# TangenX® SIUS® Gamma TFF Device

User Guide

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## Abbreviations

| | |
|---|---|
| CF | Crossflow |
| DF | Diafiltration |
| D Vol | Diavolume |
| In | Inches |
| Kg | Kilogram |
| Lbs | Pounds |
| LMH | Liters per minute per square meters |
| L/min | Liters per minute |
| ml | milliliters |
| MWCO | Molecular weight cut-off |
| N-m | Newton meter |
| Perm Vol | Permeate volume |
| PSIG | Pounds per square inch |
| TFF | Tangential Flow Filtration |
| TMP | Transmembrane Pressure |
| UF | Ultrafiltration |
| WFI | Water for injection |

# 1. Introduction

The TangenX® SIUS® Gamma TFF Device from Repligen is a flat sheet, Gamma-irradiated, closed, single-use device for tangential flow filtration (TFF). SIUS® Gamma Devices deliver:

- Fully assembled closed device with aseptic connectors
- Gamma-irradiation to minimize bacterial contamination
- Closed system to reduce contamination risk and exposure to hazardous material
- All fluid path materials meet USP Class VI requirements
- Certificate of quality included with lot certification
- Manufactured in an ISO 9001 certified facility; ISO 8 clean-room
- Validated manufacturing processes for consistent filter performance
- Biopharm Operations Group (BPOG) extractables test data

Designed and manufactured for GMP environments, TangenX® SIUS® Gamma Devices are accompanied by a detailed regulatory support file to facilitate your validation. A wide variety of molecular weight cut-offs, surface areas, membrane chemistries (ProStream and HyStream) and screen types support your diverse process needs. Potential applications include:

- Biomolecule concentration
- Buffer exchange
- Sample fractionation

## 1.1 Additional references

www.repligen.com/resources

TangenX® SIUS® Set-up Guide: **IF.PUG.021**

TangenX® SIUS® PD Filter Plate and 2-bolt Clamp Assembly: **IF.PUG.019**

TangenX® SIUS® Filter Plate and 4-bolt Clamp Assembly: **IF.PUG.018**

TangenX® Device Preservative Sodium Hydroxide Solution Safety Data Sheet: **IF.SD2-POP.1034**

TangenX® Device Air Integrity Application Note: **AN1002**

## 1.2 Intended use

The TangenX® SIUS® Gamma Device concentrates biomolecules and exchanges buffers using ultrafiltration and diafiltration processes when aseptic conditions are required. TangenX® SIUS® Gamma and TangenX® SIUS® Cassettes are both constructed with the same membrane to deliver comparable performance.

## 1.3 Abbreviations and symbols

**Table 1. Symbols used in flow diagrams**

| Symbol | Description |
|---|---|
| | Vessels |
| | Pinch clamps |
| | CPC AseptiQuik® aseptic genderless connectors |
| | Pump<br>- Diafiltration<br>- Feed |
| | Pressure sensors<br>- Retentate<br>- Feed<br>- Permeate |
| FM | Flow meter (optimization only) |
| | Inactive/Standby flow path |
| | On/Open flow path |
| | Off/Closed flow path |
| | Configuration change |
| | Open valve action |
| | Closed valve action |
| | On/Open and Off/Closed valve action |

## 1.4 Safety precautions

**Table 2. Important information before you begin**

| | |
|---|---|
| Warning: | Damage may occur as a result of the following:<br>• Dropping on hard surfaces, or other mechanical shock<br>• Poking with sharp objects on screened surfaces<br>• Excessive feed pressure<br>• Excessive permeate backpressure or pressurizing the filtrate port<br>• Exposure to harsh chemicals<br>• Freezing<br>• Excessive heat<br>• Drying out – ultrafiltration or microfiltration membrane that is allowed to dry out can permanently damage the pore structure |
| Warning: | Membrane devices must remain wet at all times to maintain product integrity and performance. Keep bag sealed until device installation. |
| Warning: | All devices are stored in 0.2 M Sodium Hydroxide. |
| Warning: | Follow standard safety procedures for handling 0.2 M Sodium Hydroxide, including the use of gloves, safety goggles, and lab coat. |
| Information: | It is recommended that you perform a device integrity test to ensure your device has been installed and clamped in the device holder properly. Reference the air integrity test procedure AN1002 and specifications listed in Table 4. |
| Information: | Devices must be equilibrated with an appropriate buffer to ensure the neutralization of the 0.2 M Sodium Hydroxide storage agent in the membrane filter. |
| Information: | It is important to pre-filter all liquids to avoid fouling the membrane or introducing contaminants into the system that could affect membrane performance and product recovery. |
| Information: | Select a pump with adequate capacity. Crossflow rate ranges (Table 7) are feed channel type and process fluid dependent. |
| Information: | Devices must be clamped in a device holder using a torque wrench and the specified torque range (Table 4). |

## 1.5 Required tools and components

The following tools and components are required to install the TangenX® SIUS® Gamma Device.

**Figure 1. Tools required**

**Scissors**

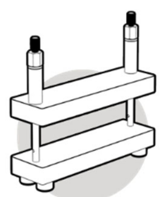
**Torque wrench**
TX019 with 11/16" socket for 0.1 $m^2$
TX026 with 1 1/4" socket for > 0.5 $m^2$

**Figure 2. Components required**

**4-bolt**

**Holder**
TSPDI-4BMC
(or equivalent)

**2-bolt**



**Holder**
TSLDI-2BMC
(or equivalent)

The following minimum components for your system are recommended. However, the exact system configuration and components will vary according to user requirements.

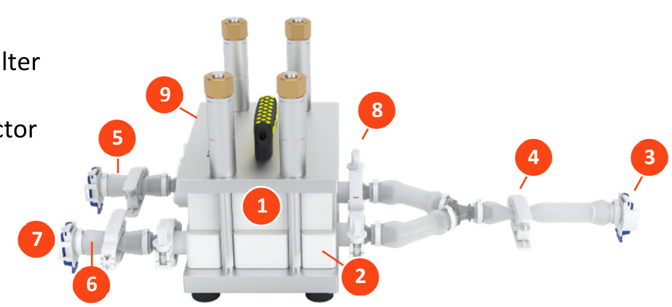
**Table 3. Minimum recommended components for system**

| Item | Description |
|---|---|
| Feed Vessel | Gamma-irradiated reservoir bag that holds the feed |
| Permeate Vessel | Gamma-irradiated reservoir bag that collects the permeate |
| Waste Vessel | Gamma-irradiated reservoir bag that collects waste |
| Water Vessel | Gamma-irradiated reservoir bag that contains water that is pushed through the feed input port |
| Buffer Vessel | Gamma-irradiated reservoir bag that contains buffer that is pushed through the feed import port |
| Collection Vessel | Gamma-irradiated reservoir bag that will be used to recover the final product |
| Inlet port | Port delivering liquid to a vessel or the device |
| Outlet port | Port transporting liquid way from a vessel or the device |
| Flow path | Gamma-irradiated assembly of tubing, reservoirs, clamps etc. that connect the TangenX® SIUS® Gamma Device to a vessel or one vessel to a second vessel |
| Pinch clamps | Clamps at each port of the vessel and TangenX® SIUS® Gamma Device that control the flow of liquid through the system |
| Pressure sensors | Sensors connected to the TangenX® SIUS® Gamma Device feed input port, retentate output port, and permeate output port |
| KrosFlo® System (or equivalent system) | Controls pumps, records sensor data and displays system status |
| Peristaltic pump x 2 | (1) Delivers feed to the TangenX® SIUS® Gamma Device feed input port and (2) second pump to deliver Diafiltration Buffer to the Retentate Vessel |
| Scale | To determine the volume of water or feed material in a vessel |
| Flow meter | To measure the flow from the retentate when defining optimal operating conditions |
| Compressed air supply | Creates pressure during air integrity test |

## 1.6 Product description

**Figure 3. TangenX® SIUS® Gamma Device and Holder**

1. TangenX® SIUS® Gamma Cassette/Filter
2. Filter plate insert manifold
3. Permeate output port with Y connector
4. Pinch clamp (3)
5. Feed input port with tubing
6. Retentate port with tubing
7. AseptiQuik® connector (3)
8. Tri-clamp connector (4)
9. Holder top plate



### 1.7 Specifications

**Table 4. Device specifications**

| Module characteristics | Surface area: 0.1 m² | Surface area: 0.5 m² | Surface area: 1.5 m² | Surface area: 2.5 m² |
|---|---|---|---|---|
| Channel path length | 16 cm | | | |
| Hold-up volume | 61 ml | 509 ml | 874 ml | 1026 ml |
| Working volume | 200 ml | 1000 ml | 3000 ml | 5000 ml |
| Max temperature | 40° C | | | |
| Max pressure (forward) | 60 psig (4 bar) | | | |
| Max pressure (reverse) | 7 psig (0.48 bar) | | | |
| Crossflow (CF) LP Screen | | 2 - 4 L/min | 6 - 12 L/min | 10 - 20 L/min |
| Crossflow (CF) EP Screen | | 3 - 6 L/min | 9 - 18 L/min | 15 - 30 L/min |
| ΔP LP Screen | 10 psig (0.7 bar) | | | |
| ΔP EP Screen | 5 psig (0.35 bar) | | | |
| Air integrity test pressure | 7.3 psig (0.5 bar) | | | |
| Max air diffusion rate | 323 ccm/m² | | | |
| AseptiQuik® connector | G | | | |
| Torque range | 120 - 180 in-lbs (13.6 - 20.3 N-m) | 300 - 450 in-lbs (33.9 - 50.8 N-m) | | |

**Table 5. Chemical compatibility**

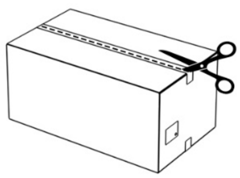
| Reagent | ProStream (pH 1 - 14) | HyStream (pH 1 - 14) |
|---|---|---|
| Acetic Acid (5%) | Yes | Yes |
| Acetic Acid (25%) | Yes | No |
| Acetone (≤ 30%) | Yes | Yes |
| Acetonitrile (≤ 15%) | Yes | No |
| Alconox (1%) | Yes | Yes |
| Aliphatic and Aromatic Esters | No | No |
| Amines | No | No |
| Ammonium Chloride (1%) | Yes | Yes |
| Ammonium Hydroxide (5%) | No | No |
| Aromatic and Chlorinated | No | No |
| Hydrocarbons | Yes | Yes |
| Butanol (70%) | Yes | No |
| Butyl Acetate (40%) | Yes | Yes |
| Butyl Cellosolve (10%) | Yes | Yes |
| Calcium chloride (5%) | Yes | Yes |
| Chloroform (0.8%) | Yes | Yes |
| Citric Acid (1%) | Yes | Yes |
| Dimethyl Acetamide (DMAC) (≤ 30%) | Yes | No |
| Dimethyl Acetamide (DMAC) (≤ 15%) | Yes | Yes |
| Dimethylformamide (≤ 40%) | Yes | Yes |
| Dimethyl Sulfoxide (≤ 40%) | Yes | Yes |
| Disodium Salt of EDTA (10%) | Yes | Yes |
| Ethanol (70%) | Yes | Yes |
| Ethers | No | No |

**Chemical compatibility - continued**

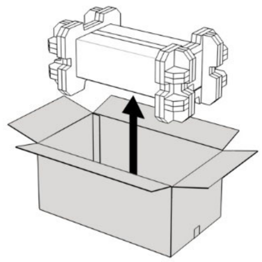
| Reagent | ProStream® (pH 1 - 14) | HyStream® (pH 1 - 14) |
|---|---|---|
| Ethyl Acetate (≤ 30%) | Yes | Yes |
| Formaldehyde (1%) | Yes | Yes |
| Formic Acid (5%) | Yes | Yes |
| Glutaldehyde (0.5%) | Yes | Yes |
| Glycerin (50%) | Yes | Yes |
| Guanidine HCl (6 M) | Yes | Yes |
| Hydrochloric Acid (0.1 N @ 25° C) | Yes | Yes |
| Hydrochloric Acid (0.1 N @ 50° C) | Yes | Yes |
| Hydrochloric Acid (1.0 N @ 50° C) | Yes | No |
| Hydrogen Peroxide (1%) | Yes | Yes |
| Isopropyl Acetate (1%) | Yes | Yes |
| Isopropyl Alcohol (25%) | Yes | Yes |
| Ketones | No | No |
| Lactic Acid (5%) | Yes | Yes |
| Mercaptoethanol (0.1%) | Yes | Yes |
| Methyl Alcohol (25%) | Yes | Yes |
| Methylene Chloride (1%) | Yes | No |
| Methyl Ethyl Ketone (1%) | Yes | No |
| N-Methyl Pyrrolidone (1%) | Yes | Yes |
| Nitric Acid (≤ 1%) | Yes | Yes |
| Oxalic Acid (1%) | Yes | Yes |
| Phenol (0.5%) | Yes | Yes |
| Phosphate Buffer (pH: 8.2) (1 M) | Yes | Yes |
| Phosphoric Acid (1 N) | No | No |
| Sodium Azide (1%) | Yes | Yes |
| Sodium Chloride (5%) (50° C) | Yes | Yes |
| Sodium Deoxycholate (5%) | No | No |
| Sodium Dodecyl Sulfate (0.01 M) | Yes | Yes |
| Sodium Hydroxide (0.1 N @ 25° C) | Yes | Yes |
| Sodium Hydroxide (0.1 N @ 50° C) | Yes | Yes |
| Sodium Hydroxide (0.5 N @ 25° C) | Yes | Yes |
| Sodium Hydroxide (0.5 N @ 50° C) | Yes | Yes |
| Sodium Hydroxide (1.0 N @ 25° C) | Yes | No |
| Sodium Hypochlorite (100 ppm) | Yes | Yes |
| Sodium Hypochlorite (400 ppm) | Yes | No |
| Sodium Hypochlorite (1000 ppm) | No | No |
| Sodium Nitrate | Yes | Yes |
| Sulfuric Acid (1 N) | Yes | |
| Terg-a-zyme (1%) | Yes | Yes |
| Tetrahydrofuran (5%) | No | No |
| Toluene (1%) | No | No |
| Tris buffer (pH: 8.2) (1 M) | Yes | |
| Triton X-100 (0.002 M) | Yes | Yes |
| Urea (25%) | Yes | Yes |
| Ultrasil 11 (1%) | Yes | |

## 2. Unpacking

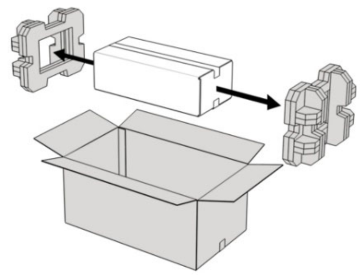
**1.** Cut open box 1.



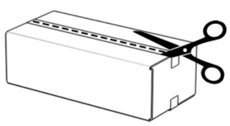
**2.** Remove box 2 from box 1.



**3.** Remove packaging ends.



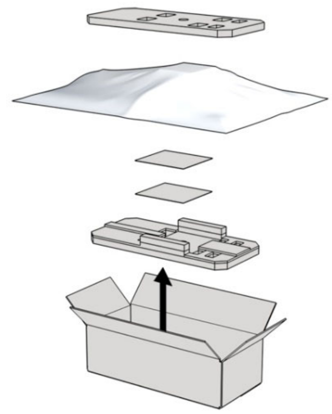
**4.** Cut open box 2
**-OR-** put in storage.



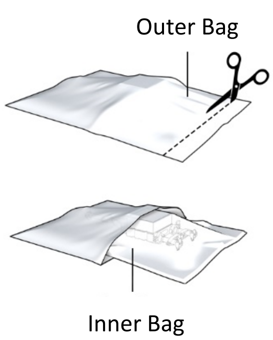
**Storage temperature:**

- 4 - 25 °C long term (> 7 Days)
- 40 °C short term (< 7 Days)
- Do not freeze cassettes

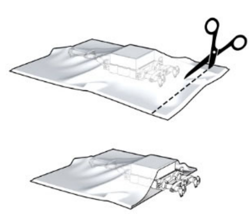
**5.** Remove contents from box 2.

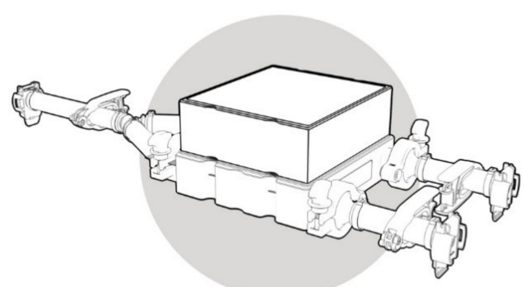


**6.** Cut open Outer Bag
and remove Inner Bag.




**7.** Cut open Inner Bag
and remove cassette.



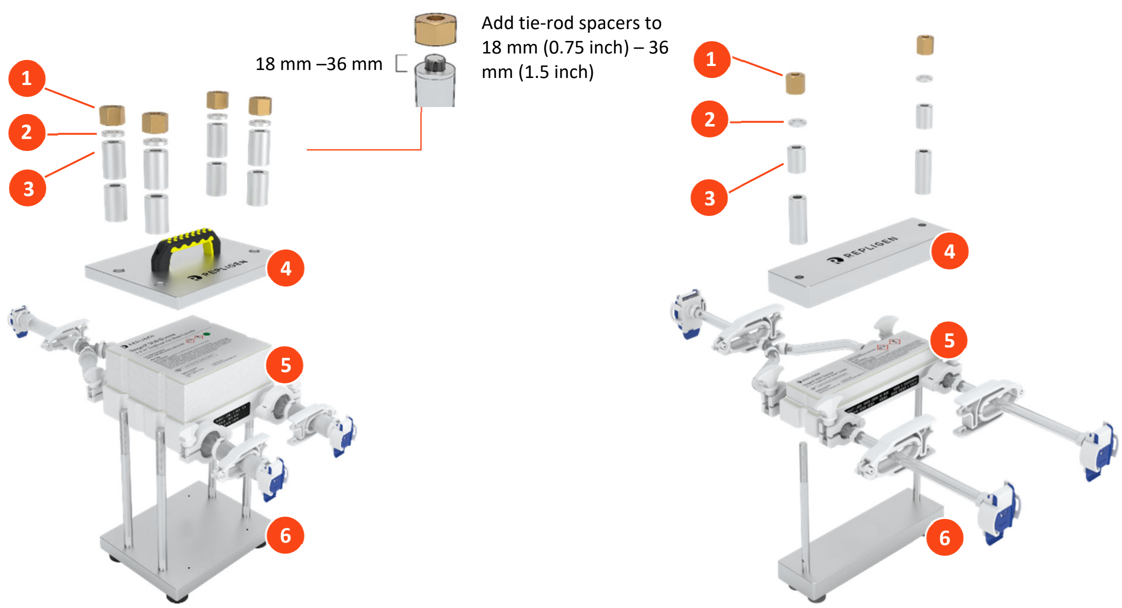


WARNING: Each cassette is stored in 0.2 M Sodium Hydroxide solution as a preservative. Follow standard safety procedures for handling 0.2 M Sodium Hydroxide, including the use of gloves, safety goggles, and lab coat.

## 3. Install the filtration device in the holder

1. Remove the hex nuts, washers and tie-rod spacers from the holder bolts.
2. Remove the holder top plate.
3. Insert TangenX® SIUS® Gamma Device into the holder with the device label facing up.
4. Plate the holder top plate on top of the device.
5. Add tie-rod spacers (if used) until the exposed thread on rod reaches ~18 mm (0.75 inch) – 36 mm (1.5 inch).
6. Add the washer.
7. Add the hex nut and tighten using the torque sequence described in Section 3.1 "Torque Sequence".

**Figure 4. TangenX® SIUS® Gamma Holder Assembly**




**SIUS® Gamma Filtration Device installed in 4-bolt holder**

**SIUS® PD Gamma Filtration Device installed in 2-bolt holder**

1. Hex nut
2. Washer
3. 2" or 1" Tie-rod spacer(s)
4. Holder top plate
5. TangenX® SUIS® Gamma Device
6. Holder bottom plate

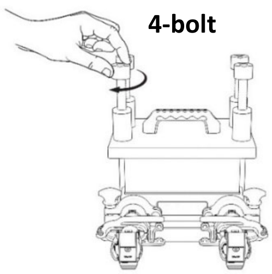
### 3.1 Torque sequence

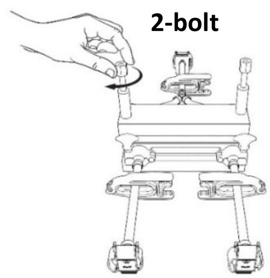
***Note:*** *The torque process should be executed at the following points.*

- *After installation of the filtration device in the holder*
- *After flushing and prior to executing concentration and diafiltration steps*
- *At any point when the device temperature has changed by more than 10° C*
- *After the device rests idle in the holder for more than approximately 4 hours.*

1. By hand, screw the nut on each bolt and hand tighten evenly by alternating from one nut to the other (Figure 5).

**Figure 5. Hand-tighten the nuts on each bolt by hand**

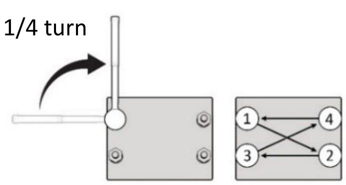



2. Using the calibrated torque wrench tighten each hex nut 1/4 turn at a time following the torque sequence illustrated in (4-bolt pictured above) or (2-bolt pictured above).
3. Further tighten the nuts using the calibrated torque wrench by turning each hex nut 1/4 turn using the torque sequence for 4-bolt (Figure 6) or 2-bolt (Figure 7) device holder.
4. Wait 5 - 10 minutes to allow the gasket to relax.
5. Re-torque each nut, using the torque sequence for your device holder.

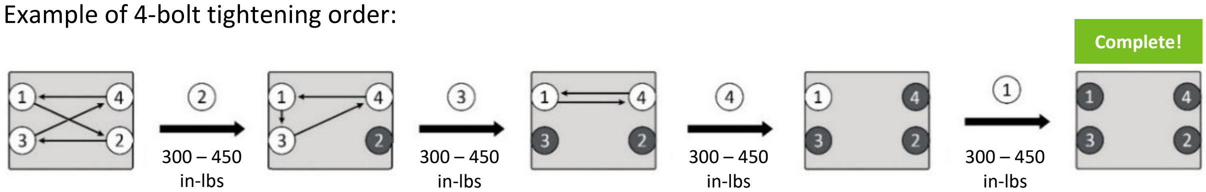
***Note:*** *Nut must be tightened uniformly using the appropriate torque sequence to avoid damaging the device. Leakage may result from non-parallel plate alignment or over-compression of the devices at one end. Discontinue tightening the nut when the torque wrench pivot point clicks.*

**Figure 6. 4-bolt torque sequence: appropriate for 0.5 m$^2$, 1.5 m$^2$, and 2.5 m$^2$ devices**



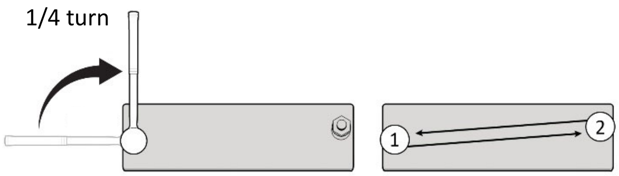

- Torque range for 4-bolt TangenX® SIUS® Cassette Holder is 300 - 450 in-lbs (33.9 - 50.8 N-m)
- Each nut should be tightened to a maximum of 450 in-lbs (50.8 N-m)
- Skip nut once 450 in-lbs is reached
- Repeat sequence one additional time when specified torque is reached (300 - 450 in-lbs) (33.9 - 50.8 N-m)

Example of 4-bolt tightening order:



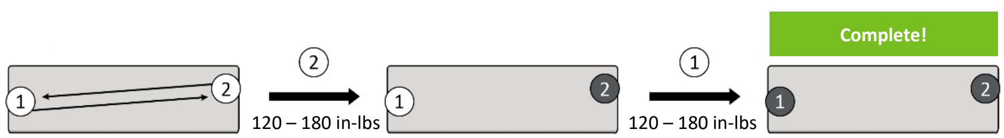

# = Numbers in grey have been tightened to 450 in-lbs.

**Figure 7. 2-bolt torque sequence: appropriate for 0.1 m$^2$ devices**




- Torque range for 2-bolt SIUS® PD Cassette Holder is 120 - 180 in-lbs (13.6 - 20.3 N-m)
- Each nut should be tightened to a maximum of 180 in-lbs (20.3 N-m)
- Skip nut once 180 in-lbs is reached
- Repeat sequence one additional time when specified torque is reached (120 - 180 in-lbs)

Example of 2-bolt tightening order:




# = Numbers in grey have been tightened to 180 in-lbs.

## 4. Aseptic connections

You have multiple options when integrating the TangenX® SIUS® Gamma Device into a closed TFF system:

1. Build a custom ProConnex® Flow Path from a library of over 400 components.
2. Order a pre-built, off-the-shelf ProConnex® Flow Path.
3. Leverage the flexibility of genderless connectors and connect to your existing flow path.

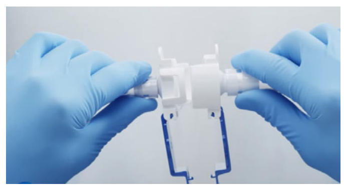
### 4.1 Using AseptiQuik® Genderless Connectors

Aseptically connect the TangenX® SIUS® Gamma Device to the TFF system using the genderless AseptiQuik® connectors from Colder Products Company. Refer to the following video to learn more on making aseptic connections:
https://products.cpcworldwide.com/en_US/ProductsCat/AseptiQuik-Sterile/AseptiQuikGConnector/

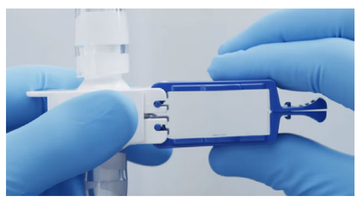
1. Flip the blue pull-tab cover down towards the hinge to expose the membrane that acts as an aseptic barrier.

***Note:*** *Do not remove the hinge or the membrane before the mating connection is made.*

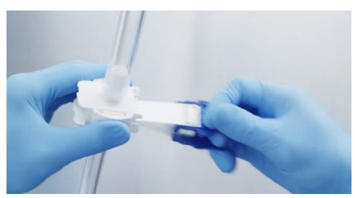
2. Align and push the two AseptiQuik® connectors together until you hear two clicks.



3. Press the CPC logo on the two blue pull-tab covers to interlock them.



4. Pull on the two blue interlocked tabs to remove the membrane from the connectors.



# 5. System set-up

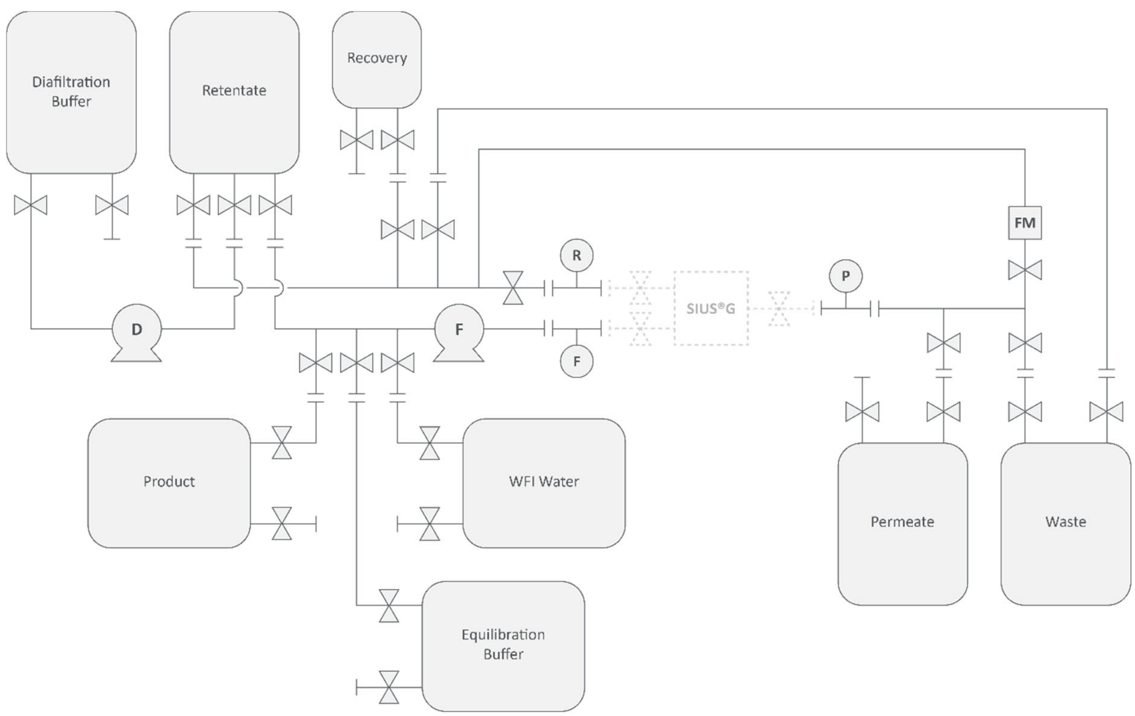
Record the following TangenX® SIUS® Gamma Device details when setting up a system:

- Part number
- Serial number
- Channel type
- MWCO
- Surface area
- Membrane type

## 5.1 TFF System flow path parts

The TangenX® SIUS® Gamma Device is often used in a closed TFF system for concentration and diafiltration operations. While the exact flow path used during these operations varies according to specific user requirements, a general example is outlined here. In this example, the flow path contains the components included in Table 3 and Figure 8.

**Figure 8. System configuration without TangenX® SIUS® Gamma Device**




Legend:

| | | |
|---|---|---|
| Vessels | Pumps | Genderless connectors |
| Pinch clamps | Pressure sensors | FM Flow meter |

For full list of symbols and descriptions see Table 1.



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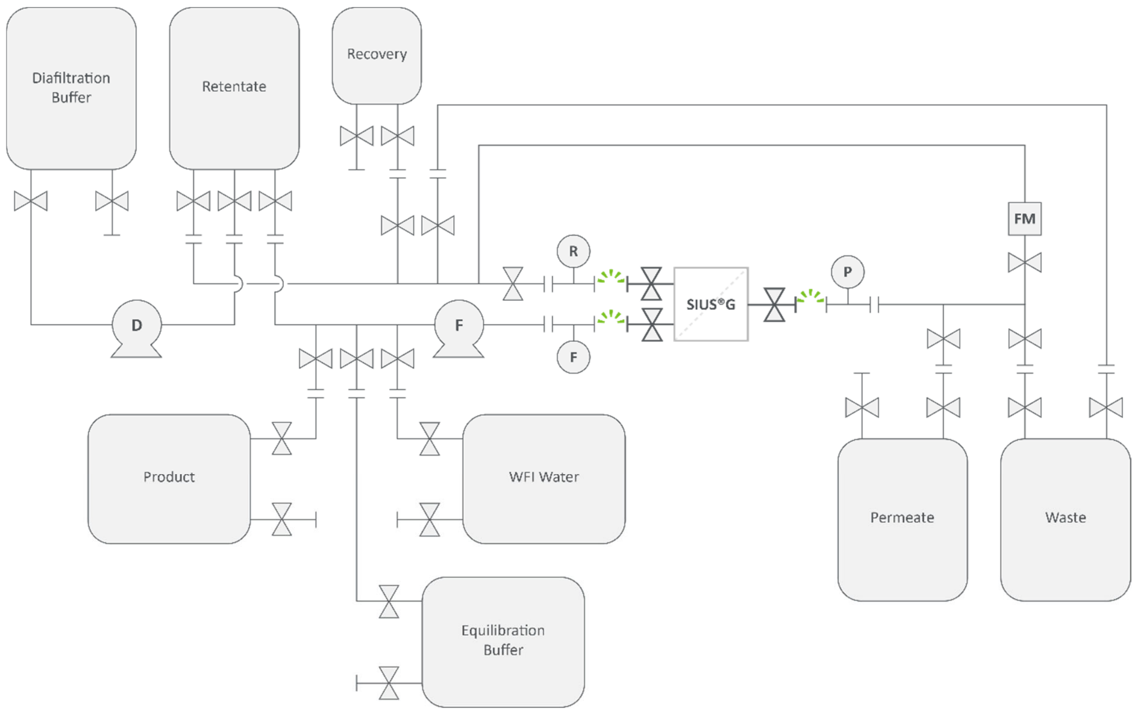
## 5.2 Connect the TangenX® SIUS® Gamma Device

Aseptically connect the TangenX® SIUS® Gamma Device to the extended tube set (Figure 9).

**Figure 9. System configuration with TangenX® SIUS® Gamma Device Installation**



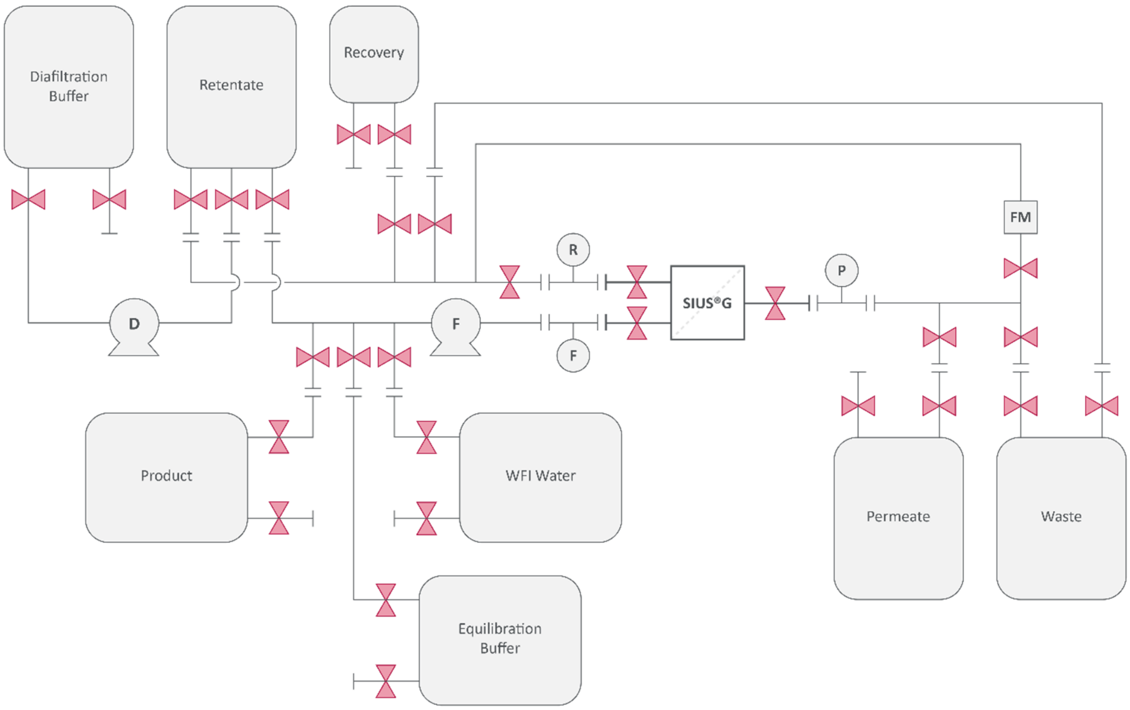

Legend:

- Vessels
- Pumps
- Configuration change
- Pinch clamps
- Pressure sensors
- On/Open flow path
- Genderless connectors
- FM Flow meter

For full list of symbols and descriptions see Table 1.

1. Connect feed inlet port on the TangenX® SIUS® Gamma Device to the feed clamp on the extended flow path with the feed pressure sensor.
2. Connect retentate outlet port on the TangenX® SIUS® Gamma Device to the retentate clamp on the extended tube set with the retentate pressure transducer.
3. Connect permeate outlet on the TangenX® SIUS® Gamma Device to the permeate clamp on the extended flow path with permeate pressure transducer.
4. Confirm that all the clamps are closed (Figure 10).

**Figure 10. System configuration with all pinch clamps closed**




Legend:

Vessels

Pumps

Flow meter

Pinch clamps

Pressure sensors

Off/Closed flow path

Genderless connectors

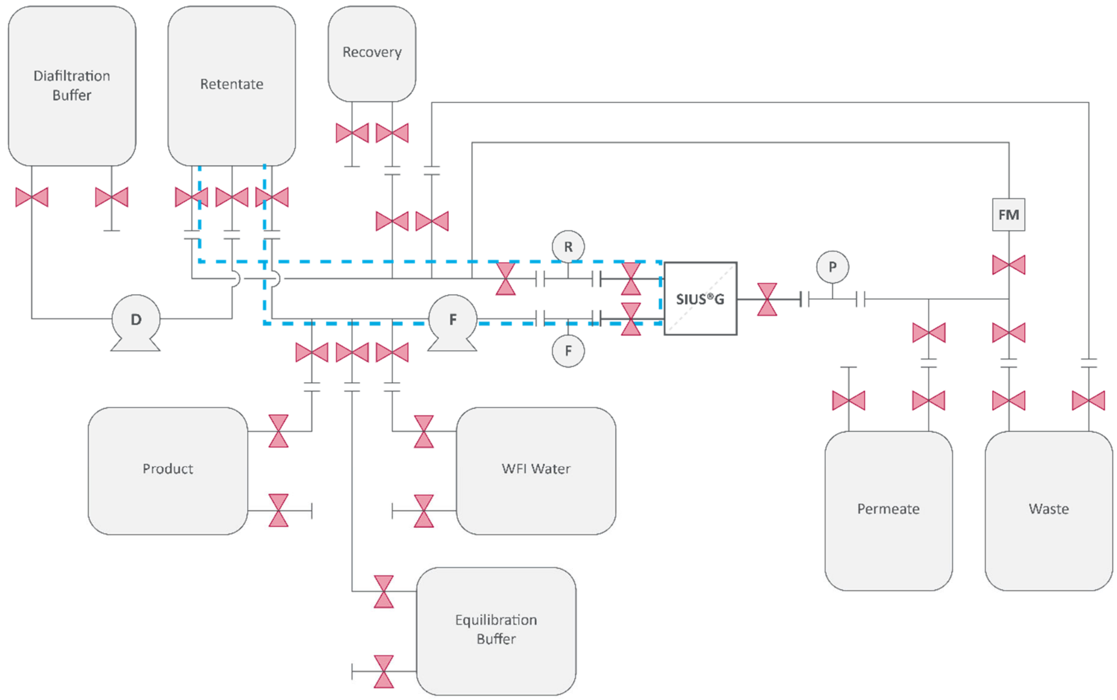
For full list of symbols and descriptions see Table 1.

## 6. Estimate hold-up volume

Estimate the hold-up volume of your system based on your engineering diagrams. Sum the volume of the tubing (Figure 11) and the hold-up volume of the TangenX® SIUS® Gamma Device (Table 4). The hold-up volume of the SIUS® Gamma Device includes the tubing that terminate with AseptiQuik® connectors. The appropriate flow path for hold-up volume estimation includes:

- From the outlet of the Retentate Vessel to the feed inlet port of the SIUS® Gamma Device
- From the SIUS® Gamma Device retentate outlet port to the inlet port of the Retentate Vessel

**Figure 11. System configuration with the hold-up volume estimation flow path**




Legend:

Vessels

Pumps

Off/Closed flow path

Pinch clamps

Pressure sensors

Hold-up volume

Genderless connectors

FM Flow meter

For a full list of symbols and descriptions see Table 1.

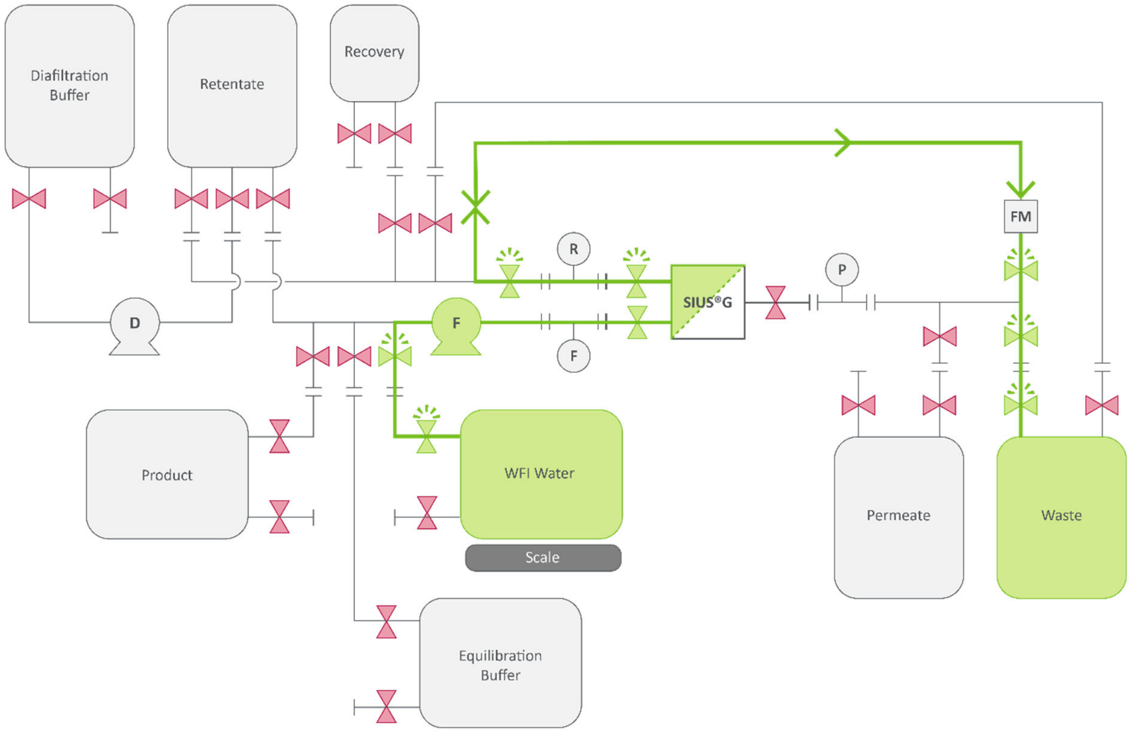
## 7. Device flush

Flushing the TangenX® SIUS® Gamma Device with water for injection (WFI) removes 0.2 M NaOH present in the shipped product.

### 7.1 Flush the SIUS® Gamma Device retentate flow path

1. Tare the scale. Place the Water Vessel on the scale and record the weight.
2. Route tubing through the feed pump.
3. Open pinch clamps to create a path from WFI Water Vessel to Waste Vessel (Figure 12).
    a. Open clamps between the WFI Water Vessel outlet port and SIUS® Gamma Device feed port.
    b. Open clamps between SIUS® Gamma Device retentate port and the Waste Vessel inlet port.
    c. Close all other clamps.

**Figure 12. System configuration for SIUS® Gamma Device retentate port WFI flush**




Legend:

- Vessels
- Pumps
- Configuration change
- Pinch clamps
- Pressure sensors
- On/Open flow path
- Genderless connectors
- FM Flow meter
- Off/Closed flow path
- Scale

For full list of symbols and descriptions see Table 1.

4. Start the pump with a CF rate based on Table 6 to begin flushing water through the SIUS® Gamma Device and into the Waste Vessel.

***Note:*** *Do not exceed feed pressure of 30 psig.*

5. Stop the pump after 5 L of water per $m^2$ surface area has left the WFI Water Vessel (Table 7).
6. If continuing directly to flushing permeate flow path go to Section 7.2. If pausing for an extended period of time, close all pinch valves.

**Table 6. Recommended crossflow (CF) rates**

| | Cross flow range | Low Crossflow | Medium Crossflow | High Crossflow | ΔP |
|---|---|---|---|---|---|
| LP Screen | 4 - 8 L/min/$m^2$ | 4 L/min/$m^2$ | 6 L/min/$m^2$ | 8 L/min/$m^2$ | 10 psig (0.7 bar)* |
| EP Screen | 6 - 12 L/min/$m^2$ | 6 L/min/$m^2$ | 9 L/min/$m^2$ | 12 L/min/$m^2$ | 5 psig (0.35 bar)* |
| *Typical ΔP measured with water and permeate closed | | | | | |

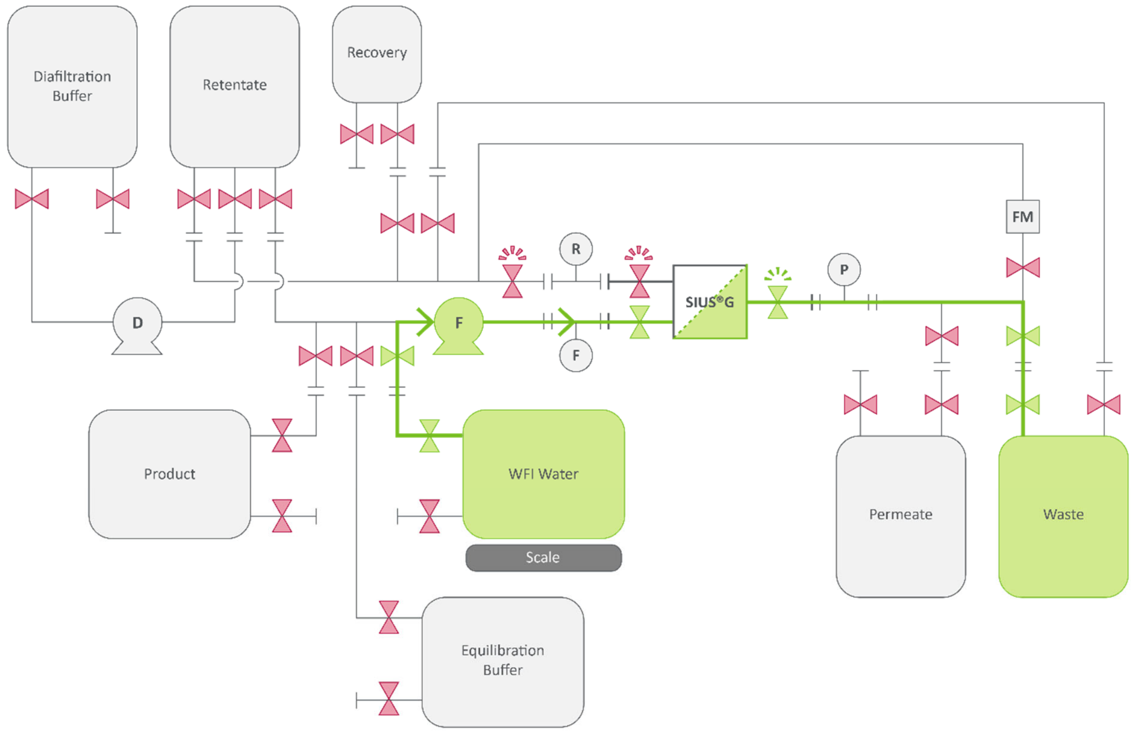
**Table 7. Normalized SIUS® Gamma Device flush volumes**

| TFF Filter surface area | Retentate to waste | Permeate to waste |
|---|---|---|
| 0.1 $m^2$ | 0.5 L | 1.0 L |
| 0.5 $m^2$ | 2.5 L | 5.0 L |
| 1.5 $m^2$ | 7.5 L | 15.0 L |
| 2.5 $m^2$ | 12.5 L | 25.0 L |

## 7.2 Flush the SIUS® Gamma Device permeate flow path

1. Tare the scale. Place the Water Vessel on the scale and record the weight.
2. Open pinch clamps to create a path from with WFI Water Vessel to Waste Vessel (Figure 13).
    a. Open clamps between the WFI Water Vessel outlet port and the SIUS® Gamma Device feed port.
    b. Open clamps between the filtration device permeate port to the Waste Vessel inlet port.
    c. Close all other clamps.

**Figure 13. System configuration for SIUS® Gamma Device permeate port WFI flush**




Legend:

| | | |
|---|---|---|
| Vessels | Pumps | Configuration change |
| Pinch clamps | Pressure sensors | On/Open flow path |
| Genderless connectors | Flow meter | Off/Closed flow path |
| Scale | | |

For full list of symbols and descriptions see Table 1.

3. Start the pump with a low CF rate (approximately 10% of recommended CF rate from (Table 6) to flush water through the permeate path of the SIUS® Gamma Device and into the Waste Vessel.
4. Increase pump CF rate slowly until the transmembrane pressure reaches 10 - 15 psig
5. Stop the pump after 10 L of water per $m^2$ surface area has left the WFI Water Vessel (Table 7).
6. If continuing directly to air integrity testing, go to Table 8. If pausing for an extended period of time, close all pinch valves.

## 8. Air integrity test

The air integrity test non-destructively and quantitatively verifies the integrity of the SIUS® Gamma Device and confirms performance to the air integrity specification. Each SIUS® Gamma Device undergoes strict release testing, including an air integrity test, to verify the integrity of the device up to the point of shipment. However, due to uncertainties associated with the shipping processes, this guarantee does not extend to point of receipt or time of use. Therefore, Repligen recommends executing an integrity test immediately prior to running your process. Integrity test specifications are shown in Table 8. A detailed procedure (AN1002) for the measurement of air integrity can be obtained at repligen.com/resources or by contacting Repligen support.

***Note:*** *An air integrity test should be performed after device flushing as it measures air diffusion through a wetted membrane.*

***Note:*** *Re-torque the nuts (Section 3.1 Torque sequence) on the device holder after measuring the hold-up volume and flushing the retentate and permeate paths.*

**Table 8. SIUS® Gamma Device air integrity specifications**

| Membrane type (1) (ProStream and HyStream) | Specification(2) |
|---|---|
| Ultrafiltration ≤ 5 kD | ≤ 323 $ccm/m^2$ at 1 bar or ≤ 30 $ccm/ft^2$ at 15 psig |
| Ultrafiltration 10 kD - 100 kD | ≤ 323 $ccm/m^2$ at 0.5 bar or ≤ 30 $ccm/ft^2$ at 7.3 psig |
| Microfiltration ≥ 0.1 µm | ≤ 323 $ccm/m^2$ at 0.2 bar or ≤ 30 $ccm/ft^2$ at 3 psig |

(1) Applies for ProStream and HyStream.
(2) 1 ccm = 1 mL/min.

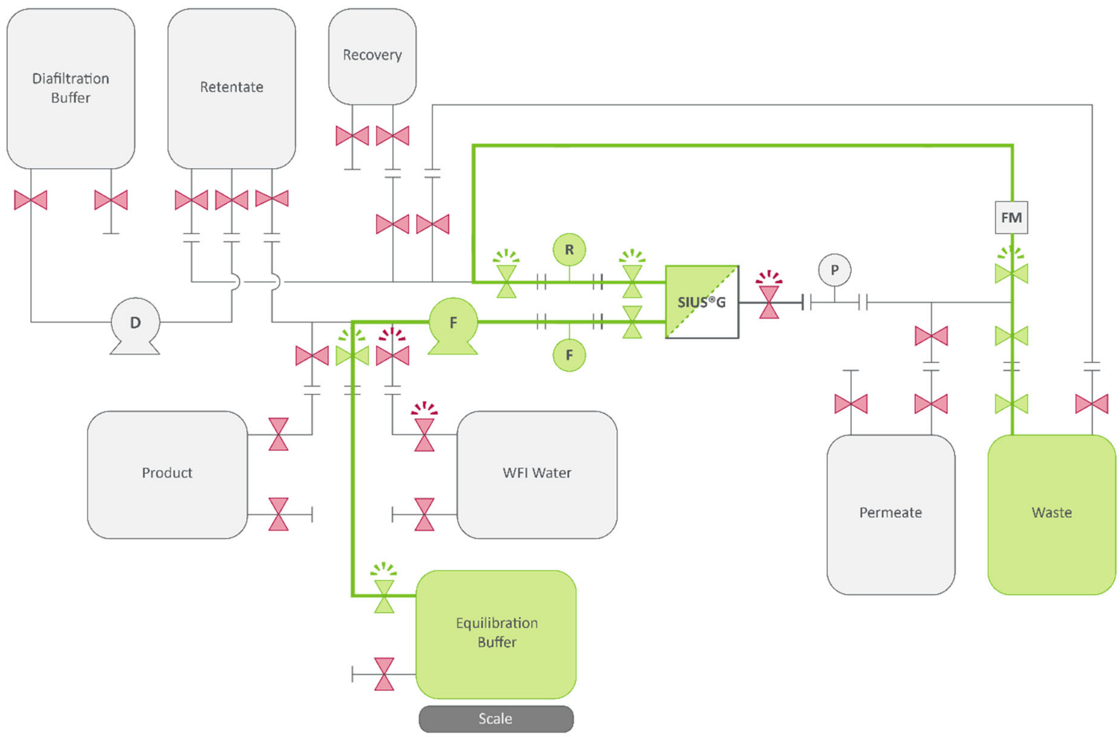
## 9. Buffer equilibration

Equilibrating the TangenX® SIUS® Gamma Device with buffer prepares the system for introduction of product.

### 9.1 Equilibrate the SIUS® Gamma Device retentate flow path

1. Tare the scale. Place the Equilibration Buffer Vessel on the scale and record the weight.
2. Open pinch clamps to create a path from the Equilibration Buffer Vessel to the Waste Vessel (Figure 14).
    - Open clamps between the Equilibration Buffer Vessel outlet port and the filtration device feed port.
    - Open clamps from the filtration device retentate port to the Waste Vessel port.
    - Close all other clamps.

**Figure 14. System configuration SIUS® Gamma Device retentate outlet port buffer equilibration**




Legend:

- Vessels
- Pumps
- Configuration change
- Pinch clamps
- Pressure sensors
- On/Open flow path
- Genderless connectors
- FM Flow meter
- Off/Closed flow path
- Scale

For full list of symbols and descriptions see Table 1.

3. Start the pump with a CF rate based on Table 6 to begin flushing equilibration buffer through the filtration device and into the Waste Vessel.

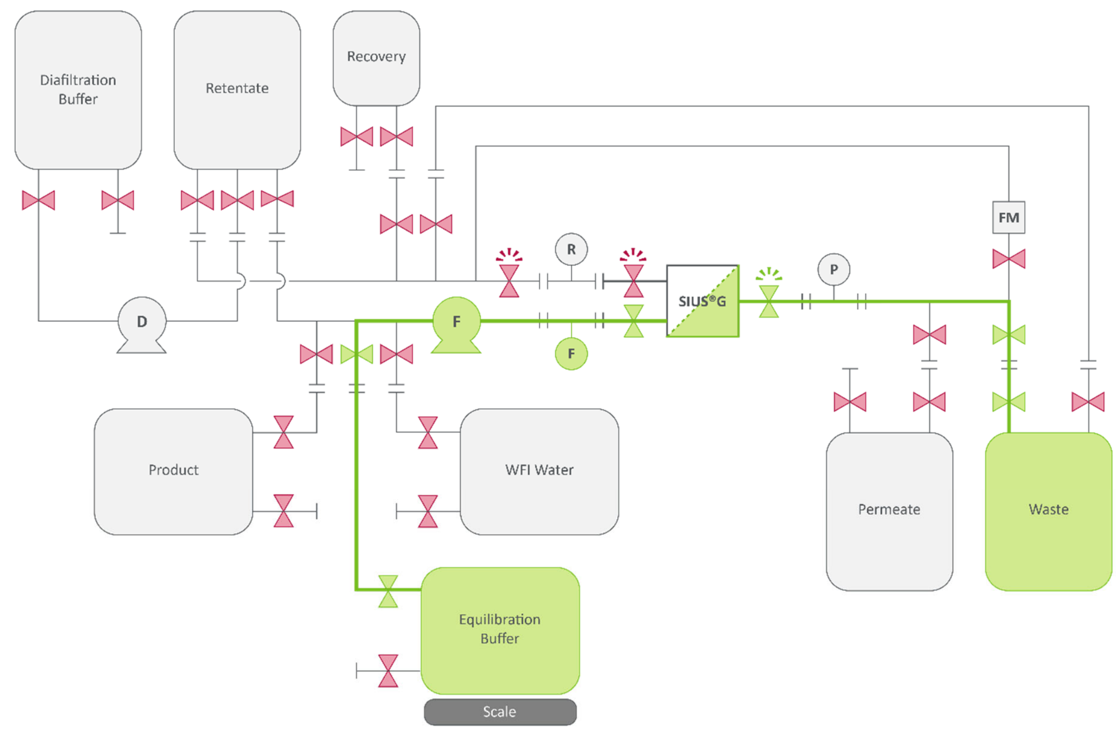
***Note:*** *Do not exceed feed pressure of 30 psig.*

4. Stop the pump after 5 L of equilibration buffer per $m^2$ surface area has left the Equilibration Buffer Vessel (Table 7).
5. If continuing directly to flushing permeate flow path with equilibration buffer go to Section 9.2. If pausing for an extended period of time, close all pinch valves.

## 9.2 Equilibrate the SIUS® Gamma Device permeate flow path

1. Tare the scale. Place the Equilibration Buffer Vessel on the scale and record the weight.
2. Open pinch clamps to create a path from with Equilibration Buffer Vessel to Waste Vessel (Figure 15).
    a. Open clamps between the Equilibration Buffer Vessel port and the filtration device feed inlet port.
    b. Open clamps between the filtration device permeate outlet port and the Waste Vessel inlet port.
    c. Close all other clamps.

**Figure 15. System configuration for filtration device permeate outlet port buffer equilibration**




Legend:

| | |
|---|---|
| Vessels | Pumps | Configuration change |
| Pinch clamps | Pressure sensors | On/Open flow path |
| Genderless connectors | Flow meter | Off/Closed flow path |
| Scale | | |

For full list of symbols and descriptions see Table 1.

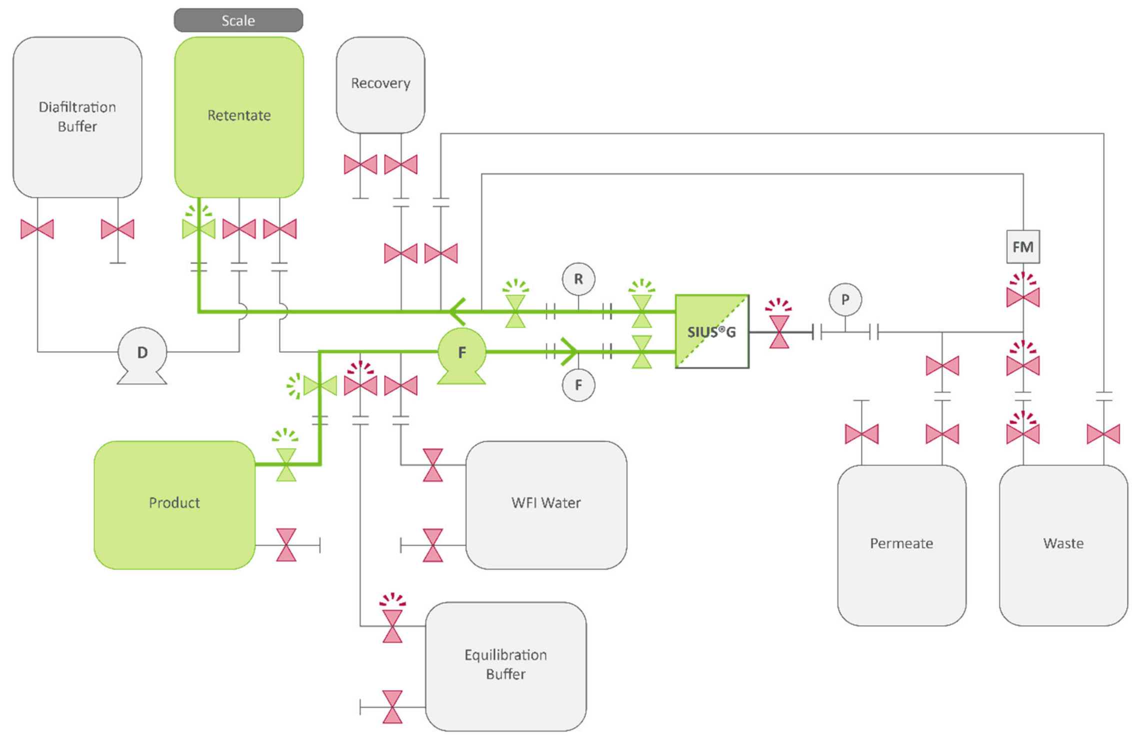
3. Start the pump with a low CF rate (approximately 10% of recommended CF rate from Table 6 to flush equilibration buffer through the permeate path of the filtration device and into the Waste Vessel.
4. Increase pump CF rate slowly until the transmembrane pressure reaches 10 - 15 psig
5. Stop the pump after 10 L of equilibration buffer per $m^2$ surface area has left the Buffer Equilibration Buffer Vessel (Table 7).
6. If continuing directly to product introduction, go to Section 10. If pausing for an extended period of time, close all pinch valves.

## 10. Product introduction

You are now ready to introduce product to your system for optimization (Section 11), concentration (Section 12), or diafiltration (Section 13) of your biological product.

1. Tare the scale. Place the Retentate Vessel on the scale and record the weight.
2. Open pinch clamps to create a path from the Product Vessel, through the filtration device and ending at the Retentate Vessel (Figure 16).
    a. Open clamps from the Product Vessel outlet port to the filtration device feed port.
    b. Open clamps from the filtration device retentate port to the Retentate Vessel inlet port.
    c. Close all other clamps.

**Figure 16. System configuration for product introduction**




Legend:

Vessels

Pumps

Configuration change

Pinch clamps

Pressure sensors

On/Open flow path

Genderless connectors

FM Flow meter

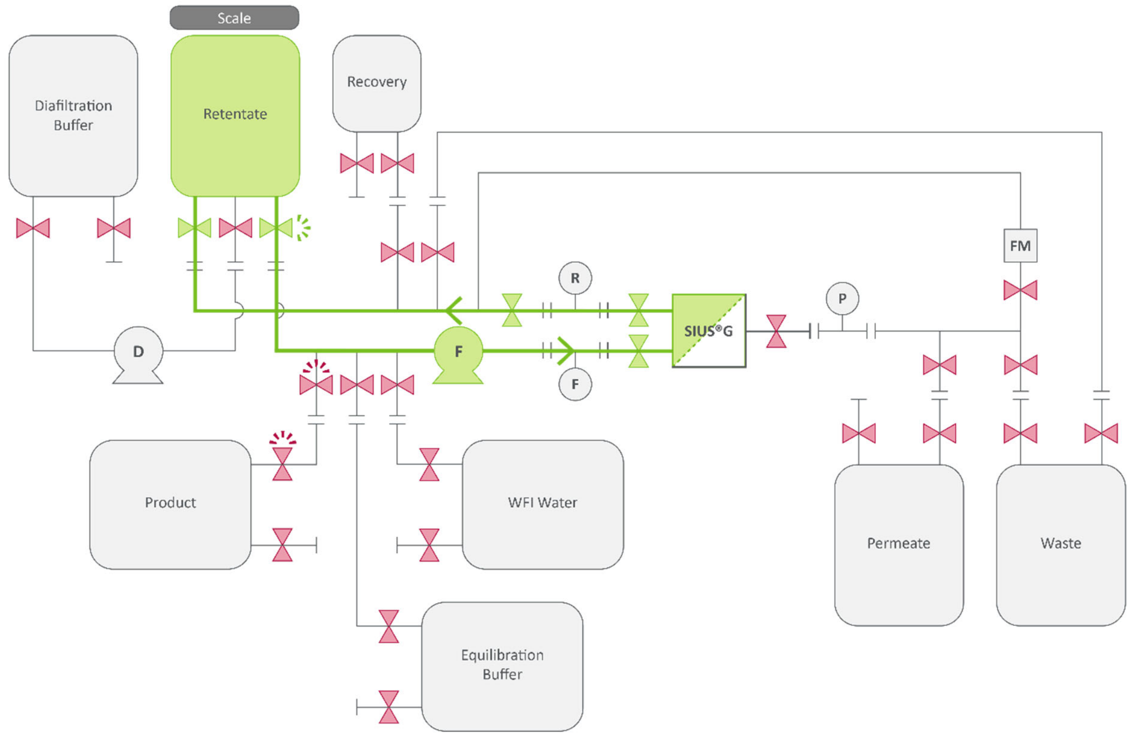
Off/Closed flow path

Scale

For full list of symbols and descriptions see Table 1.

3. Start the pump with CF rate based on Table 6.
4. Transfer all material from the Product Vessel to the Retentate Vessel.
5. Record Retentate Vessel weight.
6. Stop the feed pump to discontinue product transfer.
7. Re-torque the device holder.
8. Open pinch clamps to create a path from the Retentate Vessel, through the filtration device and returning to the Retentate Vessel (Figure 17).
    a. Close clamps between the Product Vessel and the filtration device feed inlet port.
    b. Open clamps between the Retentate Vessel outlet port and the filtration device feed inlet port.
    c. Open clamps between the Retentate Vessel inlet vessel and the filtration device outlet port.

**Figure 17. System configuration for re-circulation mode**




Legend:

Vessels | Pumps | Configuration change

Pinch clamps | Pressure sensors | On/Open flow path

Genderless connectors | FM Flow meter | Off/Closed flow path

Scale

For full list of symbols and descriptions see Table 1.

9. Start the pump with maximum CF rate based on Table 6.
10. Continue re-circulation for 10 minutes.
11. If continuing directly to optimization, concentration or diafiltration go to Section 11, 12 or 13, respectively.

## 11. Optimization

***Note:*** *This example of a TangenX® SIUS® Gamma Device is in a closed system. Optimization processes require a flow meter on the device permeate valve.*

During the development phase, you may need to optimize your process for CF rate and TMP. Optimization is performed at a steady-state so that all product is retained in the system. Sections 2 - 10 should have been completed as described in earlier sections before you start the optimization process.

### 11.1 Optimization through flux excursion

1. Open the pinch clamps to create a second loop from the filtration device permeate outlet port back to the Retentate Vessel (Figure 18).



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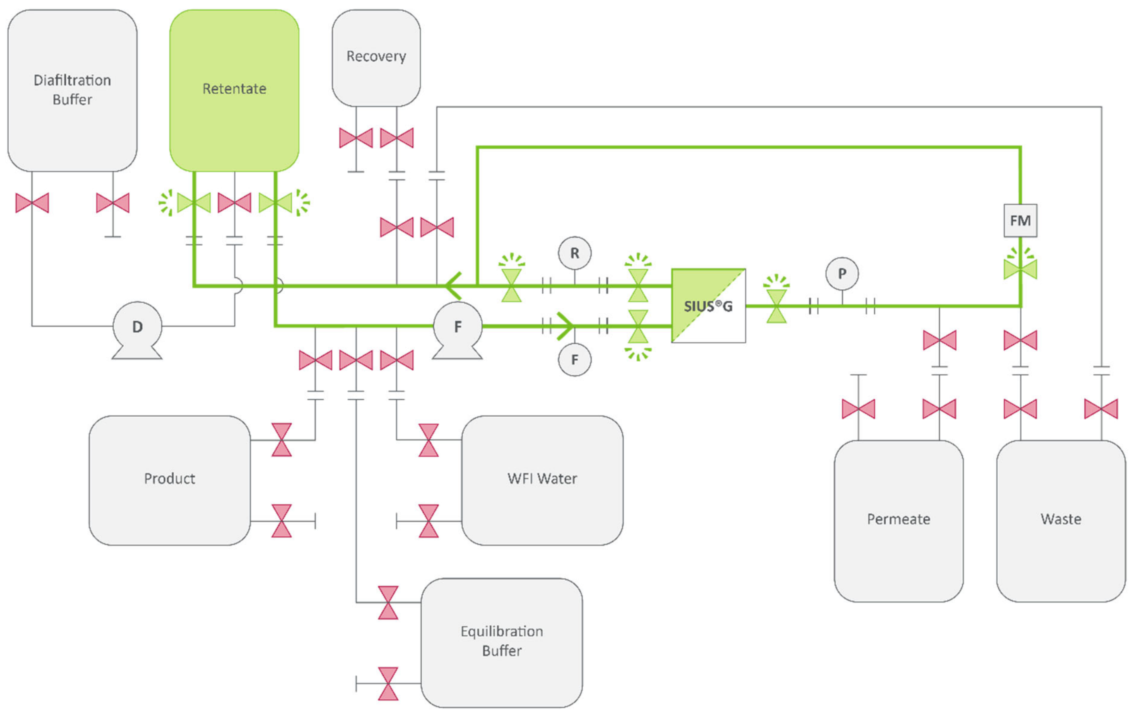
a. Open clamps between the Retentate Vessel outlet port and filtration device inlet port.
b. Open clamps between the Retentate Vessel inlet port and the filtration device retentate outlet port.
c. Close all other clamps.

**Figure 18. System configuration for optimization studies**




Legend:

Vessels | Pumps | Configuration change
Pinch clamps | Pressure sensors | On/Open flow path
Genderless connectors | FM Flow meter | Off/Closed flow path
Scale

For full list of symbols and descriptions see Table 1.

2. Set the CF at or close to the maximum value (see Table 6).
3. Adjust the TMP using the retentate clamp to the lowest desired pressure.
4. Allow the system to stabilize for 5 minutes.
5. Record the feed, retentate, and permeate pressures, and TMP, the feed flow rate (by weight), and the permeate flow rate (using the flow meter).
6. Calculate the flow from the retentate using the following equation:

   Retentate flow rate = Feed flow rate - Permeate flow rate.

7. Incrementally, increase the TMP by pinching the retentate pinch clamp. It is important to maintain a constant CF at the new TMP; adjust the flow rate of the feed pump to achieve or maintain the desired CF rate.

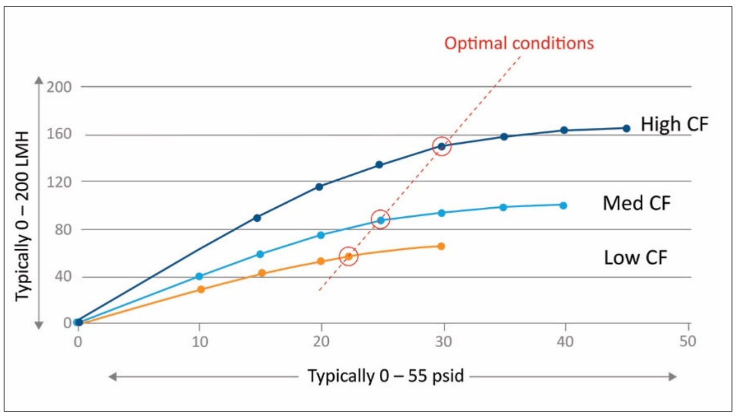
***Note:*** *Typical experiments use 3 - 5 increments to a TMP when flux response plateaus. Do not exceed the maximum operating pressure.*

8. Reduce the CF rate by 10 - 20%.
9. Repeat steps 5 - 10 until data for 3 CF rates have been collected.
    a. If using the current set-up for additional processing (concentration or diafiltration), put the system into recirculation mode as described in Figure 17.
        - Open retentate clamp completely
        - Close permeate clamp completely
        - Allow product to re-circulate with low CF rate
    b. If processing is complete, proceed to Product Recovery (Section 14).

## 11.2 Process optimization data plotting and analysis

1. Graph the TMP vs the permeate flux for each CF rate (Figure 19).
2. Perform a non-linear curve fit to each set of CF rate data.
3. Identify optimal TMP and CF based on plateau of plot.

**Figure 19. Model of a graph plotting TMP against LMH at 3 different CF rates**



## 12. Concentration

Prior to concentration, Sections 2 - 10 steps should be performed and the system should be in re-circulation mode.

*Concentration Factor (CF) = Retentate volume Initial/Retentate volume final*

1. Continue running until the required concentration factor is achieved. The concentration factor formula is:

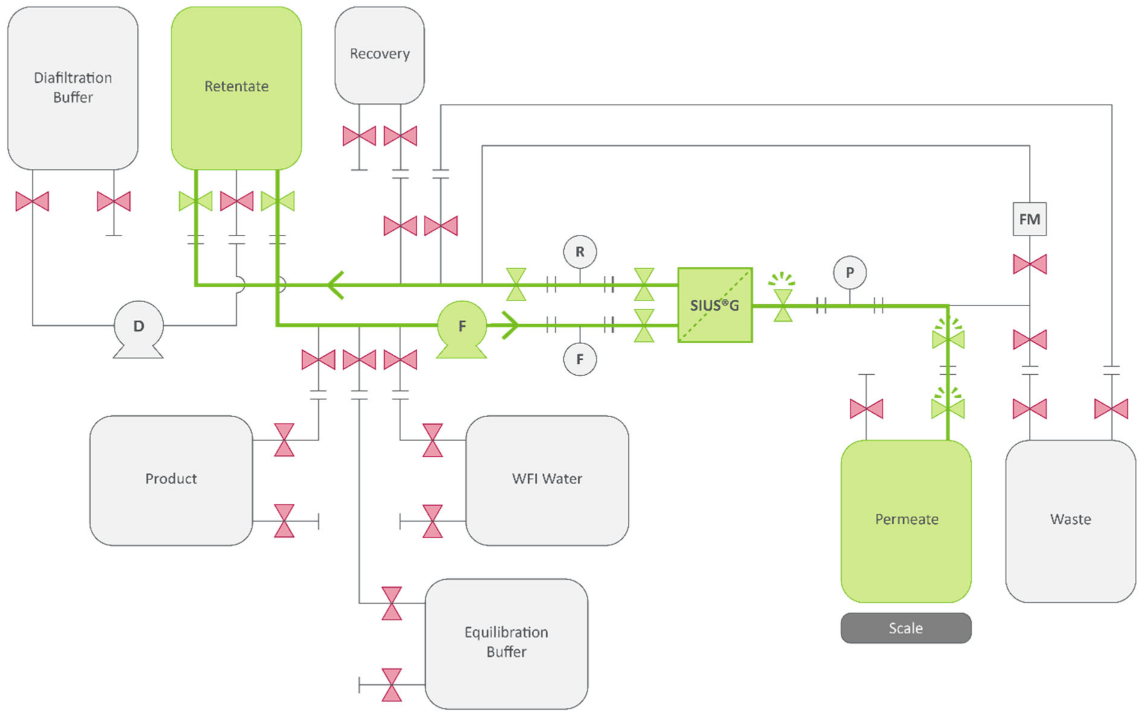
$$CF\ (X)_{Batch} = \frac{Feed_{starting\ volume}}{Concentrate_{left\ in\ reservoir}} = \frac{Feed_{starting\ volume}}{Feed_{starting\ volume} - Permeate_{Total\ removed}}$$

**Example:** If there was originally 2000 ml of material in the retentate vessel and the goal is to concentrate the sample by 10X, run the process until there is 200 ml of material left. Determine this by collecting 1800 ml of solution in the retentate vessel plus the hold-up volume plus the hold-up buffer volume in the retentate tubing.

To compensate for dilution that occurs during product recovery (Section 14), you may elect to over-concentrate the material by a proportional amount during the concentration step.

1. Place the Permeate Vessel on the scale and tare the scale.
2. Open the pinch clamps to create a loop between the Retentate Vessel and the filtration device and a path from the filtration device to the Permeate Vessel (Figure 20).
    a. Open clamps between the Retentate Vessel outlet port and the filtration device feed inlet port.
    b. Open clamps between the filtration device retentate port and the Retentate Vessel inlet port.
    c. Open clamps between the filtration device permeate port and the Permeate Vessel inlet port.
    d. Close all other clamps.

**Figure 20. System configuration for product concentration**




Legend:

- Vessels
- Pumps
- Configuration change
- Pinch clamps
- Pressure sensors
- On/Open flow path
- Genderless connectors
- FM Flow meter
- Off/Closed flow path
- Scale

For full list of symbols and descriptions see Table 1.

3. Adjust or confirm feed pump to the desired CF rate determined during optimization.
4. Set the TMP to the pressure determined during optimization by partially closing the retentate port pinch clamp. The CF rate will drop. Adjust the feed pump accordingly to maintain a constant TMP.
5. Record the increase of material in the Permeate Vessel and visually inspect the Retentate Vessel until the desired concentration is achieved.
6. If you plan to continue with either diafiltration or product recovery, open the retentate clamp completely and close the permeate clamp completely. Otherwise, turn off the pump and close all the clamps.

## 13. Diafiltration

During constant-volume diafiltration, buffer is added to the Retentate Vessel as material is circulated from the Retentate Vessel through the filter and back to the Retentate Vessel. Product is retained on the retentate side of the membrane while salts and buffers pass through the membrane to the permeate. A dedicated peristaltic pump introduces diafiltration buffer to the system. Diafiltration is typically performed after concentrating the retentate.

### 13.1 Prepare the TFF System for diafiltration

Calculate target retentate/permeate volume.

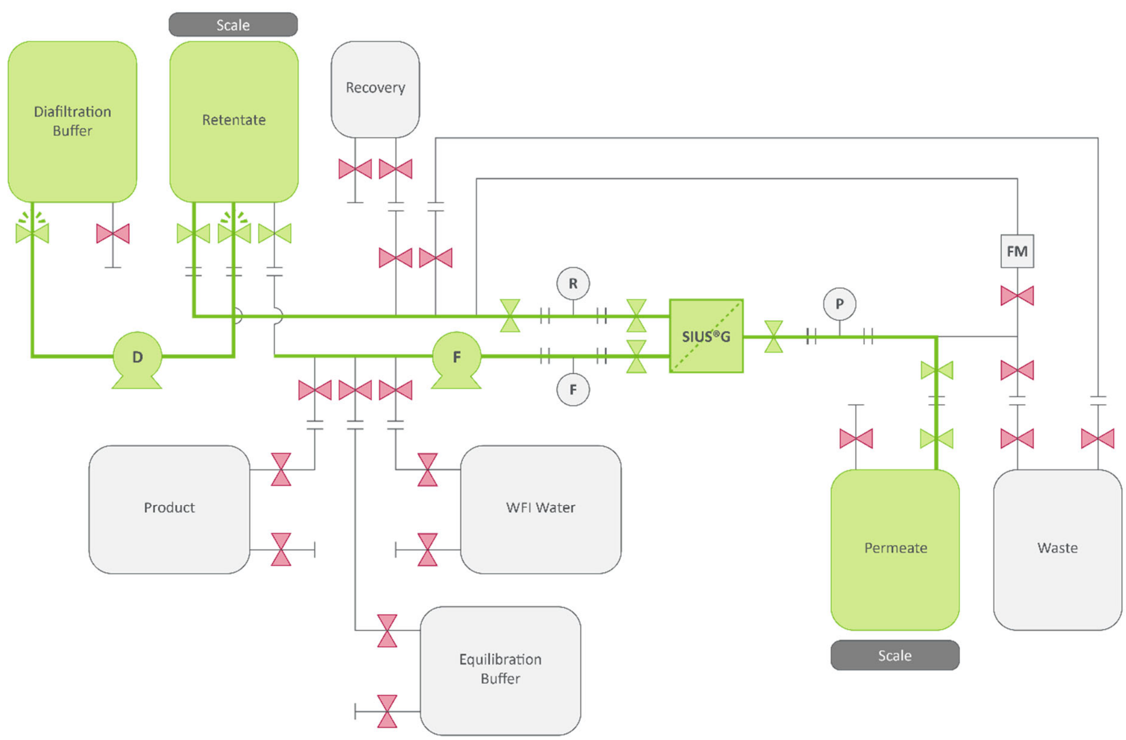
**Dvol = Ret volume (see Figure 22 for % removal)**

**Perm vol = #DVs x Ret vol**

1. Place Permeate Vessel on a scale. Tare the scale.
2. Start feed pump at desired CF rate determined during optimization and/or used during product concentration.
3. Open pinch clamps to create a loop between the Retentate Vessel and the filtration device, a path from the Diafiltration Buffer Vessel to the Retentate Vessel and a path from the filtration device permeate port to the Permeate Vessel (Figure 21).
   a. Open clamps between the Retentate Vessel outlet port and the filtration device feed inlet port
   b. Open clamps between the filtration device retentate port and the Retentate Vessel inlet port.
   c. Open clamps between the Diafiltration Buffer outlet port and the Retentate vessel inlet port
   d. Open clamps between the filtration device permeate port and the Permeate Vessel.
   e. Close all other clamps.

***Note:*** *Once the permeate valve is opened, steps 5 - 8 become time sensitive.*

**Figure 21. System configuration for diafiltration**



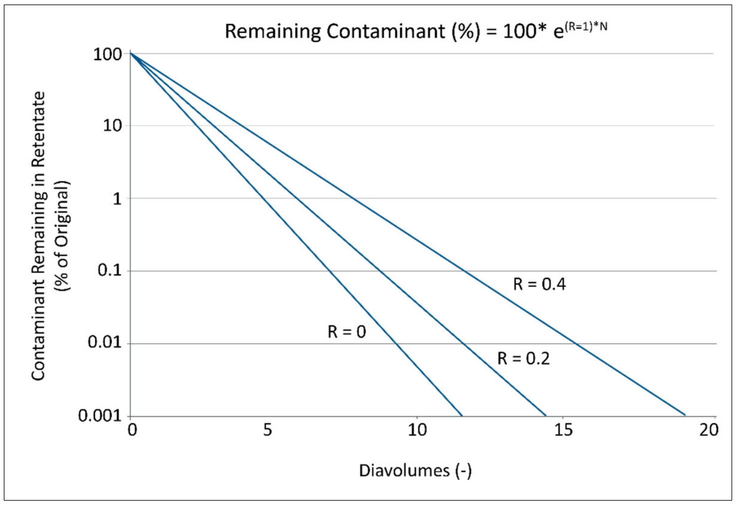

Legend:

- Vessels
- Pumps
- Configuration change
- Pinch clamps
- Pressure sensors
- On/Open flow path
- Genderless connectors
- FM Flow meter
- Off/Closed flow path
- Scale

For full list of symbols and descriptions see Table 1.

4. Turn on the diafiltration pump. Adjust the flow rate to approximate the current permeate flow rate.
5. Adjust the retentate pinch clamp to obtain the desired TMP.
6. Adjust the feed pump flow rate to obtain the desired CF rate.
7. Adjust diafiltration pump rate so that diafiltration buffer transfer flow rate approximates the permeate flow rate. The retentate volume (or mass) should remain constant.
8. Continue the diafiltration process until the number of desired diavolumes has been achieved (Figure 22). One diafiltration volume is defined by the sum of the Retentate Vessel volume and the loop dead volume.

**Figure 22. Relationship between remaining contaminant and diavolumes**




## 13.2 Stop the diafiltration process

1. Open the retentate clamp to reduce the backpressure.
2. Simultaneously, close the permeate outlet and turn off the diafiltration pump (Figure 23).



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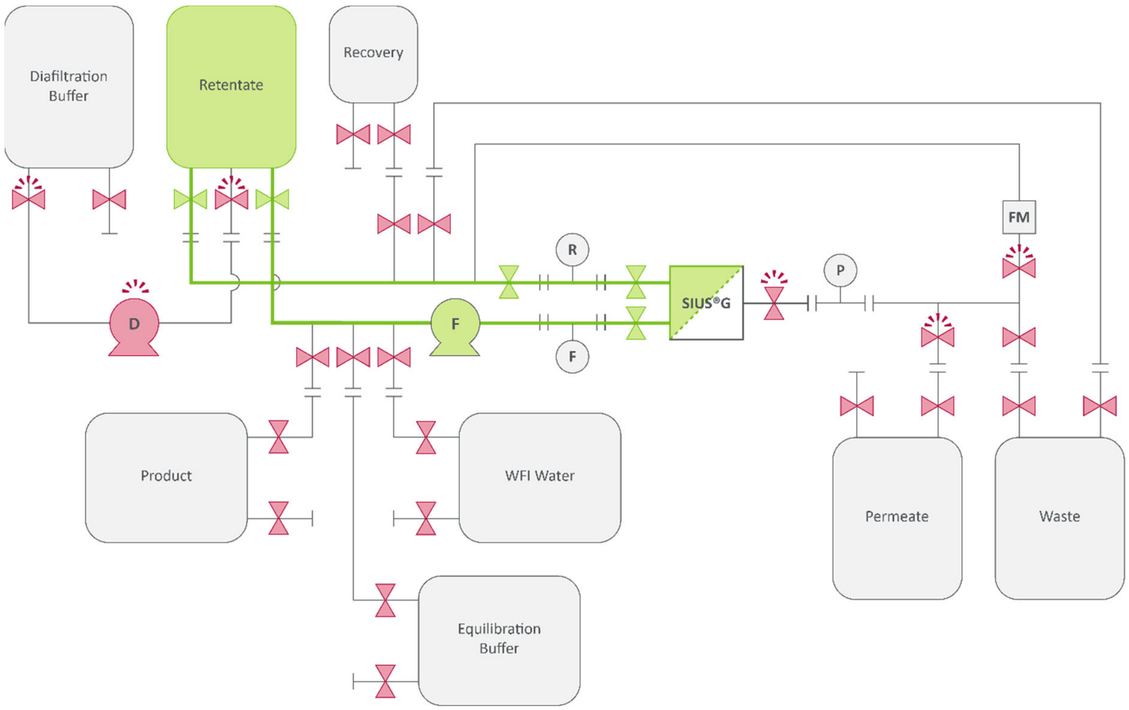
**Figure 23. System configuration for stopping diafiltration**




Legend:

Vessels

Pumps

Configuration change

Pinch clamps

Pressure sensors

On/Open flow path

Genderless connectors

FM Flow meter

Off/Closed flow path

Scale

For full list of symbols and descriptions see Table 1.

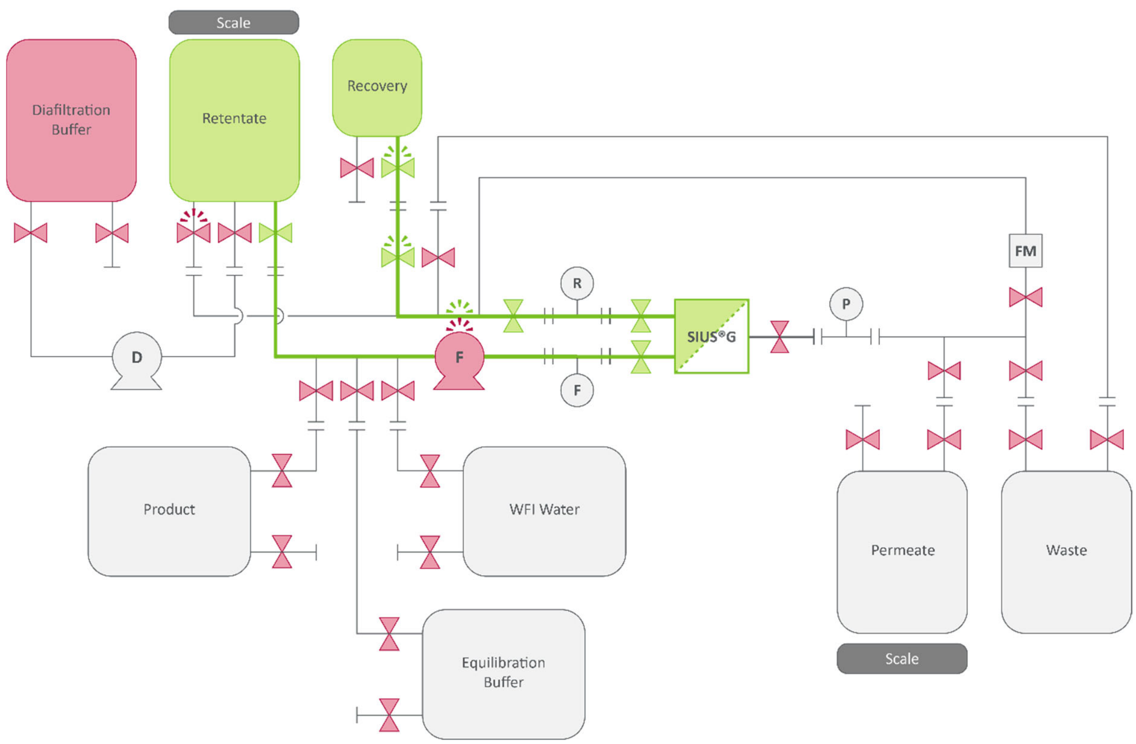
3. Close clamps between Diafiltration Buffer Vessel outlet port and Retentate Vessel inlet port.
4. Decrease feed pump CF rate to return the system to re-circulation mode.
5. Proceed with your next process step:
    a. If continuing with product recovery, proceed to Section 14.
    b. If continuing with additional concentration, proceed to Section 12.

## 14. Product recovery

Product recovery transfers material from the tubing and filtration device to the Recovery Vessel, maximizing the amount of product in the Retentate Vessel. One hold-up volume of diafiltration buffer should be used to displace product from the tubing and filtration device into the Retentate Vessel. This process will lead to dilution of the product in the Retentate Vessel. To compensate for the dilution, you may elect to over-concentrate the material by a proportional amount during the concentration step.

1. Re-circulate the material between the Retentate Vessel and filtration device for 5 - 10 minutes (Figure 20).
2. Open a path between Retentate Vessel and Recovery Vessel and execute product recovery (Figure 24).
    a. Stop the feed pump.
    b. Open a path from the filtration device retentate port to the Recovery Vessel.
    c. Close pinch clamps at the Retentate Vessel inlet port.
    d. Turn feed pump to 10% - 25% of the CF rate used during concentration and diafiltration.
    e. Stop the feed pump when the Retentate Vessel is visibly empty.

**Figure 24. System configuration for product recovery step 2**




Legend:

Vessels | Pumps | Configuration change

Pinch clamps | Pressure sensors | On/Open flow path

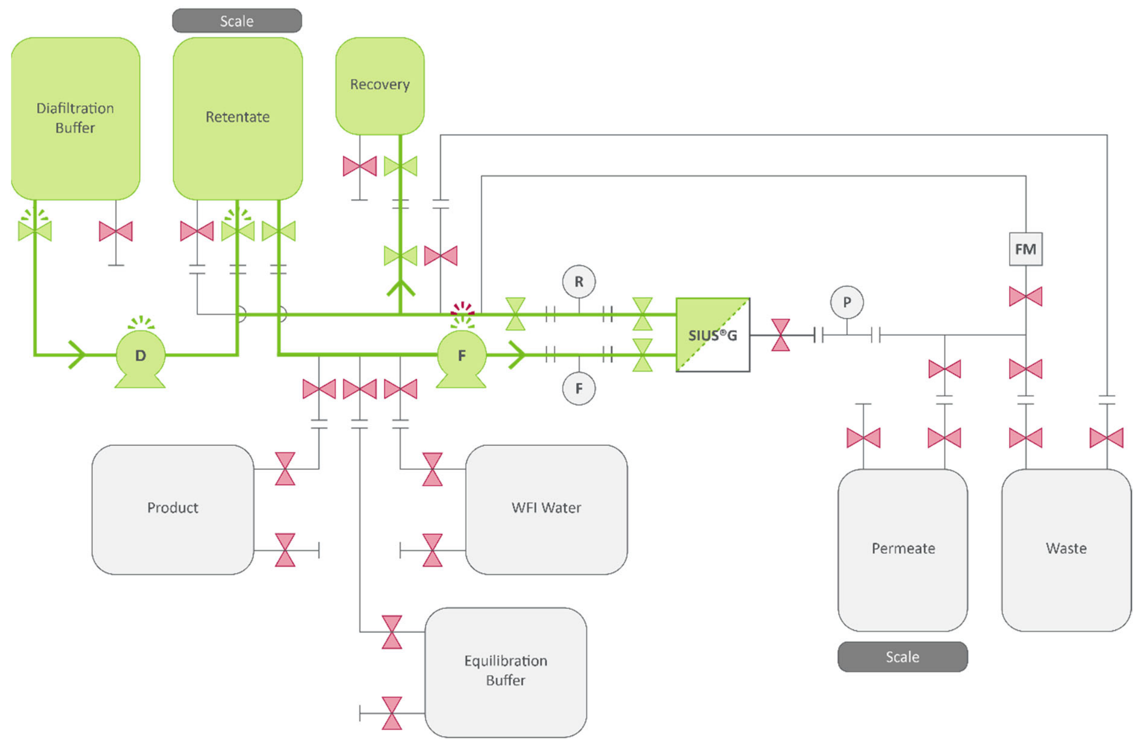
Genderless connectors | FM Flow meter | Off/Closed flow path

Scale

For full list of symbols and descriptions see Table 1.

3. Open a path and execute a buffer purge (Figure 25).
    a. Open a path from the Diafiltration Buffer Vessel outlet port to the Retentate Vessel inlet port.
    b. Turn on diafiltration pump.
    c. Add a volume of diafiltration buffer to the Retentate Vessel that equals the hold-up volume of the filtration device.
    d. Turn off the diafiltration pump.
    e. Turn feed pump to 10% - 25% of the CF rate used during concentration and diafiltration.
    f. Transfer all liquid from the Retentate Vessel and the hold-up volume to the Recovery Vessel.
    g. Stop the feed pump.
4. Close all pinch clamps.

**Figure 25. System configuration for product recovery step 3**




Legend:

| | | |
|---|---|---|
| Vessels | Pumps | Configuration change |
| Pinch clamps | Pressure sensors | On/Open flow path |
| Genderless connectors | FM Flow meter | Off/Closed flow path |
| Scale | | |

For full list of symbols and descriptions see Table 1.

## 15. Product removal

After the Recovery Vessel has been sealed with a pinch clamp, either cut the tubing above the Recovery Vessel to remove it from the system – OR – use a tube welder to seal the connections before severing the tubing and removing the Recovery Vessel from the system.

## 16. Inactivation and disposal

The system is still closed if the product outlet clamp is closed prior to removing the Recovery Vessel to the system.

1. Close the remaining valves in the system.
2. Use pinch clamps to close the permeate, feed, and retentate ports to the TangenX® SIUS® Gamma Device.
3. Remove the feed tubing from the pump.
4. Remove the device from the holder.
    a. Use the torque wrench to remove the nuts from the holder.
    b. Remove the spacers and the top plate.
    c. Take the device out of the holder.
    d. Reassemble the device holder and set aside. This has not contacted any fluid and can be used later.
5. Dispose of the remaining portion of the system according to your company's safety guidelines:
    - Transducers
    - Retentate Vessel and tubing
    - Diafiltration Buffer Vessel
    - TangenX® SIUS® Gamma Device
    - Permeate Vessel
    - Buffer Vessel
    - Water Vessel

## 17. Index