffer consumption.
4. During flushing, open the permeate 1 valve for 30 seconds. Repeat every 5 minutes.
5. Stop the feed pump after 15 L/m<sup>2</sup> of buffer passes through the module.

*Part 1 — Pre-use Conditioning* is now complete, and the user is ready to process the product using single-pass TFF following the procedures in *Part 2 — Processing*.

## 5.4 Part 2 – Processing

### Procedure I – Process Product

Before completing product processing procedures, review the following recommended operating parameters.

#### *Recommended Operating Parameters*

Operating parameters are specific to each application.

Feed pressures during processing typically range from 2.1 to 4.1 barg (30 to 60 psig).

Design the process so that the retentate pressure is greater than 0.3 barg (5 psig).

#### *Recommended Operating Pressures, Temperatures, and pH*

The following list provides the recommended operating limit specifications for pressure, temperature, and pH for Cadence single-pass TFF modules:

- Processing temperature range: 4 to 40 °C
- Maximum operating pressure: 6 barg (87 psig) @ 23 °C or 4.1 barg (58 psig) @ 55 °C
- pH range: 2 to 13

#### *Steps for Concentrating a Protein*

The following steps describe a typical single-pass TFF application — a 10X concentration of the feed material where the retentate is the product of interest.

1. Connect the feed line to the feed solution.
2. Place the retentate and permeate lines into separate collection vessels.
3. Fully open the feed, retentate, and permeate 1 and 2 valves.
4. Start the feed pump and increase the pump speed until the process design feed pressure is obtained.
5. Once the feed design pressure is reached, adjust the retentate flow control valve to apply enough backpressure on the retentate to attain the targeted concentration factor (concentration factor =  $Q_{\text{FEED}}$  divided by  $Q_{\text{RETENTATE}}$ ). For example, if the feed flow rate is 100 mL/min, adjust the retentate flow rate to 10 mL/min to achieve a concentration factor of 10X.
6. After processing the protein solution, stop the feed pump.

### Procedure J – Recover Product from the Cadence Module

Follow these steps to recover product from the module:

1. Close both permeate valves.
2. Remove the retentate line from the collection vessel and place it in another collection vessel.
3. Connect the feed line to a buffer solution that is compatible with the product.
4. Start the feed pump on slow speed and adjust the speed to maintain a feed pressure of less than 1.4 barg (20 psig). Do not exceed a feed pressure of 1.5 barg (22 psig) when the permeates are fully closed.
5. Pump one hold-up volume through the module and into a separate retentate vessel. Repeat this step, each time pumping one hold-up volume into a separate retentate vessel. The number of recovery flushes performed should be defined by the application and the targeted recovery yield. This recovery step should be optimized for each application.
6. Stop the pump(s).

**Table 2***Minimum Hold-up Volumes for Cadence Modules*

<b>Module size</b>	<b>Minimum Module Hold-Up Volume</b>
T01 (4-in-series through 9-in-series)	20 – 60 mL
T02 (4-in-series through 9-in-series)	40 – 110 mL
T12 (4-in-series through 9-in-series)	250 – 650 mL
T06 (4-in-series through 9-in-series)	1 – 3.5 L

The system minimum hold-up volumes may vary depending on the plumbing and modules being used. The system hold-up volume should be determined prior to processing. Refer to *Procedure B – Remove Air and Flush the Cadence Module with Purified Water*.

*Part 1 – Pre-use Conditioning* and *Part 2 – Processing* are now complete. The user is ready to begin *Part 3 – Post-use Conditioning*, where the Cadence module will be cleaned, characterized, and prepared for storage.

## 5.5 Part 3 – Post-use Conditioning

After completing product recovery, clean, test, and prepare the Cadence module for storage or reuse. Document and validate the cleaning process for biopharmaceutical applications.

### Procedure K – Clean the Cadence Module

If a sufficient amount of product has been removed from the module with buffer flushing during product recovery, proceed directly to Cleaning Steps below.

If product must be flushed from the module with purified water or buffer before beginning the cleaning step, follow *Procedure B (Flush the Cadence Module)*. Then go to the Cleaning Steps below.

#### *Cleaning Steps*

Follow these steps to clean the Cadence module and system:

1. Connect the feed line to a supply of cleaning solution (i.e., 0.1 N NaOH) at ambient temperature.
2. Direct the retentate and permeate lines to drain.
3. Fully open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve. Bypass the retentate pump during the cleaning step.
4. Start the feed pump and adjust the feed pump speed to deliver a feed pressure of 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during cleaning may be set to lower than 4.1 barg (60 psig).
5. Open the permeate 1 valve for 30 seconds and then close for the remainder of the cleaning step.
6. Stop the feed pump after 45 L/m<sup>2</sup> of cleaning solution passes through the module.



*The effectiveness of this cleaning procedure to remove product residue will depend on the nature of the product. The user must evaluate and validate the effectiveness of the cleaning process with respect to time, cleaning agent, and operating conditions.*

Between uses, modules are typically stored in a caustic solution (0.1 N NaOH). During this time, remaining foulants on the membrane may be degraded and removed when the module is flushed prior to next use. As a result of membrane relaxation and static cleaning during storage, it is not unusual for the module NWP, measured after storage, to increase relative to the module NWP measured immediately after cleaning.

### **Procedure L – Flush the Cadence Module with Purified Water**

Flush the cleaning solution from the Cadence module and system with purified water following these steps:

1. Connect the feed line to a supply of purified water.
2. Direct the retentate and permeate lines to a collection vessel or waste.
3. Open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve.
4. Start the feed pump and slowly increase the pump speed until the feed pressure reaches 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during flushing may be set to lower than 4.1 barg (60 psig) to minimize water consumption.
5. During flushing, open the permeate 1 valve for 30 seconds. Repeat every 5 minutes.
6. Stop the feed pump after 25 L/m<sup>2</sup> of water passes through the module or when the pH reaches  $\leq 7$ .

*Helpful Information: The effectiveness of this flushing protocol will depend on the composition and volume of chemicals in the module and minimum acceptable levels in effluent at the end of the flush. The user must evaluate and validate the effectiveness of the process with respect to time, temperature and other operating conditions.*

### **Procedure M – Determine Cadence Module Normalized Water Permeability (NWP)**

Determine the membrane NWP following the steps in *Procedure E – Determine Cadence Module Normalized Water Permeability (NWP)*. Note: Use the same volumetric concentration factor noted during the initial water permeability test.

### **Procedure N – Test Integrity of the Cadence System and Module (Optional)**

Test the integrity of the Cadence system and module following the steps in *Procedure G – Test the Integrity of the Cadence System and Module*.

### **Procedure O – Add Storage Solution to the Cadence Module**

The objective for proper storage of the Cadence module is to ensure the module remains wet and to prevent microbial growth during storage.

If *Procedure N – Test Integrity of the Cadence System and Module* was performed, then follow the procedure *Remove Air from the Cadence Module* provided in Procedure B. If Procedure N is not performed, then continue with the procedure *Adding Storage Solution to the Cadence Module* provided below.

#### ***Adding Storage Solution to the Cadence Module***

1. Connect the feed line to a supply of storage solution.
  - a. Use a storage solution of 5% ethanol and 1% sodium diacetate to store the module for more than three months.
  - b. Use a storage solution of 0.1 N NaOH to store the module for less than three months.
2. Open the feed, retentate, and permeate 2 valves. Close the permeate 1 valve.
3. Direct retentate and permeate lines to collection vessel or drain.
4. Start the feed pump and adjust the pump speed to deliver a feed pressure of 4.1 barg (60 psig). For shorter path length module configurations (i.e., 4-in-series), the feed pressure used during flushing may be set to lower than 4.1 barg (60 psig).



5. Open the permeate 1 valve for 30 seconds and then close for the remainder of the procedure.
6. Stop the feed pump after 10 L/m<sup>2</sup> of storage solution passes through the module.
7. Disconnect the module from the Cadence system and cap the ports while keeping module full of storage solution.
8. Place the module in a sealed plastic bag.
9. Store module in a temperature-controlled room, optimally at a temperature of 4 to 25 °C.

## 6. Appendices

### 6.1 Recommended Storage of New Cadence Single-pass TFF Modules

Cadence modules can be expected to perform within specifications if, when new, they are stored and handled in a manner consistent with the parameters below:

- Store modules, unopened in the original packaging in a dry environment at 4 to 25 °C.
- Protect the module from direct sunlight, radiation, or weather conditions.
- Prevent physical damage while handling.
- Protect the module from thermal shock.

### 6.2 Delta Membrane Chemical Compatibility Chart

The chemical compatibility of Cadence modules with Delta membrane can be described in terms of changes in physical characteristics due to continuous contact with a chemical solution for several hours. Changes can affect dimensions, hardness, swelling, the integrity of internal seals, and membrane integrity. Changes can also be described in terms of the functional characteristics of the membrane (such as water permeability, and retention characteristics).

Table 3 illustrates the compatibility of different Cadence modules with Delta membrane at 20 °C, unless otherwise noted, with respect to physical characteristics. The table should only be used as a guide. Modules should be tested in the appropriate solvent and product under actual operating conditions for an appropriate time to determine compatibility for a specific application.

**Table 3**

*Cadence Modules with Delta Membrane Chemical Compatibility Chart*

Reagent	Compatible
Acetic Acid (5%)	•
Alconox <sup>◆</sup> (1%)	•
Citric acid (1%)	•
Ethanol (20%)	•
Glycerine (20%)	•
Phosphoric acid (0.1 N)	•
Sodium dodecyl sulfate (0.01 M)	•
Sodium hydroxide (0.25 N @ 25 °C) <sup>1</sup>	•
Terg-a-zyme <sup>◆</sup> (1%)	•

<sup>1</sup> Data for module membrane and components at 25 °C, 20 hours cumulative exposure. There may be changes in porosity and/or selectivity of membrane.

Pall offers validation services to test compatibility of membranes and modules with different solvents. Contact your local Pall representative for information on Validation Services.

## 6.3 Alternative Cleaning Agents

**Table 4**  
*Alternative Cleaning Agents*

Agent	Mode of Cleaning	Foulant
Alkalies	High pH hydrolysis Solubilization	Biomolecules, fats, proteins, starches
Acids	Solubilization	Inorganic salts
Surfactants	Wetting, emulsification, dispersion, solubilization	Biomolecules, fats, oils, proteins, insoluble particles
Solvents	Solubilization	Oils, fats, grease, proteins, biomolecules
Enzymes	Enzymatic digestion	Proteins

**Table 5**

*Acids*

Type	Foulant	Condition
Phosphoric Acid (H <sub>3</sub> PO <sub>4</sub> ) <sup>1</sup>	Inorganic, Nucleic Acids	0.1 – 0.25 N, 25 – 45 °C, pH ~2
Citric Acid	Iron	1%, 25 – 45 °C, pH ~3

<sup>1</sup> Long-term storage (> 7 days) in Phosphoric Acid (H<sub>3</sub>PO<sub>4</sub>) may cause slight yellowing of the module's polyurethane encapsulant.

**Table 6**

*Alkalies and Oxidizers*

Type	Foulant	Condition
Sodium Hydroxide (NaOH)	Proteins, enzymes, vaccines, viruses, bacterial cells and lysates, polysaccharides, organic colloids, pyrogens, lipids	0.25 N, 25 °C, 20 hrs

**Table 7**

*Surfactants*

Type	Foulant	Condition
Sodium Dodecyl Sulfate (SDS), Alconox, Terg-a-zyme	Bacterial whole cells and lysate, lipids, oils, antifoams, polysaccharides, and precipitated proteins	0.1%, 25 – 45 °C, pH 4 – 9

## 6.4 Glossary

Access Pall's Tangential Flow Filtration (TFF) Glossary online at [www.pall.com/biopharm](http://www.pall.com/biopharm).



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