TangenX® SIUS® TFF Cassettes

Regulatory Support File

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Abbreviations

µg	microgram
µm	micron
µS	microSiemens
BPOG	BioPhorum Operations Group
BSA	bovine serum albumin
BSE	bovine spongiform encephalopathy
C	Celcius
ccm	cubic centimeter per minute
CFU	colony forming units
cGMP	current Good Manufacturing Practice
CIP	clean-in-place
cm	centimeter
cm^2	centimeter squared
CMC	Chemistry, Manufacturing & Controls
DI	deionized
DMAC	dimethyl acetamide
EDTA	ethylenediaminetetraacetic acid
EP	extra low pressure
EPDM	ethylene propylene diene monomer
ERP	enterprise resource planning
EtOH	ethanol
EU	endotoxin units
FDA	Food and Drug Administration
ft^2	feet squared (square feet)
FTIR	Fourier-transform infrared spectroscopy
g	gram
GC/MS	gas chromatography/mass spectrometry
GMP	Good Manufacturing Practice
H_3PO_4	phosphoric acid
HDPE	high density polyethelene
HPLC	high-performance liquid chromatography
HPLC/DAD	high-performance liquid chromatography/photodiode-array detection
ICP/MS	inductively coupled plasma/mass spectrometry
in	inch
in-lb	inch-pounds
IQ	Installation Qualification
ISO	International Organization for Standardization
J	open channel
kD	kilodalton
L	liter
LMH	liter per square meter per hour
LP	low pressure
LPB	low protein binding
M	molar
m^2	meter squared (square meter)
mAU	milli-absorbance units
MEM	minimum essential medium
mg	milligram

mL	milliliter
mm	millimeter
mPES	modified polyethersulfone
MW	molecular weight
MWCO	molecular weight cut-off
N	normal
NaCl	sodium chloride
NaOH	sodium hydroxide
nm	nanometer
N-m	Newton-meter
NMWL	nominal molecular weight limit
NVR	non-volatile residue
NWP	normalized water permeability
OQ	Operation Qualification
PBS	phosphate buffered saline
PP	polypropylene
ppm	parts per million
psi	pounds per square inch
psig	pounds per square inch gauge
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
RSF	Regulatory Support File
SOP	Standard Operating Procedure
TFF	tangential flow filtration
TMP	transmembrane pressure
TOC	total organic carbon
TSE	transmissible spongiform encephalopathy
UF	ultrafiltration
USP	United States Pharmacopeia
UV	ultraviolet
VMP	Validation Master Plan
VPL	Validation Document Package
WFI	water for injection

Note: *TangenX TFF Cassettes is a product line of Repligen. Listed below are the previous and current product names:*

Previous Name	Current Name
SIUS Cassettes	TangenX SIUS Cassettes
SIUS-LS Cassettes	TangenX SIUS PD Cassettes
NovaSet™ Cassettes	TangenX PRO Cassettes
NovaSet™-LS Cassettes	TangenX PRO PD Cassettes

1. Introduction

The Regulatory Support File (RSF) for TangenX SIUS Tangential Flow Filtration (TFF) Cassettes is intended to be used as:

- A guide for appropriate application use in process development, clinical, and commercial purification processes
- A guide to validation in manufacturing processes
- A support reference for CMC submissions for regulatory license approval
- A guide for supplier audits
- In place of a Drug Master File submission

Repligen is committed to providing all relevant technical, manufacturing, and quality information; however, only non-confidential information is presented in this document. Confidential details may be made available upon request through a formal confidentiality agreement or as part of a supplier audit.

1.1 Safety Notices

- Follow all local regulations for safe disposal
- For laboratory and manufacturing production only

1.2 Responsible Official

The individual designated responsible for quality and regulatory affairs for Repligen, and to whom all correspondence or requests for audits should be addressed.

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2. Quality Documentation

Repligen Corporation has over 50 years of experience providing products that meet the quality required in bioprocessing applications. We can satisfy the quality needs of customers with particular application requirements. Full compliance with regulatory requirements and meeting customer needs are the driving forces for the Repligen higher standard of quality. A copy of the Repligen quality policy can be found at repligen.com in the Quality System Management Documents section.

2.1 Quality Standards

To meet the needs of GMP manufacturing, TangenX SIUS Cassettes are manufactured in the USA under the following quality standards:

- TangenX SIUS Cassettes are manufactured in a facility whose Quality Management System is approved by an accredited registering body to the ISO® 9001 2015 Quality System Standard.
- TangenX SIUS Cassettes are manufactured in a facility that adheres to current Good Manufacturing Practices (cGMP).
- All fluid paths meet USP <88> Biological Reactivity Tests for Class VI Plastics criteria.

3. Product Description

TangenX SIUS Cassettes are the first truly single-use TFF cassettes for the biopharmaceutical industry. These single-use cassettes have been designed to offer comparable performance to reusable products at a fraction of the cost. The TangenX SIUS Cassette product family is completely interchangeable with existing cassette hardware available on the market.

The TangenX SIUS Single-use TFF Cassette is a membrane device that is used to concentrate, diafilter, and fractionate a wide range of macromolecules (e.g., enzymes, proteins, oligonucleotides). The retentate recirculates across the membrane surface, minimizing membrane fouling. The TangenX SIUS TFF Cassette is a rigid, flat, rectangular filter comprised of multiple layers of permeable membrane and polypropylene screens. Fluid is pumped through the feed channel, tangentially to the membrane surface. Pressure generated by the pumping process is used to drive the filtration operation. Each cassette arrives pre-sanitized, packaged in 0.2 M sodium hydroxide (NaOH). The TangenX SIUS Cassette is simply installed and conditioned with buffer to neutralize the pH and

it is ready for the process. Additionally, validation of membrane cleaning studies and performance studies after reuse are eliminated.

Figure 1. TangenX SIUS PD Cassettes

The TangenX SIUS PD Cassettes for lab scale and pilot applications are available in a range of membrane pore sizes: 1 kD – 300 kD in ProStream and 5 kD – 0.65 µm in HyStream mPES membrane chemistries. The TangenX SIUS PD Cassettes are also available in 0.01, 0.02, and 0.1 m^2 surface areas, and three channel configurations. The L-Screen (also referred to as LP-screen) channel is ideal for low to medium viscosity streams where high flux and lower recirculation rates are desired. The E-Screen (also referred to EP screen) channel is ideal for medium to high viscosity streams while still maintaining a beneficial cross flow rate. Finally, 0.5 mm (J) open channel cassettes are ideal for streams with a very high viscosity or ones containing particulates.

Figure 2. TangenX SIUS Cassettes

The TangenX SIUS Cassettes for process applications are available in a wide range of membrane pore sizes from 1 kD – 300 kD in ProStream and 5 kD – 0.65um in HyStream mPES membrane chemistries. TangenX SIUS Cassettes are available in 0.5, 1.5, and 2.5 m^2 surface areas, and L-Screen, E-Screen, and J channel options. Like the TangenX SIUS PD, TangenX SIUS process cassettes are available in numerous configurations that are directly scalable from 0.01 m^2 through 2.5 m^2, and beyond. TangenX SIUS Cassettes are designed for processing volumes from tens to thousands of liters.

The TangenX SIUS Cassette family is designed to deliver optimal performance as well as exceptional batch-to-batch reproducibility. Each cassette undergoes rigorous QA lot release testing to verify it meets specification. Cassettes are tested for both air integrity and for their hydrodynamic performance. This testing ensures cassette-to-cassette consistency, scalable process development, and reproducible manufacturing.

4. Product Information

4.1 Cassette Design

TangenX SIUS Cassettes are designed and constructed using FDA approved materials that have been validated for use in demanding biopharmaceutical applications. Each cassette is manufactured using a fully validated and documented manufacturing process according to the principles of cGMP and meets specific release criteria. The TangenX SIUS PD laboratory scale and TangenX SIUS process scale TFF Cassettes are purpose-built for single-use processing with optimal performance that is equivalent to the TangenX PRO reusable product line.

Table 1. TangenX SIUS PD and TangenX SIUS Cassette Materials of Construction

Component	Material
Membrane	Modified Polyethersulfone (mPES)
Membrane support	Polypropylene (PP)
Channel configurations: L-Screen Channel (Feed/retentate channel) E-Screen Channel (Feed/retentate channel) Filtrate channel (both L-Screen and E-Screen)	 High Density Polyethylene (HDPE) medium woven PP Screen High Density Polyethylene (HDPE) spacer with coarse woven PP Screen Medium woven PP Screen, Polyurethane
Encapsulant: Feed/retentate channel Filtrate channel	 Class VI approved Silicone Class VI approved Polyurethane
Cassette gasket	Ethylene Propylene Diene Monomer (EPDM)

Table 2. TangenX SIUS PD Cassette Physical Dimensions

Size (Approximate)
Length: 8.1 inch (20.6 cm)
Width: 2.2 inch (5.6 cm)
Height: 0.12 – 0.64 inch (0.3 – 1.6 cm) 0.01 m^2 filter area: L-screen 0.12 inch (0.32 cm); E-screen, J-channel 0.13 inch (0.34 cm) 0.02 m^2 filter area: L-screen 0.16 inch (0.41 cm); E-screen, J-channel 0.17 inch (0.44 cm) 0.1 m^2 filter area: L-screen 0.60 inch (1.53 cm); E-screen, J-channel 0.64 inch (1.62 cm)

Table 3. TangenX SIUS PD Cassette Hold-up Volumes

Surface Area	Channel Type		
	L-Screen	E-Screen	Open Channel (J)
0.01m^2 (0.11 ft^2)	1.2 mL	2.2 mL	5.4 mL
0.02m^2 (0.22 ft^2)	2.1 mL	3.8 mL	8.0 mL
0.1m^2 (1.1 ft^2)	8.7 mL	15.7 mL	28.7 mL

Table 4. TangenX SIUS Cassette Physical Dimensions

Size (Approximate)
Length: 8.3 inch (21.1 cm)
Width: 7.9 inch (20.1 cm)
Height: 0.6 – 2.66 inch (1.53 – 6.75 cm) 0.5 m^2 filter area: L-screen 0.60 inch (1.53 cm); E-screen, J-channel 0.64 inch (1.62 cm) 1.5 m^2 filter area: L-screen 1.50 inch (1.62 cm); E-screen, J-channel 1.59 inch (4.05 cm) 2.5 m^2 filter area: L-screen 2.50 inch (6.35 cm); E-screen, J-channel 2.66 inch (6.75 cm)

Table 5. TangenX SIUS Cassette Hold-up Volumes

Surface Area	Channel Type		
	L-Screen	E-Screen	Open Channel (J)
0.5 m^2 (5.41 ft^2)	38 mL	68 mL	136 mL
1.5 m^2 (16.2 ft^2)	114 mL	205 mL	385 mL
2.5 m^2 (26.9 ft^2)	190 mL	342 mL	633 mL

4.2 Product Contents

TangenX SIUS PD Cassette product contents

Package includes the following:

- One (1) TangenX SIUS PD TFF packet or cassette in one of the following sizes:
  - Packet 0.01 m^2 or 0.02 m^2
  - Cassette 0.1 m^2
- Two (2) gaskets (EDPM)
- Certificate of Conformance

TangenX SIUS Cassette product contents

Package includes the following:

- One (1) TangenX SIUS TFF cassette or block in one of the following sizes:
  - Cassette 0.5 m^2
  - Block 1.5 m^2
  - Block 2.5 m^2
- Two (2) gaskets (EDPM)
- Certificate of Conformance

4.3 Important Information Before Use

Cassettes

- TangenX SIUS Cassettes are compatible with all TangenX Cassette Holders.
- Cassettes may be stacked to increase filtration surface area; however, only one membrane molecular weight cut-off should be use at a time. Do not install a mixture of cassettes with different pore sizes in the same hardware.
- Cassettes must be equilibrated with an appropriate buffer (e.g., phosphate buffered saline) to ensure the neutralization of the 0.2 M NaOH storage agent in the membrane filter. It is important to use pre-filtered buffer to avoid fouling the membrane or introducing contaminants into the system that could affect membrane performance and product recovery.

Gaskets

- Gaskets should be used once. It is recommended by Repligen that gaskets are replaced with each cassette changeover. Repligen supplies two gaskets per cassette. Installation of the first cassette requires two gaskets; stacking additional cassettes requires only one gasket. Extra gaskets should be saved to replace worn or damaged gaskets.

Pump

- When using TangenX SIUS Cassettes, select a pump with adequate capacity. Crossflow rate ranges are dependent on feed channel type and process fluid characteristics.

4.4 TangenX SIUS Cassette Installation

1. Lift the end plate off and install a TangenX Filter Plater Insert (FPI). The FPI acts as the fluid manifold.
2. Rinse the EDPM gaskets with deionized (DI) water or water for injection (WFI). Place a rinsed gasket flat against the FPI, ensuring that the holes in the gasket line up with the holes in the FPI.
3. Using scissors carefully open the cassette bag and remove cassette.

4. Place the cassette into the holder flat against the gasket. Place another gasket on top of the cassette. Ensure that the holes in the manifold, both gaskets, and the cassette are completely aligned.

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

5. If using multiple cassettes, continue the same gasket/cassette/gasket pattern, ending with a gasket between the last cassette and the end plate. Place an isolation plate (provided with the FPI) between the gasket and the holder end plate.
6. Place the end plate on top of the isolation plate.
7. Install the tie-rod spacers (if used) and washers on each bolt leaving a minimum of 18 mm (0.75 inch) of thread exposed on the rod. By hand, screw the nut on each bolt and hand tighten evenly by alternating from one nut to the other.
8. Bolts must be further tightened to within the recommended torque values as shown in Table 6 using a calibrated manual torque wrench.

Table 6. Recommended Torque Ranges

Holder Type	Number of Bolts	Recommended Torque Range	
		in-lb	N-m
TangenX SIUS PD	2	120 – 180	14 – 20
TangenX SIUS	4	300 – 450	35 – 50
TangenX SIUS	2	600 – 900	70 – 100

9. **TangenX SIUS PD 2-bolt torque sequence:** Using the calibrated torque wrench with an 11/16-inch deep socket, pick a bolt and place the socket over the nut and tighten ¼ turn. Next, move the wrench across to the other bolt and tighten the nut ¼ turn. Alternate back and forth between bolts. Repeat this sequence until the wrench clicks without turning the nuts, indicating that the the nut has reached the torque set point.
10. **TangenX SIUS 4-bolt torque sequence:** Using the calibrated torque wrench with a 1¼ inch deep socket, pick a bolt (B1) and place the socket over the nut and tighten ¼ turn. Next, move the wrench to the next bolt (B2) diagonally across and tighten the nut ¼ turn. Next, move the wrench back across the cover to the other bolt (B3) and tighten the nut ¼ turn. Finally, move to the last of the four bolts (B2) and tighten the nut ¼ turn. Alternate back and forth using this crisscross pattern until the torque wrench clicks without turning the nuts indicating that the nut has reached the torque set point.
11. **TangenX SIUS 2-bolt** holders are hydraulic holders that are automatically torqued.
12. Wait 5 – 10 minutes to allow the gaskets to relax. Check each nut by attempting to tighten ¼ turn with the torque wrench at the appropriate set point per Table 6. If necessary, continue to re-torque until each nut clicks. Do not exceed the maximum torque limit.

For more information regarding installation and use of TangenX SIUS Cassettes, visit the User Guide section in the Quality Management Center on the Repligen website (https://www.repligen.com/support/quality-documents).

CAUTION: Nuts must be tightened uniformly to avoid damaging the cassette. Leakage may result from non-parallel plate alignment or over compression at one end.

NOTE: *Torque may change during processing as the TangenX SIUS Cassettes may compress, or as the cassettes expand or contract with temperature changes. Periodically check the torque of the bolts and adjust torque as needed.*

4.5 Equilibration of TangenX SIUS Cassettes

TangenX SIUS Cassettes must be equilibrated with an appropriate buffer (e.g., phosphate buffered saline) to ensure the neutralization of the 0.2 M NaOH storage agent in the membrane filter. Verify that the pH of the effluent from the cassette is neutralized to minimize any possible interaction with your application. For most applications, further sanitization is not required.

4.6 Cleaning of the TFF Cassette System

TangenX SIUS Cassettes are intended for single use only; post-use cleaning and reuse is not recommended. To clean the TFF system, recirculate a 0.5 M NaOH solution through the system with all valves open. TangenX SIUS Cassettes are left in place during the system cleaning procedure to provide a flow path for the cleaning solution. Alternatively, the cassettes may be removed, and a spacer gasket is put in place of the used cassettes. Upon completion of the cleaning cycle, flush the system with WFI, or DI water prior to draining and discarding the TangenX SIUS Cassettes.

4.7 Disposal of Used TangenX SIUS Cassettes

TangenX SIUS Cassettes are removed from the holder by reversing the cassette installation procedure. If the cassettes are difficult to separate from the stainless-steel holder, a thin plastic spatula can be slid under the edge of the cassette to break the seal. TangenX SIUS Cassettes can then be disposed of in a similar fashion to other disposable process equipment.

4.8 Storage of Unused TangenX SIUS Cassettes

Membrane cassettes must remain sealed in their original packaging prior to use to maintain their characteristics and integrity and to prevent microbial growth. Below are critical factors to remember when storing unused TangenX SIUS Cassettes:

Recommended storage temperature:

- 15°C – 25°C (optimal)
- 30°C (maximum)
- Do not freeze cassettes

4.9 Membrane Operating Characteristics

Take care to use the membrane at the lowest pressure possible while still producing consistent permeate flow. Although higher operating pressures initially improve flow rate, they also promote increased concentration polarization and membrane compaction, which ultimately limit flow. With very low molecular weight cut-off (MWCO) membranes, lower operating pressure may also reduce the retention of salts and very low molecular weight species.

4.10 Catalog and Serial Numbering System

Serial number system (Figure 3).

Decade Code

- 2000 thru 2009: 1
- 2010 thru 2019: 2
- 2020 thru 2029: 3

Year Code

- 1-digit(last digit of current year): 0 through 9

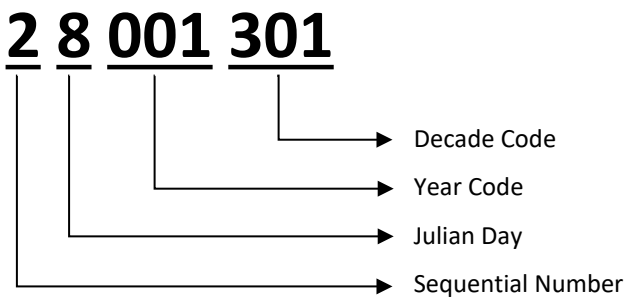
Julian Day

- 3-digit: 001 through 366

Sequential number

- 3-digit: 001 through 999

Figure 3. Serial Number System



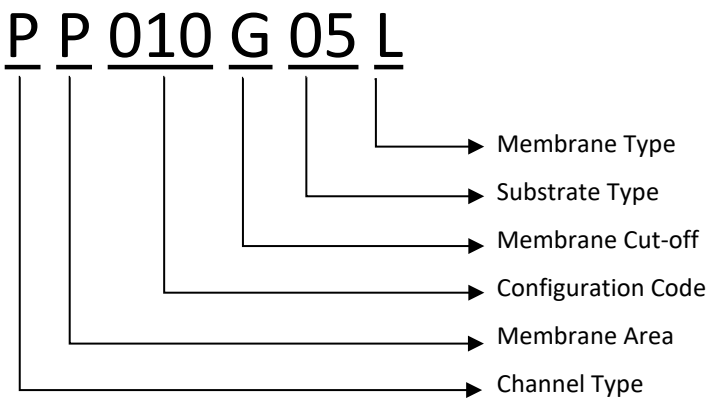
Cassette batch numbers

Cassette batch numbers are printed on each cassette label. The batch number is the eight (8) digit manufacturing process order number assigned by the ERP system. A batch is defined as a group of consecutively serialized cassettes manufactured on the same day, built from up to six (6) raw material lots and generated from the same ERP process order. Batch traceability is maintained on the batch record and in the ERP system.

Table 7. Product Code Description

Description			Batch Number Code
Membrane type:			
ProStream: mPES, Low protein binding (LPB)			P
HyStream: mPES, Ultra-hydrophilic and LPB			X
Substrate type:			
Polypropylene			P
Unsupported			U
Membrane cut-off:			
1 kD			001
3 kD			003
5 kD			005
10 kD			010
30 kD			030
50 kD			050
100 kD			100
300 kD			300
0.1 µm			M10
0.2 µm			M20
0.45µm			M45
0.65 µm			M65
Configuration code:	TangenX,		
TangenX SIUS PD	Pall,	Single use (lab/pilot)	L
TangenX SIUS PD	Millipore,	Single use (lab/pilot)	M
TangenX SIUS	Sartorius	Single use (process)	G
Membrane area:		Available Configuration:	
0.01 m^2 (0.11 ft^2)		L, M	P1
0.02 m^2 (0.22 ft^2)		L, M	P2
0.1 m^2 (1.1 ft^2)		L, M	01
0.5 m^2 (5.4 ft^2)		L, M, G	05
1.5 m^2 (16.2 ft^2)		G	15
2.5 m^2 (26.9 ft^2)		G	25
Channel type:			
LP Screen Channel		Medium woven	L
EP Screen Channel		Coarse woven	E
J Open Channel		0.5 mm	J

Figure 4. Catalog Part Number System



5. Product Performance

5.1 Membrane Performance

Designed specifically for use in a wide range of biopharmaceutical applications, especially those that are protein based, TangenX ProStream and HyStream membranes represent the latest in development of modified polyethersulfone (mPES). In contrast to conventional composite mPES, UF membranes are made in multi-step manufacturing processes that often include a post-casting surface modification. The TangenX mPES membranes have been developed from state-of-the-art technology including two unique features that deliver significant user benefits:

1. Manufactured in a single-cast, uniquely controllable process.
    - Reduced numbers of manufacturing steps equal lower cost and excellent consistency and reliability.
    - Balanced flux and selectivity. This highly controllable manufacturing process enables tight control of the microporous/UF transition interface. The macroporous and UF zones of this membrane are a finely controlled continuum. This controlled transition ensures no breakthrough of the UF skin, which maximizes selectivity performance.
2. Integral cast modification of the membrane chemistry.
    - This is achieved by the addition of a second polymer into the pre-casting membrane solution and ensures total and consistent surface modification that delivers:
        - Very low protein binding due to the membranes neutral charge.
        - Excellent chemical resistance.

The result is an application-focused membrane with a finely balanced performance profile combining:

- The flux of a highly porous UF membrane substructure with the retention and selectivity of a composite structure.
- Highly desirable low protein binding properties that maximize recovery and chemical resistance comparable to unmodified polymeric membranes.

Water Flux data was generated using membrane cut to 44.5 mm discs in stirred cells at 50 psig and purified water at 20°C. Purified water was used to measure the membrane water permeability. The TangenX ProStream and HyStream mPES membranes demonstrate comparable water permeability.

Many membranes are formulated for either retention or flux. The TangenX ProStream membrane has been designed and balanced to provide both. The following figures show the retention and rejection data for each membrane in the molecular weight cut-off (MWCO) series. When reviewed in conjunction with the MWCO series normalized water permeability (NWP) data in Figure 5, the user can specifically select a membrane that best balances flux and retention for a specific application.

Under specified test conditions using stirred cells, purified proteins and molecular weight markers were used to challenge the membranes. The TangenX mPES membranes demonstrate excellent selectivity as shown in Figure 6. Membranes above 0.1 µm are

characterized using latex particles (not a marker with a defined MW) and are therefore not included in the figure plotting molecular weight versus percent retention. Retention of the latex particles is shown in Figure 24.

Figure 5. ProStream and HyStream Membrane Cut-off (MWCO) vs. Normalized Water Permeability

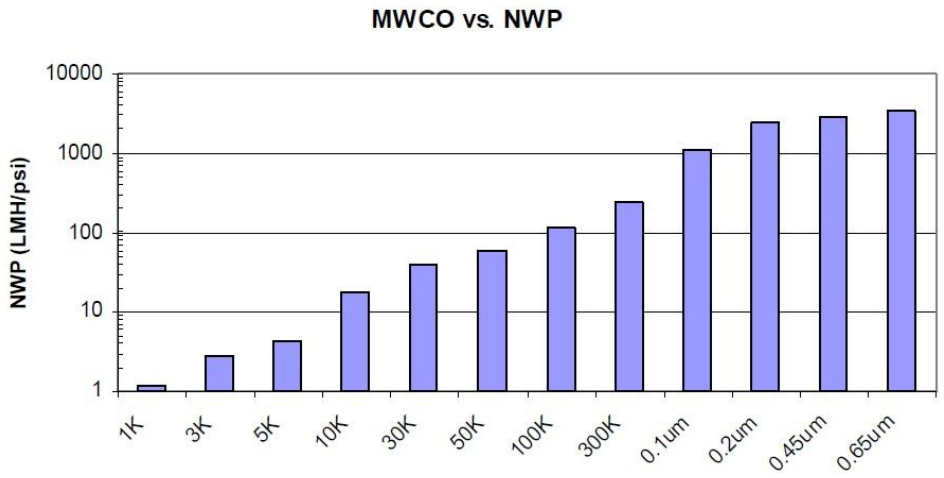
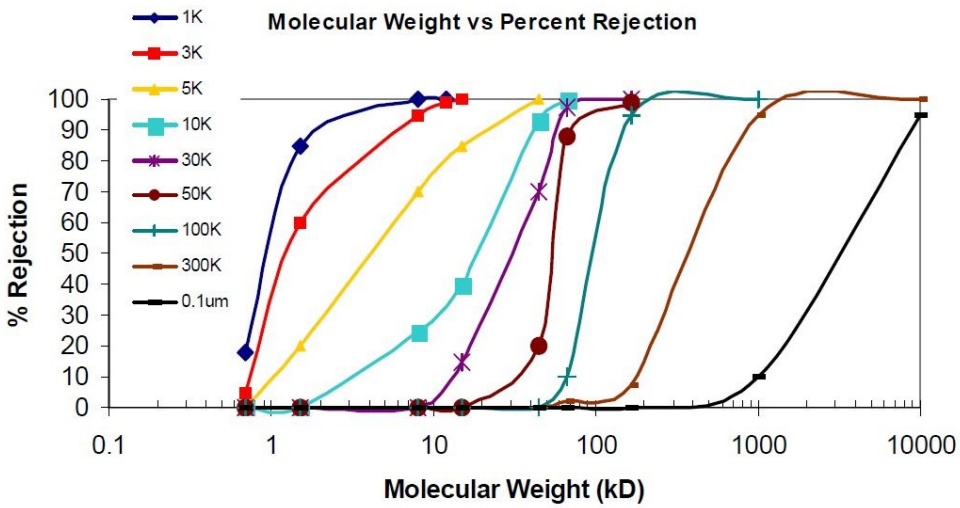


Figure 6. Membrane Selectivity Performance



5.2 Non-specific Protein Binding

The protein binding study was conducted to quantify the level of non-specific protein binding of two polyethersulfone membrane chemistries manufactured by Repligen. Non-specific protein binding is defined as the adsorption of a protein to a surface by one or more modes of attraction (e.g., charge effect, hydrophobic interaction). Non-specific protein binding tends to lead to the undesirable effects of yield loss and membrane fouling.

The approved test procedure provides methods for evaluating the membranes for non-specific protein binding. The study was applied to both the ProStream and HyStream membranes. One membrane of each type was chosen since the membrane chemistry is the same for each pore size. The 5 kD molecular weight cut-off membranes were ideal, as they retained each of the proteins tested. Each membrane was challenged with a protein solution, and the amount of protein bound to the membrane was measured by absorbance at 280 nm. Several proteins were used as models. Each protein was significantly different in molecular weight, structure, and isoelectric point.

Once the membranes had been challenged with protein and the measurements made, the amount of protein bound was quantified. The results were tabulated and are compared to the binding potential of an unmodified polyethersulfone membrane used as a control.

Table 8. Non-specific Protein Binding Test Results

Membrane type	BSA binding (µg/cm²)	IgG binding (µg/cm²)	Cyto-C binding (µg/cm²)
5 kD PES Control	<0.1	11.34	36.73
5 kD ProStream	<0.1	2.99	1.36
5 kD HyStream	<0.1	3.29	9.21

Table 8 summarizes the results from the final set of experiments. Each point represents an average of three sets of data. The results show the PES membrane control binds the highest amount of protein while the mPES binds significantly less protein. Lower protein binding is a desirable attribute of these membranes as lower binding leads to higher product recovery. Additionally, lower protein binding reduces the chances of a secondary boundary layer forming on the membrane surface, reducing productivity. Based on the information gathered, it may be claimed that the modified PES membranes manufactured by Repligen are considered low protein binding as compared to unmodified polyethersulfone (PES) membranes.

5.3 Cassette Hydraulic Performance

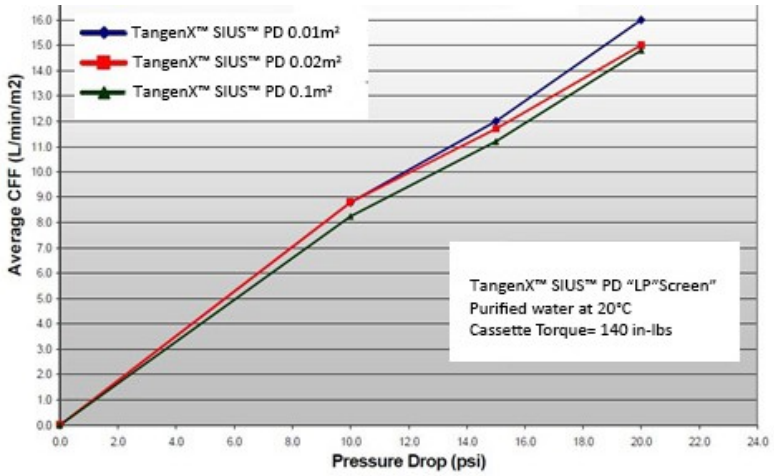
Scale-up performance is critical for successful process development and can be demonstrated by evaluating hydraulic performance using purified water. The TangenX SIUS Cassettes are manufactured with specific channel geometries and hydrodynamic characteristics. These hydraulic performance characteristics have a direct impact on process performance. It is important for the end user to select the proper channel type and that the cassette exhibits scalable performance. This leaves the end user with two primary factors to consider:

- The effect of channel type on the process flux and selectivity profile.
- Scalability (the performance at less than 0.1 m^2 scaling linearly to multiple m^2 filter area).

The TangenX SIUS PD Cassettes address these factors. Optimized channel geometry, with enhanced rigidity, ensures hydraulic performance is maintained when scaling up through the TangenX SIUS PD Cassette and TangenX SIUS Cassette family resulting in optimal and reproducible scaling performance. Additionally, each cassette undergoes rigorous QA release testing to verify specificationsm are met. Cassettes are tested for both air integrity and hydrodynamic performance, ensuring cassette-to-cassette consistency. The result is scalable process development and reproducible manufacturing. Data support the scale-up between the TangenX SIUS PD and TangenX SIUS products.

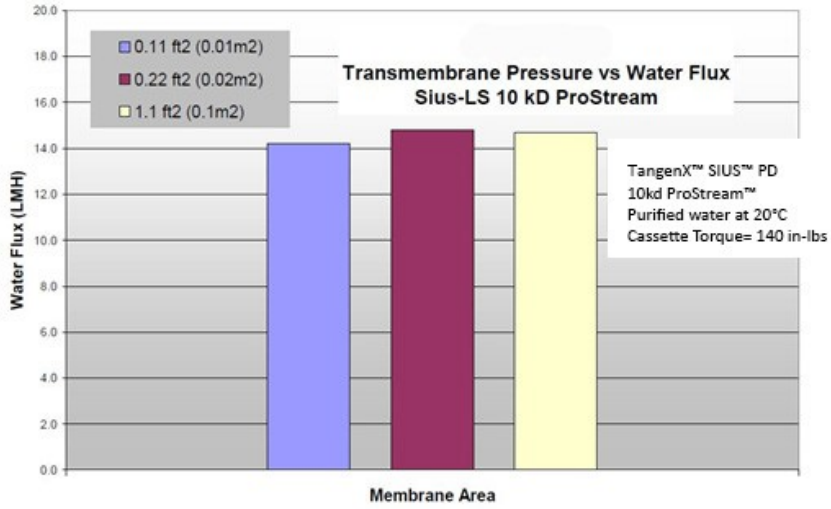
The hydraulic scalability of a cassette can be demonstrated using purified water under controlled conditions. Typically, the pressure drop between the feed and the retentate is measured at various crossflow rates. This information can then be generated for each cassette size as well as cassettes stacked together in parallel. Figure 7 shows the pressure drop versus crossflow flux for the TangenX SIUS PD Cassettes.

Figure 7. Pressure Drop vs. Crossflow Flux: TangenX SIUS PD L-Screen



Cassette hydraulic scalability can also be evaluated using purified water to measure normalized water permeability (NWP). NWP data can be used to support scalability of the TangenX SIUS TFF Cassette product line as well. Although not applicable to TangenX SIUS Cassette, NWP is typically used for reusable cassettes in characterizing cassettes before use and then after post-use cleaning. The NWP recovery demonstrates whether the clean-in-place (CIP) procedure effectively removes foulants deposited on the membrane surface during use. Figure 8 shows the transmembrane pressure (TMP) versus water flux for the 10 kD TangenX SIUS PD Cassettes through scale up from 0.02 – 0.5 m^2.

Figure 8. Transmembrane Pressure vs. Water Flux



Normalized water permeability (NWP) is dependent on its molecular weight cut-off (MWCO). Therefore, there is a range of permeability rates for each cassette of a given MWCO. It is important to note that external influences such as manifolds, piping, and valves create restrictions and can affect the measured NWP. Therefore, it is important to measure the initial NWP of your cassette in its designated system. Typical NWP ranges for a given MWCO are shown in Table 9. These values may be used as a guide to determine if the NWP is within specification.

Table 9. Typical NWP Ranges for TangenX SIUS Cassettes

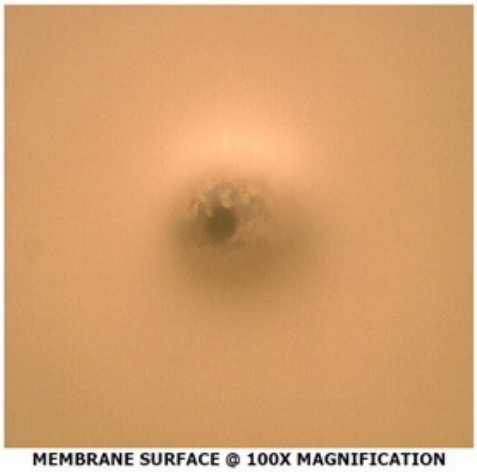
MWCO	Typical NWP Range (LMH/psi)
1 kD	0.8 – 1.5
3 kD	1.5 – 3.8
5 kD	2.6 – 5.7
10 kD	8.6 – 20
30 kD	24 – 41
50 kD	34 – 56
100 kD	32 – 91
300 kD	82 – 129
0.1 µm	112 – 225
0.2 µm	138 – 284
0.45 µm	152 – 312
0.65 µm	180 – 370

5.4 Cassette Integrity

The purpose of the cassette integrity testing is to provide a non-destructive method to verify the integrity of a tangential flow filtration (TFF) cassette. Each cassette manufactured by Repligen undergoes strict release testing, including an air integrity test. Release testing at Repligen follows a validated test method for cassette QC testing. This procedure refers to ultrafiltration and microfiltration cassettes manufactured by Repligen.

To demonstrate the sensitivity of the air diffusion test, a cassette was tested for integrity in which the upstream side of the cassette was pressurized with air. The integral membrane did not allow a significant amount of air to pass through the membrane due to the surface tension of the liquid in the pores. The result of the initial integrity test is found in Table 10. The effectiveness of the method was demonstrated by creating a pinhole in a cassette and measuring airflow before and after the pinhole was created.

Figure 9. Sensitivity of Air Integrity Test



The air diffusion specifications are found in Table 10 and the result of the integrity test following the defect being added to the cassette is found in Table 11. The pinhole defect in the membrane allowed air to pass through the membrane and the flow was

measured. The difference in the airflow between the initial sample and the modified sample was nearly 100 times greater. The difference was specific to the air diffusion rate and not the liquid crossflow rate. The difference between the two liquid flow rates was not affected and no difference in liquid flow was detected.

Table 10. Cassette Integrity Specifications (Air Diffusion Rate)

Cassette Channel Type	Membrane Type	Specification
L-Screen	Ultrafiltration (1 – 5 kD)	≤323 ccm/m^2 at 1 bar ≤30 ccm/ft^2 at 15 psi
E-Screen	Ultrafiltration (10 – 300 kD)	≤323 ccm/m^2 at 0.5 bar ≤30 ccm/ft^2 at 7.3 psi
J Open	Microfiltration (≥0.1 µm)	≤323 ccm/m^2 at 0.2 bar ≤30 ccm/ft^2 at 3 psi

Table 11. Cassette Integrity Test Results

Cassette Serial Number	Cassette Status	Results		Results within spec (Y/N)	Difference Observed (Y/N)	
		Air Diffusion Rate (ccm)	Liquid Flow Rate (mL/min)		Air Diffusion Rate	Liquid Flow Rate
17213102	Initial	24	621	Yes	N/A	N/A
	Modified	2196	620	No	Yes	No

5.5 Cassette Pre-flushing Study

The pre-flushing study was conducted to verify that the purified water pre-flushing procedure performed on all TangenX SIUS Cassettes successfully removes storage agents prior to sanitization with 0.2 M NaOH and final packaging. Membranes used in the cassettes are treated with 20% glycerin and 0.1% sodium azide as a storage agent during the membrane manufacturing process. After assembly, each TangenX SIUS Cassette is flushed with purified water prior to sanitization and final packaging. The storage agents removed by flushing would be considered unwanted leachables by the user if not sufficiently removed by the specified rinse and sanitization procedures. The following summary outlines the steps taken to determine the ideal conditions under which to remove the membrane storage agents (leachables) prior to final packaging.

Several cassettes were evaluated for storage agent leachables in duplicate. Each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. Due to comparable materials of construction and manufacturing processes, one cassette type was chosen to represent the TangenX SIUS product line: TangenX SIUS PD 0.1m^2 L-Screen Channel Cassette with 10 kD ProStream membrane. A set of devices, accounting for membrane chemistry, pore size, cassette configuration, and cassette channel type, was selected.

A TangenX LS Pilot System was assembled, sanitized with 0.5 M NaOH and rinsed with DI water. The cassettes were installed in the cassette holder, then flushed with purified water, and the effluent was analyzed for pH, conductivity, and absorbance, using a calibrated pH probe, conductivity meter, and UV spectrophotometer. The data show that the UV absorbance at 214 nm (Figure 10) and the conductivity (Figure 11) drop after 0.5 L of water is flushed through the retentate and 1.5 L through the permeate. The pH of the stream is neutral and the conductivity less than 2 µS. The UV absorbance at 214 nm falls below 0.05 mAU or approximately 10 parts per million (ppm) glycerin. The following results represent an average of duplicate cassette tests.

In conclusion, the storage agents are effectively flushed from the cassettes using 2 liters of purified water per square foot (20 L/m^2) membrane area. This flushing procedure is incorporated into the cassette final release procedure SOP-0482 for all TangenX SIUS Cassettes prior to sanitization with 0.2M NaOH.

Figure 10. Pre-flush Absorbance @ 214 nm

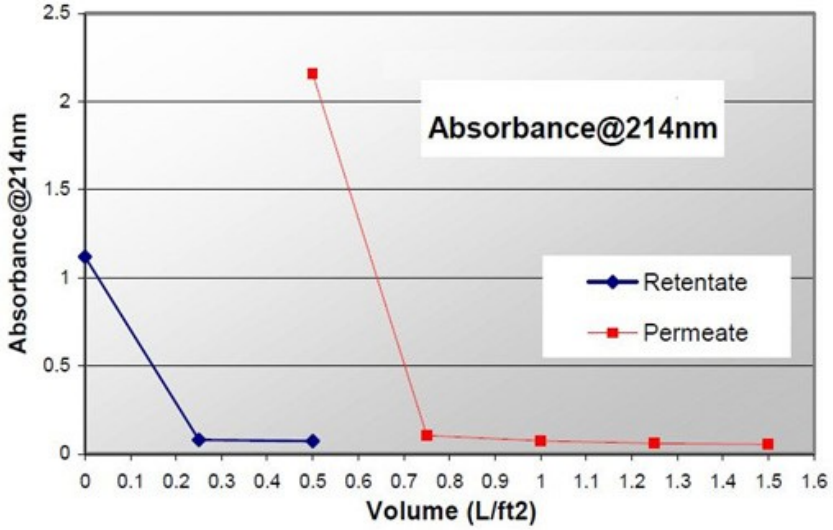
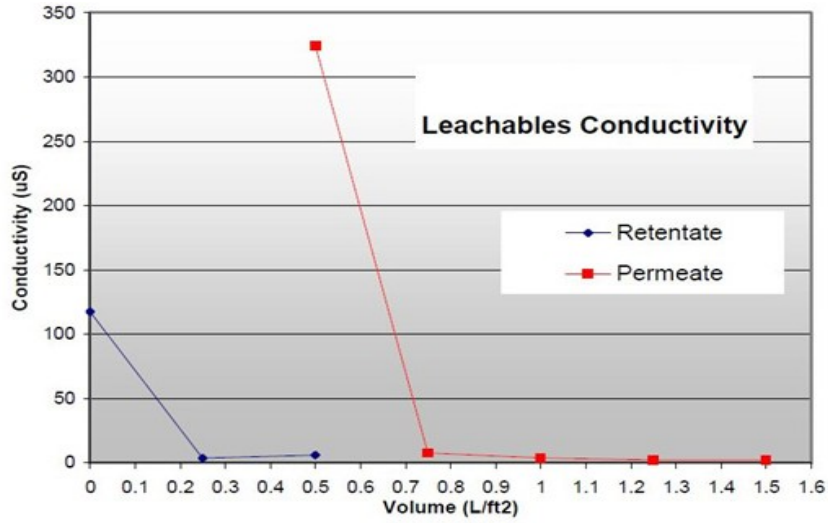


Figure 11. Pre-flush Conductivity



5.6 Cassette Leachables

Although TangenX SIUS Cassettes are shipped ready-to-use, they must be equilibrated with a buffered aqueous solution prior to use. At the Repligen manufacturing facility, each cassette is flushed with purified water, sanitized, and packaged in 0.2 M NaOH. Trace impurities not removed from the cassettes during the flushing procedure would be considered leachables, if found by the end user. The following summary outlines the steps taken to demonstrate that the process used by Repligen to flush, sanitize, and neutralize the cassette storage solution is effective. The results quantify the amount of leachables present in a typical TangenX SIUS Cassette.

TangenX SIUS PD Cassettes with 10 kD ProStream and 10 kD HyStream membrane chemistries were selected for use in this study The cassettes were manufactured and evaluated in triplicate. Each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. Due to comparable materials of construction and manufacturing processes, one cassette type accurately represents the TangenX SIUS product line. Both membrane chemistries were chosen since each represents a product line with unique filtration characteristics. Moreover, this study was carried out on a specific set of devices that represents the entire product range and accounts for membrane chemistry, pore size, cassette configuration, and cassette channel type.

The following SOPs were followed as part of the study:

1. TangenX SIUS PD Cassettes were prepared using SOP-0522.
2. These cassettes were tested in QC using SOP-0482.

A TangenX LS Pilot System was assembled, sanitized with 0.5 M NaOH, and then flushed with DI water. Each cassette was individually installed in the cassette holder, then equilibrated with 1 liter of PBS buffer (10 L/m^2). The PBS buffer was then drained from the system and 1 L of fresh PBS buffer was recirculated through the system for two hours. This PBS buffer was analyzed for pH, conductivity, absorbance at 214 nm, and TOC. The methods specified in TX1001-POQ-135 were used to conduct the leachables studies and are referenced in supporting development reports.

The procedure was repeated three times for each of the two chemistries for a total of six cassette samples. The results shown represent an average of three cassettes for each type. Measurements were taken using a calibrated pH probe, conductivity meter, and UV spectrophotometer The results from the PBS buffer recirculation are reported in Figure 12, Figure 13, and Figure 14. The data show the pH of the buffer remains constant at 7.4. The conductivity increased by 0.2 µS before reaching a steady state after approximately 15 minutes. This slight increase in conductivity is likely related to a trace amount of NaOH remaining in the system, however, not enough to affect the pH. The absorbance at 214 nm reaches a maximum value of 0.070 mAU representing the highest level of leaching into the buffer after 2 hours. This absorbance corresponds to a total organic carbon (TOC) value of approximately 0.04 mg/cm^2. Figure 15 shows the TOC value of the PBS buffer after 2 hours of recirculation for each of the cassettes tested.

The results of the leachables study show the 0.2 M NaOH storage solution was effectively neutralized using 10 L/m^2 of PBS buffer. TOC analysis of the PBS buffer stream demonstrated the level of leachables to be ≤0.04 mg/cm^2.

Figure 12. Leachables Study: pH

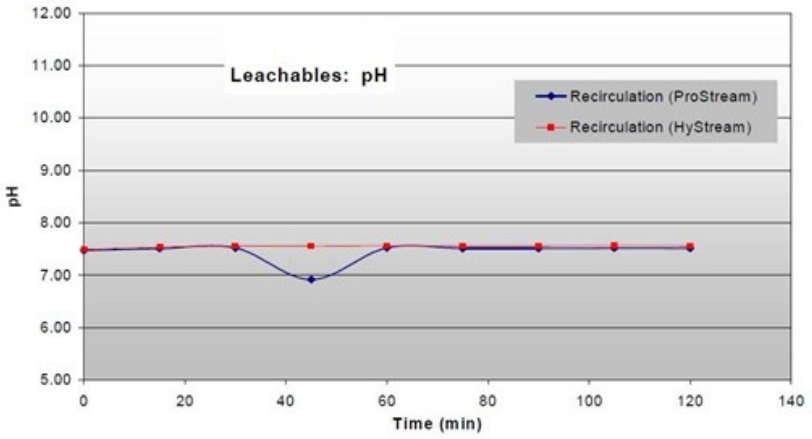


Figure 13. Leachables Study: Conductivity

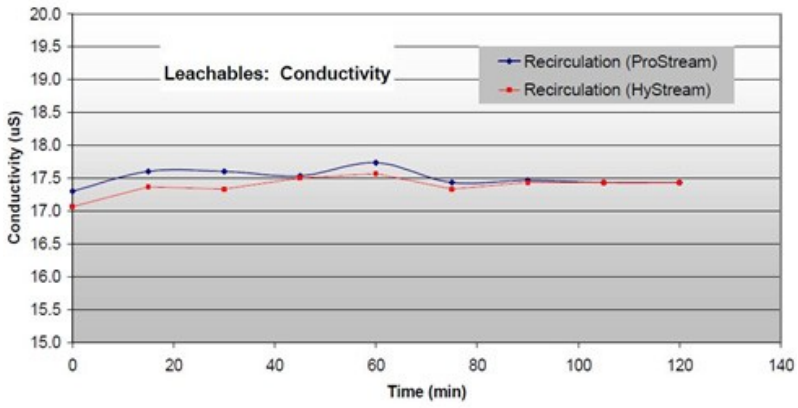


Figure 14. Leachables Study: Absorbance @ 214 nm

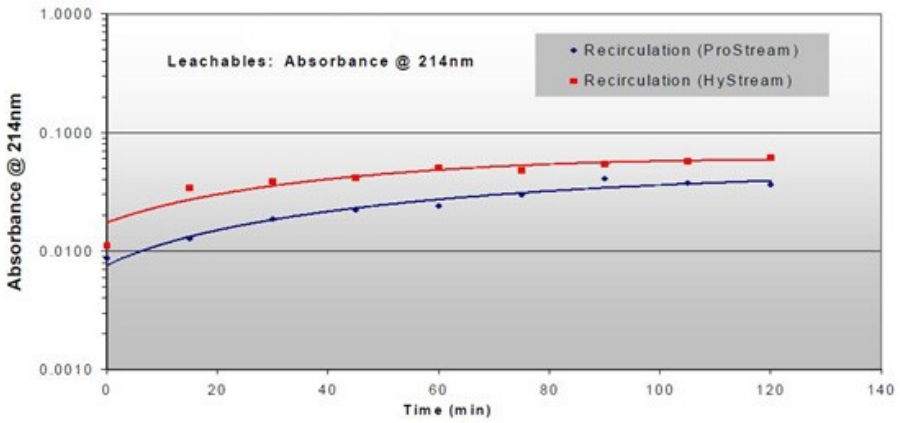
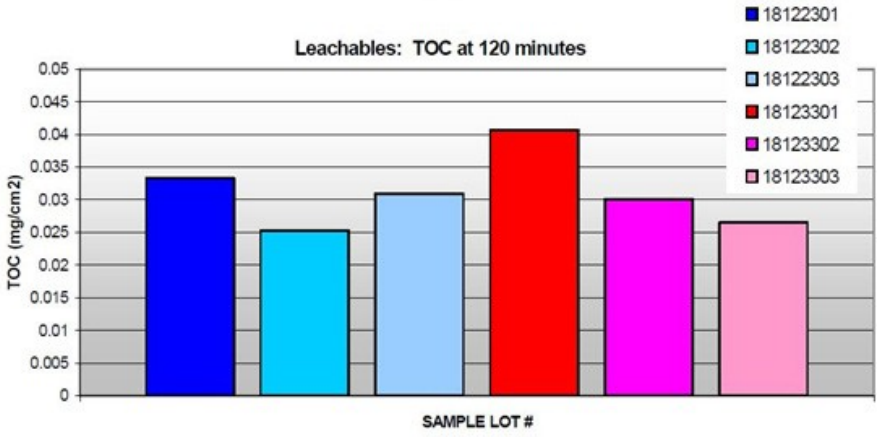


Figure 15. Leachables Study: TOC at 120 Minutes



5.7 Robustness Study

The purpose of this study was to evaluate the robustness of the TangenX SIUS Single-use TFF Cassettes by demonstrating the ability of the cassettes to operate under stressful conditions. Parameters such as time, temperature, pressure, flow rate, and buffer conditions can contribute to the stress on TFF cassettes. The following steps were used to validate the robustness of the TangenX SIUS Single-use Cassettes under aggressive operating conditions.

1. TangenX SIUS PD Cassettes were prepared using SOP-0522.
2. Cassettes were tested and released following procedure SOP-0482.

Several 0.1 m^2 TangenX SIUS PD Cassettes were manufactured and evaluated for robustness both individually as well as stacked to 0.5m^2. Each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. Due to comparable materials of construction and manufacturing processes, one cassette type was chosen as it accurately represents the entire TangenX product line: TangenX SIUS PD 0.1m^2 L-Screen Channel Cassette with 10 kD ProStream membrane. In addition, this study was carried out on a specific group of devices that are intended to represent the entire product range and account for membrane chemistry, pore size, cassette configuration, and cassette channel type. Procedure TX1001-POQ-133 provides the methods used to evaluate robustness of the cassettes.

Each cassette was evaluated at two temperatures, 4°C and 40°C. Testing was conducted on both a single cassette and a stack of five cassettes. The cassettes were removed from the packaging, installed in the cassette holder, and equilibrated with PBS. The baseline air integrity, NWP, and pressure drop were measured and recorded. Next, PBS buffer, pH 7.4, was recirculated through the system for 8 hours at 4°C and again at 40°C. Once this recirculation was complete, the cassettes were retested for air integrity, NWP, and

pressure drop and had to meet the original release specifications. The test conditions and results for the testing are summarized in Table 12.

Table 12. Robustness Testing

Study Conditions	0.1 m^2 Single Cassette	0.1 m^2: 5 Cassette Stack
Time	8 hours	8 hours
Temperature 1	4°C	4°C
Temperature 2	40°C	40°C
Pressure	100 psi	100 psi
Flow Rate	0.7 L/min	3.8 L/min
Buffer Conditions	PBS pH 9.0	PBS pH 9.0
Results	PASS	PASS

The results of the robustness testing showed that the cassettes withstood extended run time, temperature extremes, high pressure, and flow rate and passed all release criteria specified in SOP-0482. The TangenX SIUS Cassettes have demonstrated their robustness and will withstand at least one process cycle as designed.

5.8 Shelf-life Study

5.8.1 Membranes

A shelf-life study for ultrafiltration and microfiltration membranes manufactured by Repligen was performed over five years. Following casting, the membranes are stored for up to five years prior to being incorporated into a cassette product.

Several lots of membranes were cast during process validation. Each membrane was prepared using current SOPs and reflected the standard membrane manufacturing process at Repligen. The following steps were taken as part of the study:

1. Membranes were prepared using SOP-0448 and SOP-0564.
2. Membranes were sampled and tested following TX1001-POQ-115.

The results for the shelf-life study, including interim sampling and testing, of the ProStream and HyStream membranes manufactured at Repligen are provided. The membrane storage study procedure TX1001-POQ-115 was applied to both the mPES ProStream (BioFlo) and HyStream (HyFlo) membranes. One membrane of each type was chosen to represent the product line. These membranes correspond to those in the cassette shelf-life study. Each membrane was tested following the standard QC release procedure SOP-0463.

The shelf-life study consisted of two conditions, one at ambient temperature and the other at 50°C. The standard shelf-life study at ambient temperature simulated exposure at a normal or median temperature conditions for five (5) years. The accelerated shelf-life study was conducted at 50°C, the maximum temperature limit of the product, for one (1) month. A study at lower temperatures was not conducted.

Each membrane sample sheet, at a given time point, was evaluated in triplicate. In the event one membrane failed during the study, a failure analysis would have been conducted through the deviation procedure (SOP-0847). The mode of failure and impact on product quality would have then been assessed. If the membrane were deemed to be an anomaly, the study would continue as planned. The documented failure would accompany the final report. If all membranes failed during any one time point, the endpoint of the study would have been considered reached and the study concluded. A detailed analysis of the membranes that did not meet release criteria would have been included in the final report.

This study was reported on in an interim summary and after five years.

The results show both the ProStream and HyStream membranes meet or exceed all release specifications following both standard shelf-life study and the accelerated study. Membrane performance, based on water permeability, rejection, and integrity, was not affected in either study. The membrane shelf-life study demonstrated that the membrane storage under ambient conditions is stable for five years.

Table 13. Membrane Shelf-life Study Acceptance Criteria

Description	Specifications
Normalized water permeability	**NWP**
NWP (LMH/psi)	9.5 – 22.0 LMH/psi
Percent Deviation	15%
Passing Molecular weight marker	**PVP C-15 (~15 kD)**
Flux (LMH)	140 – 250 LMH
Percent Rejection	30% – 60%
Retaining molecular weight marker	**PVP C-30 (~45 kD)**
Flux (LMH)	70 – 110 LMH
Percent Rejection	>85%
Integrity test	**Air diffusion @ 15 psi**
Total number of discs with air diffusion	≤6 (of 18 discs)

Table 14. Membrane Shelf-life Study Results: 50°C

Time point	Normalized Water Permeability	Passing Molecular Weight Marker	Retaining Molecular Weight Marker	Integrity test
Time Initial	Pass	Pass	Pass	Pass
1 Week	Pass	Pass	Pass	Pass
1 Month	Pass	Pass	Pass	Pass

Table 15. Membrane Shelf-life Study Results: Ambient Temperature

Time Point	Normalized Water Permeability	Passing Molecular Weight Marker	Retaining Molecular Weight Marker	Integrity Test
Time Initial	Pass	Pass	Pass	Pass
3 months	Pass	Pass	Pass	Pass
6 months	Pass	Pass	Pass	Pass
1 year	Pass	Pass	Pass	Pass
2 years	Pass	Pass	Pass	Pass
3 years	Pass	Pass	Pass	Pass
4 years	Pass	Pass	Pass	Pass
5 years	Pass	Pass	Pass	Pass

5.8.2 Cassettes

A shelf-life study for cassettes manufactured by Repligen was performed over three years. Following manufacturing, the cassettes are stored prior to shipping and, once shipped, may remain unopened prior to use. The maximum projected duration for the TangenX SIUS Cassette shelf life has been determined to be three years.

Several different cassettes were manufactured and evaluated. Each cassette was prepared using current SOPs and reflected the standard membrane manufacturing process at Repligen. The following steps were taken as part of the study:

1. Cassettes were prepared using SOP-0522.
2. Cassettes were sampled and studied following TX1001-POQ-134.

One cassette type and two membranes was chosen for this study to represent the entire product line: the TangenX SIUS PD 0.1 m^2 L-Screen cassette using 10 kD ProStream and 10 kD HyStream membranes. The 0.1 m^2 TangenX SIUS PD Cassettes were chosen as they accurately represent the construction of the entire product line, including the 0.5 m^2 – 2.5 m^2 TangenX SIUS Cassettes. The two membrane chemistries represent each membrane type. A separate membrane storage study was used to evaluate the effect of aging on various membane types and pore sizes (TX1001-POQ-115 and interim report DR-09-010). Each cassette was initially evaluated following the standard QC release procedure SOP-0482.

The shelf-life study consisted of two conditions, one at ambient temperature and the other at 50°C. The standard shelf-life study at ambient temperature simulated exposure at normal or median temperature conditions for three (3) years. The accelerated shelf-life study was conducted at 50°C, the maximum temperature limit of the product, for one (1) month. A study at lower temperatures, below ambient, was not conducted.

Each cassette type at a given time point was evaluated in triplicate. In the event one cassette failed during the study, a failure analysis would have been conducted through the deviation procedure (SOP-0847). The mode of failure and impact on product quality would have then been assessed. If the cassette were deemed to be an anomaly, the study would continue as planned. The documented failure would accompany the final report. If all cassettes failed during any one time point, the endpoint of the study would have been considered reached and the study concluded. A detailed analysis of the cassettes that did not meet release criteria would have been included in the final report.

The acceptance criteria for each of the cassettes are shown below and include standard release testing, normalized water permeability/rejection, and testing for leachables.

The results show that the TangenX SIUS Cassettes meet or exceed all release specifications following both the standard shelf-life study and the accelerated study. Cassette performance, based on water permeability and rejection, was not affected over three (3) years. Finally, the cassette leachables profile has not increased and endotoxin is within acceptable limits.

Table 16. Cassette Shelf-life Study Acceptance Criteria

Description	Specifications
Water cross-flow rate: Flow rate (liter per minute) @ Pressure drop (psi)	0.4 – 0.8 LPM @ 10 ± 0.5 psi (0.7 ± 0.03 bar)
Air diffusion rate: Rate (ccm)	≤30 ccm @ 7.3 ± 0.5 psi
Visual inspection: Lot number	Matches Data Sheet
Particulates	≤10 particles
Standard Release Testing	Follow the TangenX SIUS Cassette storage study procedure TX1001-POQ-134 and test the cassettes for final release using SOP-0482.

Table 17. Cassette Shelf-life Study Results: 50°C

Time Point	Standard Release Testing	Normalized Water Permeability and Rejection	Leachables
Time Initial	Pass	Pass	Pass
1 Week	Pass	Pass	Pass
1 Month	Pass	Pass	Pass

Table 18. Cassette Shelf-life Study Results: Ambient Temperature

Time Point	Standard Release Testing	Normalized Water Permeability and Rejection	Leachables
Time Initial	Pass	Pass	Pass
3 Months	Pass	Pass	Pass
6 Month	Pass	Pass	Pass
1 Year	Pass	Pass	Pass
2 Years	Pass	Pass	Pass
3 Years	Pass	Pass	Pass

5.9 Chemical Compatibility

Table 19. ProStream and HyStream Chemical Compatibility

Reagent	ProStream	HyStream
pH range	1-14	1-14
Acetic acid (5%)	√	√
Acetic acid (25%)	√	x
Acetone (≤30%)	√	√
Acetonitrile (≤15%)	√	x
Alconox® (1%)	√	√
Aliphatic and aromatic esters	x	x
Amines	x	x
Ammonium chloride (1%)	√	√
Ammonium hydroxide (5%)	x	x
Aromatic and chlorinated hydrocarbons	x	x
Butanol (70%)	√	√
Butyl acetate (40%)	√	x
Butyl cellosolve™ (10%)	√	√
Calcium chloride (5%)	√	√
Chloroform (0.8%)	√	√
Citric acid (1%)	√	√

Reagent	ProStream	HyStream
Dimethyl acetamide (DMAC) (≤30%)	√	x
Dimethyl acetamide (DMAC) (≤15%)	√	√
Dimethylformamide (≤40%)	√	√
Dimethyl sulfoxide (≤40%)	√	√
Disodium salt of EDTA (10%)	√	√
Ethanol (70%)	√	√
Ethers	x	x
Ethyl acetate (≤30%)	√	√
Formaldehyde (1%)	√	√
Formic acid (5%)	√	√
Glutaraldehyde (0.5%)	√	√
Glycerin (50%)	√	√
Guanidine HCl (6 M)	√	√
Hydrochloric acid (0.1 N @ 25°C)	√	√
Hydrochloric acid (0.1 N @ 50°C)	√	√
Hydrochloric acid (1.0 N @ 50°C)	√	x
Hydrogen peroxide (1%)	√	√
Isopropyl acetate (1%)	√	√
Isopropyl alcohol (25%)	√	√
Ketones	x	x
Lactic acid (5%)	√	√
Mercaptoethanol (0.1%)	√	√
Methyl alcohol (25%)	√	√
Methylene chloride (1%)	√	x
Methyl ethyl ketone (1%)	√	x
N-methyl pyrrolidone (1%)	√	√
Nitric acid (≤1%)	√	√
Oxalic acid (1%)	√	√
Phenol (0.5%)	√	√
Phosphate buffer (pH 8.2, 1 M)	√	√
Phosphoric acid (1 N)	x	x
Sodium azide (1%)	√	√
Sodium chloride (5%, 50°C)	√	√
Sodium deoxycholate (5%)	x	x
Sodium dodecyl sulfate (0.01 M)	√	√
Sodium hydroxide (0.1 N @ 25°C)	√	√
Sodium hydroxide (0.1 N @ 50°C)	√	√

Reagent	ProStream	HyStream
Sodium hydroxide (0.5 N @ 25°C)	√	√
Sodium hydroxide (0.5 N @ 50°C)	√	√
Sodium hydroxide (1.0 N @ 25°C)	√	x
Sodium hypochlorite (100 ppm)	√	√
Sodium hypochlorite (400 ppm)	√	x
Sodium hypochlorite (1000 ppm)	x	x
Sodium nitrate	√	√
Sulfuric acid (1 N)	√	x
Tergazyme® (1%)	√	√
Tetrahydrofuran (5%)	x	x
Toluene (1%)	x	x
Tris buffer (pH: 8.2) (1 M)	√	√
Triton™ X-100 (0.002 M)	√	√
USP Class VI	√	√
Ultrasil™ 11 (1%)	√	√

√ = Compatible: no significant changes in either rejection or flow rate.
x = Not Compatible: significant change observed.

6. Safety Information

6.1 USP Class VI

The purpose of USP Class VI testing is to verify the biological safety of each of the components used in the TangenX SIUS Cassette product line. Samples for USP Class VI testing consisted of each of the five components of the TangenX SIUS TFF Cassette. Each component used to construct the cassettes is listed in Figure 16. Sample dimension, sample mass and test regime are identified as well.

Figure 16. USP Testng Results

TangenX Sample Matrix **USP Testing** **Vendor: Toxikon**

	Component Description	Composition	Minimum Sample Mass	Sample Dimensions	Tests to be Conducted
1	Cassette Encapsulant	Polyurethane	~ 45 grams from 3 lots	25mm x 25mm x 5mm (1)	A,B,C
2	Screen Spacer	Polyolefin	~ 45 grams from 3 lots	25mm (diameter) x 0.8mm (1)	A,B,C
3	HyStream Membrane	Polyethersulfone	~ 45 grams from 3 lots	25mm (diameter) x 0.2mm (1)	A,B,C
4	ProStream Membrane	Polyethersulfone	~ 45 grams from 3 lots	25mm (diameter) x 0.2mm (1)	A,B,C
5	EPDM Gasket	EPDM	~ 45 grams from 3 lots	25mm (diameter) x 1mm (1)	A,B,C
6	Silicone PSA w/screen	Silicone & Polypro	~ 45 grams from 3 lots	25mm (diameter) x 0.8mm (1)	A,B,C
(1) Must also include 1mm x 1mm x 10mm sample					

Test ID	Test Description	Sample Mass	Sample Dimensions	Total Qty
A	MEM Elution per USP <87>	4 grams	(see above)	7
B	Class VI per USP <88>	16 grams, plus additional pieces ~10g (1)	(see above), plus 12 pieces 1mmx1mmx10mm	7
C	Hemolysis - Indirect with rabbit blood	15 grams	(see above)	7

Samples for both USP and extractables testing required preparation prior to analysis. Each sample was rinsed with WFI, sanitized with 0.5 M NaOH, and then rinsed again with WFI. The purpose of this sample preparation is two-fold:

1. To simulate the sanitization procedure the end user may perform prior to use of the cassette.
2. To sanitize the sample so as not to allow external contamination to interfere with the USP testing.

Approved procedures were followed during preparation of samples and used for USP and Class VI testing. The procedure was used to provide a record of the samples to be prepared, as well as the method of preparation. Experimental deviations were recorded in a laboratory notebook and a copy attached to the final report. They were used to describe the deviations, to determine ways to rectify them, and to record whether they would significantly affect the result of the experiment.

Results and discussion

The results of the studies show that all component materials meet:

- Current requirements for USP Class VI biological testing for plastics.
- The test article(s) meets the test requirements as defined in the USP guidelines: USP 30, NF 25, 2007, <788> Particulate Matter in Injections.

All components materials used in cassettes manufactured by Repligen have been independently tested for USP safety and were shown to be safe according to:

- L929 MEM Elution per USP <87>
- Class VI per USP <88>
- Hemolysis – Indirect with rabbit blood

The study proposal for the USP testing conducted with Toxikon is found in Toxikon laboratory proposal #07-2-26TF7757 and #08-5-8TF9874. The study reports the results generated by Toxikon in a complete USP report that can be provided by Repligen. A summary of the test results is below.

Figure 17. Summary of USP Testing Results (1)

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Test Summary

Date: Oct.27, 2008
Sponsor: TangenX Technology Corp.
Contact: Mark Pereault

Test Article Number: 08-2554
Test Material: EPDM Gasket

Test Name	Project #	Status / Results
MEM Elution-USP	08-2554-G1	PASS – Report Complete
Class 6 (includes implant)	08-2554-G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	08-4577-G1	PASS – Report Complete

Test Article Number: 08-2555
Test Material: Silicone PSA with Screen

Test Name	Project #	Status / Results
MEM Elution-USP	08-2555 -G1	PASS – Report Complete
Class 6 (includes implant)	08-2555 -G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	08-2555 –G3	PASS – Report Complete

Test Article Number: 07-1878
Test Material: ProStream (BioFlo) PES Membrane

Test Name	Project #	Status / Results
MEM Elution-USP	07-1878-G1	PASS- Report Complete
Class 6 (includes implant)	07-1878-G2	PASS - Verbal 5/29PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1878-G3	PASS – Report Complete

Figure 18. Summary of USP Testing Results (2)

TOXiKON
Leaders in Life Science and Technology

Test Article Number: 07-1880
Test Material: Screen Spacer

Test Name	Project #	Status / Results
MEM Elution-USP	07-1880-G1	PASS – Report Complete
Class 6 (includes implant)	07-1880-G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1880-G3	PASS – Report Complete

Test Article Number: 07-1881
Test Material: Channel Spacer

Test Name	Project #	Status / Results
MEM Elution-USP	07-1881-G1	PASS – Report Complete
Class 6 (includes implant)	07-1881-G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1881-G3	PASS – Report Complete

Test Article Number: 07-1882
Test Material: Cassette Encapsulent

Test Name	Project #	Status / Results
MEM Elution-USP	07-1882-G1	PASS – Report Complete
Class 6 (includes implant)	07-1882-G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1882-G3	PASS – Report Complete

Test Article Number: 07-1885
Test Material: HyStream (HyFlo) PES Membrane

Test Name	Project #	Status / Results
MEM Elution-USP	07-1885-G1	PASS – Report Complete
Class 6 (includes implant)	07-1885-G2	PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1885-G3	PASS – Report Complete

Note: *Test article identified as BioFlo is ProStream. Test article identified as HyFlo is HyStream.*

6.2 Extractables

A controlled extraction study was performed on the TangenX SIUS TFF Cassettes using solvents and extraction techniques across a broad range of polarities. The methodology utilized was described in the study plan M-TANGENX-210301 and results generated are summarized in Study Report 11510.3777. The analysis was based on the 2014 BPOG recommended study conditions. They present a worst-case scenario, since neither the temperature nor dissolution properties of the solvents used during this investigation are more aggressive compared to the solvents used during routine component exposure.

Three composite batches of TangenX SIUS TFF cassettes were manufactured and evaluated for extractables following the guidance outlined by BPOG. Each cassette was prepared using releases standard operating procedures and met release criteria established by Repligen.

Test samples were initially received by the contracted laboratory, flushed with purified water to remove the storage solution, then equilibrated with the extraction solution. Extraction of the test samples was performed using 50% ethanol in USP purified water, 1% polysorbate-80, 5 M NaCl, 0.5 M NaOH, 0.1 M H_3PO_4, and purified water (WFI). Samples were extracted for 24 hours and 21 days at 40°C. Each cassette sample was composed of three different lots of membrane forming a composite sample. The test articles were agitated using a rocking table for the duration of the extraction. Once the time points were reached, the extraction fluid was drained from the cassette device and analyzed for extractables. The following is a summary of the testing performed.

HPLC/DAD/MS was performed on selected component extracts according to the conditions described in the study plan. All sample extracts were analyzed for antioxidants and additives by HPLC-DAD/MS with the DAD operating at the 220 nm wavelength, and the MS operating in ESI (±) and APCI (±) modes. A number of both known and unknown extractable peaks were identified in all sample

extracts. Concentrations of BPA were quantified using the response of an authentic reference standard. Concentrations for all other analytes were determined using the response factor for the internal standard for each sample injection. All peaks greater than 0.1 µg/mL that were detected in the sample extracts at levels 1.5x higher than in the associated method control are reported as extractables. Results are available in study report 11510.3777 (Tables 3 – 15).

GC/MS was performed on selected component extracts according to the conditions described in the study plan. All sample extracts were assayed for semi-volatiles by GC/MS. A number of both known and unknown analytes were detected in the sample extracts. Concentrations of 1,3-di-tert-butylbenzene, and 2,4-di-tert-butylphenol were quantified using the response of an authentic reference standard. Concentrations of all other analytes were determined using the response factor for the internal standard for each sample injection. Results are available in study report 11510.3777 (Tables 17 – 29).

Headspace GC/MS was performed on selected component extracts according to the conditions described in the study plan. All sample extracts were assayed for volatiles by HS-GC/MS. A number of tentatively identified analytes were detected in the 50% ethanol and 1% PS-80 sample extracts. Concentrations of 1,3-di-tert-butylbenzene were quantified using the response from an authentic reference standard. Concentrations of all other analytes were determined using the response factor for the internal standard for each sample injection. Results are available in study report 11510.3777 (Tables 31 – 43).

Induction Coupled Plasma (ICP)/MS was performed on selected component extracts according to the conditions described in the study plan. All sample extracts were outsourced to Chemical Solutions, Ltd. for metals analysis by ICP/MS. Results are available in study report 11510.3777 (Tables 47 – 49).

TOC, pH, and Non-Volatile Residue analyses were performed on selected component extracts according to the conditions described in the study plan. Results for total organic carbon, pH, and non-volatile residue are provided in Table 20, Table 21, and Table 22 For TOC analysis of the 5 M NaCl extracts, a dilution was required due to an adverse matrix effect on the instrumentation. For all other sample extracts, dilutions were required to be within the calibration curve.

Table 20. TOC Results

Sample Description	Extraction Solvent	Results			
		1 day		21 days	
		µg/mL	µg/cm²	µg/mL	µg/cm²
Cassette	WFI	286	25.5	1.11×10^3	99.3
	0.1 M H_3PO_4	286	25.5	1.21×10^3	108
	0.5 N NaOH	278	24.8	1.24×10^3	110
	5 M NaCl	3.41	0.304	9.26	0.826

Table 21. pH Results

Sample Description	Extraction Solvent	Results	
		1 day	21 days
Cassette	WFI	10.5	10.1
	0.1 M H_3PO_4	1.9	1.9
	0.5 N NaOH	13.3	13.3
	5 M NaCl	9.9	9.4

Table 22. Non-volatile Residue Results

Sample Description	Extraction Solvent	Results			
		1 day		21 days	
		µg/ml	µg/cm²	µg/mL	µg/cm²
Cassette Assembly	WFI	748	66.8	2.88×10^3	257
	50% EtOH	1.29×10^3	115	3.57×10^3	319

6.2.1 Acceptance Criteria

The extractables testing is compliant when the study has reached its 21-day conclusion. Information gathered is presented in a report format and was reviewed to ensure study protocols were followed. Failure to follow protocols as written would require a deviation to justify that the results of the extractables testing is still valid.

- Operators must follow approved protocols.
- All other test components must perform their function as described in the protocols.
- Instrument control test results must be valid.

6.3 Endotoxin

TangenX SIUS Cassettes produced by Repligen are flushed, packaged, and stored in 0.2 M NaOH prior to shipment. The careful preparation of these cassettes allows them to be used in a biopharmaceutical process following a brief buffer equilibration step; no additional sanitization of the cassette is required. The endotoxin study was conducted to verify that TangenX SIUS Cassettes do not contain endotoxin that could potentially contaminate a process stream. This study quantifies the amount of endotoxin transferred to a PBS solution that was recirculated through a 0.1 m^2 TangenX SIUS Cassette. A minimal volume of phosphate buffered saline was used for the recirculation so as not to significantly dilute the sample. The buffer was then evaluated for endotoxin count by a contract lab.

The endotoxin analysis was conducted under USP 30, NF 25, 2007. <85> Bacterial Endotoxin Test, Guidance on Validation of the Limulus Amebocyte Test as an End-Product Endotoxin Test for Human and Parenteral Drugs, Biological Products, and Medical Devices, December 1987.

Two membrane chemistries and one cassette type were chosen to represent the entire product line:TangenX SIUS PD 0.1 m^2 L-Screen Channel Cassette with 10 kD ProStream and 10 kD HyStream membrane. The 0.1m^2 TangenX SIUS PD L-Screen Cassette was chosen as it accurately represents the construction of the entire product line including the TangenX SIUS Cassette. Cassettes were manufactured and evaluated in triplicate. Each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. The procedure used for this endotoxin study is found in the approved study procedure TX1001-POQ-135. The system was prepared and sanitized as specified and approved procedural steps were followed during the study. The system was initially assembled, sanitized with 0.5 M NaOH, and flushed with DI water. The first line of Table 23 shows the results of the filtration system alone with no membranes installed. The data show the system did not significantly contribute to the cassette results. Once a baseline was generated for the filtration system, the experiments for a set of three cassettes containing ProStream membrane and a set of three cassettes containing HyStream membrane began. One 0.1 m^2 cassette was installed in the hardware and equilibrated with 1 L of phosphate buffered saline. The buffer was drained from the system and then 1 L of phosphate buffered saline was recirculated for 8 hours. The buffer was analyzed, and the results reported in Table 23. The results of the endotoxin count study show that the level of endotoxin was below the detection limit and below acceptable limits when compared to industry standards.

Table 23. Endotoxin Count Study Results (Dilution)

Sample Number	Dilution	Reported Result (EU/mL)
POQ-135_Control	Neat	<0.00500
POQ-135-S1	Neat	<0.00500
POQ-135-S2	Neat	<0.00500
POQ-135-S3	Neat	<0.00500
POQ-135-S4	Neat	<0.00500
POQ-135-S5	Neat	0.00979
POQ-135-S6	Neat	<0.00500

Once the results were generated, the concentration of endotoxin was then multiplied by the sample volume and then divided by the area of the filtration cassette to give a normalized result, as shown in Table 24. The relationship between endotoxin level and filtration area can be used to determine the amount of endotoxin in a single filter or a group of stacked filters prior to use.

Table 24. Endotoxin Count Study Results (Recirculation)

Sample Number	Recirculation Volume (mL)	Cassette Area (cm^2)	Normalized Result (EU/cm^2)
POQ-135-S1	1000	1000	<0.00500
POQ-135-S2	1000	1000	<0.00500
POQ-135-S3	1000	1000	<0.00500
POQ-135-S4	1000	1000	<0.00500
POQ-135-S5	1000	1000	0.00979
POQ-135-S6	1000	1000	<0.00500
Average			**<0.00500 EU/cm^2**

6.4 BSE-Free Materials

Raw materials used in the manufacture of these products have been accepted for use in accordance with standard operating procedures and meet all incoming release criteria. Repligen certifies that the components used in the production of both membranes and filtration cassettes are BSE free.

The raw materials used in the manufacture of Repligen membrane and filtration cassettes do contain traces of animal derived material. Process stabilizers required to produce several of the polymer-based materials are made using stearic acid. This originates from tallow, a rendered form of beef lard.

However, risk is minimized using this tallow-based stabilizer. Tallow derivatives for industrial, cosmetic, or pharmaceutical uses are considered safe regarding the risk of contracting TSE/BSE when certain inactivation conditions are met as follows:

- The beef tallow used is TSE/BSE free, as the beef tallow is supplied together with a certificate from the authorities responsible, which conform that the tallow originates from healthy animals (ante and postmortem).
- The processing conditions meet the requirements of the “Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products” EMEA/410/01 Rev. 3, effective July 1, 2011.

- The above document(s) define an inactivation method and a hydrolysis process of at least 200°C under an approximate pressure for 20 minutes. These conditions are far exceeded in the production of stabilizer as the tallow is hydrolyzed at about 230°C under 30 bar for at least 6 hours.
- The stearic acid does not come from high risk countries.

6.5 Particulates

The following study was conducted to quantify the particulate count from an initial flush from the tangential flow filtration cassettes. A minimal volume of water for injection was used to perform the flush so as not to dilute the sample. The experiment was performed in triplicate where each cassette was flushed with 100 mL of water, displacing the storage solution. The storage solution flush was then evaluated for particulate matter by a contract lab. The following report outlines steps that were taken to determine the ideal conditions under which to remove the storage solution. The information gathered from this study was used to draft portions of the cassette's certificate of conformance and will be referenced in other supporting documents.
Several cassettes were manufactured and evaluated in triplicate; each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process. The following steps were taken as part of the study:

- Cassettes were prepared using approved procedures.
- Cassettes were flushed and the liquid analyzed.

This section summarizes the results generated while evaluating the cassettes manufactured at Repligen for endotoxin count. Each cassette was tested and released using approved QC procedures.

One cassette type was chosen for this study to represent the entire product line: TangenX PRO PD 0.1 m^2 L-Screen Channel Cassette with 10 kD ProStream membrane. The 0.1m^2 TangenX PRO PD Cassette was chosen as it accurately represents the construction of the entire product line, including TangenX SIUS Cassettes. Each cassette was evaluated in triplicate. The cassettes were installed in the TangenX PRO PD Cassette holder and evaluated for particulate count. The cassettes were flushed with water and analyzed for particulate count.

The procedure used for the particulate count study was adapted from TX1001-POQ-118. The system was prepared and sanitized as specified. Only the initial cassette flush was performed at a reduced volume: 100 mL. The particulate count analysis was conducted under USP 30, NF 25, 2007. <788> Particulate Matter in Injections.

The results of the particulate count study show the test articles meet the test requirements as defined in the USP guidelines. The sample complies with the test if the average particles present in the units tested do not exceed 12 particles/mL equal to or greater than 10 µm and 2 particles/mL equal to or greater than 25 µm.

The results of the study are summarized in Table 25. The control data was for the filtration system alone, with no membranes installed. The system was assembled, sanitized with 0.5 M NaOH, and then flushed with DI water. The control sample consisted of 100 mL of water for injection flushed through the empty system as a baseline control. The data show the system contributes to a portion of the particles found in the test samples but did not contribute to a failure. The cassettes were then evaluated in triplicate. The raw data from each set of cassettes may be found in the completed development report.

In conclusion, the particulate count study showed the test articles meet the test requirements as defined in the USP guidelines <788> for particulate matter in injections. It was shown that the cassette manufacturing process minimizes the particulate count prior to shipment of the cassette products.

Table 25. Particulate Count Study Results

Sample Number	Particles 10 – 25 Microns	Particles >25 Microns	Fibers >100 Microns
Control-01	0.178 per/mL	0.022 per/mL	0.000 per/mL
CA7331-01	0.011 per/mL	0.000 per/mL	0.022 per/mL
CA7331-02	0.178 per/mL	0.000 per/mL	0.022 per/mL
CA7331-03	0.067 per/mL	0.033 per/mL	0.022 per/mL

7. Qualification

7.1 Equipment Qualification

IQ and OQ were performed for each piece of critical equipment utilized in the production of the membrane and cassette assembly. The IQ/OQ were executed with documented results and a written report. The following equipment was qualified before the validation of the membrane and cassette assembly process:

- Casting machine
- Post-treatment skid
- Drying machine
- Vacuum pump
- Urethane dispensing machine

Production and Quality Assurance were responsible for the qualification and documentation of the equipment.

7.2 Qualification of QC Instruments

The instruments used in the QC testing of the membranes and cassettes were calibrated as required. The instruments were qualified during the Membrane QC Testing and Cassette QC Testing Procedures qualification.

7.3 Qualification of Critical Utilities

The term critical utility is understood at minimum to be the utilities, which might have an impact on product quality or are in contact with the product:

- Water system
- Compressed air

Non-compliances/deviations may lead to a change and might require revalidation of a step of the process.

8. Manufacturing Process Validation

Validation of the process was carried out on three membrane lots per membrane chemistry and four cassette product groups, all of which were produced and found to be compliant with the process specifications. Before process validation began, the following tests were performed and concluded positively:

- Class VI testing
- Extractables testing
- Leachables testing
- Protein binding study
- Membrane storage study
- Cassette storage study

Validation was carried out according to the approved validation plan and results recorded in the Validation Document Package. The Validation Document package includes the validation procedure, test results and validation report.

Product Validation Matrix:

- Low Pressure Screen Channel (L-Screen)
- Extra Low Pressure Screen Channel (E-screen)
- 0.5 mm Open Channel (J Channel)

Within the framework of the validation, QC methods, the utilities, equipment, and personnel were qualified. Quality Assurance approved the VMP of the production process, and the following information included:
Object and field of application

- Reference documents
- Responsibilities

- Prerequisites
- Description of the interfaces (suppliers)
- Description of the equipment used and the building
- Summary of all the data (R&D studies, previous production if applicable)
- Flowchart of the process
- Risk analysis
- Definition of the validation lots and project planning
- Type of validation, i.e., prospective, or retrospective validation
- Revalidation conditions

The results of a risk analysis allowed for the drafting of the sampling plan, defining the intervals between the sampling and the number of samples to be taken, in addition to what is described in the Validation Document Package. The VPL was also approved by the Quality Assurance Department prior to starting validation.

During validation, all information providing traceability for the membranes and cassettes was compiled in a batch production file, an analytical lot file, and the corresponding VPL. A final validation report that summarizes all the production and quality control data for the membranes and cassettes was written and approved by Quality Assurance.

8.1 Membrane Process Validation

The process validation of the ultrafiltration and microfiltration membranes produced at Repligen was carried out as specified. The validation included approved procedures for the casting solution preparation, membrane casting procedure, 20% glycerin/0.05% sodium azide procedure, membrane QC testing procedure, and the corresponding forms.

The individual procedures were combined and executed as one validation lot. Three consecutive lots were manufactured as part of the validation for each membrane type. Two membrane chemistries, HyStream 10 kD and ProStream 10 kD were each validated since these membranes represent the Repligen membrane product line. The validation was considered successful as the three lots of each membrane type were in conformance with the defined specification.

The required condition for validation of the membrane production process was the manufacturing of three (3) consecutive compliant lots. A lot was certified as compliant once it had been manufactured in accordance with:

- Development documents
- Product specifications
- Associated procedures

Validation of the process was performed in two distinct stages: validation as performed on the membrane production process and on the cassette production process. Once the individual procedures were combined and executed as one validation lot, the membranes manufactured were evaluated for their performance using approved SOPs. Three consecutive lots were manufactured as part of the validation for each membrane type.

Each of the membrane lots was found to meet product specifications following approved SOPs and found to be within compliance. The membrane validation is complete and the membrane manufacturing process at Repligen is considered validated.

Figure 19. ProStream Membrane Validation Data Summary

Membrane: **ProStream 10 kD** Lot Number: **F7267A (1 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	17.5 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	193.3 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	48.7 %	30 – 60 %	Yes
Solute Flux (Passing)	100.4 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	91.8 %	> 85 %	Yes

Membrane: **ProStream 10 kD** Lot Number: **F7268A (2 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	18.1 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	199.6 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	40.5 %	30 – 60 %	Yes
Solute Flux (Passing)	90.0 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	90.9 %	> 85 %	Yes

Membrane: **ProStream 10 kD** Lot Number: **F7269A (3 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	19.5 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	201.9 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	37.6 %	30 – 60 %	Yes
Solute Flux (Passing)	96.9 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	87.9 %	> 85 %	Yes

Figure 20. HyStream Membrane Validation Data Summary

Membrane: **HyStream 10 kD** Lot Number: **F7267B (1 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	20.1 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	201.2 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	51.0 %	30 – 60 %	Yes
Solute Flux (Passing)	102.2 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	91.1 %	> 85 %	Yes

Membrane: **HyStream 10 kD** Lot Number: **F7268B (2 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	17.6 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	171.6 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	56.1 %	30 – 60 %	Yes
Solute Flux (Passing)	100.2 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	91.3 %	> 85 %	Yes

Membrane: **HyStream 10 kD** Lot Number: **F7269B (3 of 3)**	MEASURED VALUE	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
Normalized Water Permeability	20.0 LMH/psi	9.5 – 27 LMH/psi	Yes
Solute Flux (Passing)	176.7 LMH	140 – 250 LMH	Yes
Solute Rejection (Passing)	48.0 %	30 – 60 %	Yes
Solute Flux (Passing)	90.9 LMH	75 – 110 LMH	Yes
Solute Rejection (Passing)	89.2 %	> 85 %	Yes

8.2 Cassette Process Validation

The process validation of the tangential flow filtration cassette produced at Repligen was carried out as specified. The validation included the following approved procedures: Urethane Part A Mixing Procedure, Lamination Procedure, Die Cutting Procedure, Mold Release Mixing Procedure, Single-Use Cassette Assembly Procedure, Final Packaging Procedure, Cassette QC Testing Procedure, and the corresponding forms.

The individual procedures were combined and executed as one validation group where three consecutive serialized cassettes were manufactured. Two cassette types, TangenX SIUS PD 0.1 m^2 L-Screen Channel and TangenX SIUS 0.5 m^2 L-Screen Channel, with two membranes, ProStream 10 kD and HyStream 10 kD, were validated. These cassette configurations represent the entire TangenX SIUS Cassette product line.

The validation was considered successful since the three cassettes in each group of each cassette/membrane type were in conformance with the defined specifications. Twelve cassettes were manufactured during the validation. The cassettes were divided into four (4) groups by product type (TangenX SIUS PD Cassettes vs. TangenX SIUS Cassettes) and membrane combinations, as follows:

- 3 each, TangenX SIUS PD, 0.1 m^2 with L-Screen Channel and ProStream 10 kD membrane
    - Lot #'s 18309304, 18309305, 18309306
- 3 each, TangenX SIUS PD Cassette, 0.1 m^2 with L-Screen Channel and HyStream 10 kD membrane
    - Lot #'s 18309301, 08309302, 18309303
- 3 each, TangenX SIUS Cassette, 0.5 m^2 with L-Screen Channel and ProStream 10 kD membrane
    - Lot #'s 18310304, 18310305, 18310306
- 3 each, TangenX SIUS Cassette, 0.5 m^2 with L-Screen Channel and HyStream 10 kD membrane
    - Lot #'s 18310301, 18310302, 18310303

Each group contained three consecutively serial numbered cassettes where each cassette was individually tested according to the approved Cassette QC Testing Procedure and QC Release specifications. Each cassette was tested in the cassette QC test area for liquid volume flow rate and air mass flow rate. The test results for each cassette are found in Figure 21 and Figure 22.

Following the validation, Quality Assurance conducted a review of the test data, verifying the adherence to set specifications. Quality Assurance was responsible for the final review of the executed validation procedures and test results.

The Cassette Assembly process was validated separately from the Membrane Manufacturing process (VPL-PRO-101-TX1001) where each had a separate Validation Report written. VPL-PRO-102- TX1001 applies to the cassette production process only. This process was validated if the specifications defined in the VMP-PRO-102-TX1001 and VMP-PRO-102-TX1001-ADDENDUM were met.

A total of twelve cassettes in two configurations types and two membrane chemistries, as defined by the validation plan, were manufactured, and tested. The following tables provide the measured QC results versus the QC specifications for each of the twelve products manufactured. All were found to be in conformance with the defined expectations.

Figure 21. TangenX SIUS PD Cassette Process Validation Summary

MEMBRANE	CASSETTE LOT #	MEASURED VALUE	SOLUTION TEMP °C	NORMALIZED VALUE *	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
ProStream 10 kD	18309304	0.540 L/min	22	0.516 L/min	0.4 to 0.8 L/min	YES
		7.5 ccm			≤ 30 ccm	YES
	18309305	0.570 L/min	22	0.544 L/min	0.4 to 0.8 L/min	YES
		20 ccm			≤ 30 ccm	YES
	18309306	0.600 L/min	22	0.573 L/min	0.4 to 0.8 L/min	YES
		10 ccm			≤ 30 ccm	YES
HyStream 10 kD	18309301	0.576 L/min	22	0.550 L/min	0.4 to 0.8 L/min	YES
		4 ccm			≤ 30 ccm	YES
	18309302	0.588 L/min	22	0.562 L/min	0.4 to 0.8 L/min	YES
		7 ccm			≤ 30 ccm	YES
	18309303	0.558 L/min	22	0.533 L/min	0.4 to 0.8 L/min	YES
		22 ccm			≤ 30 ccm	YES

Figure 22. TangenX SIUS Cassette Process Validation Summary

MEMBRANE	CASSETTE SERIAL #	MEASURED VALUE	SOLUTION TEMP °C	NORMALIZED VALUE *	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
ProStream 10 kD	18310304	2.65 L/min	22	2.53 L/min	2.0 to 4.0 L/min	YES
		40 ccm			≤ 150 ccm	YES
	18310305	2.67 L/min	23	2.49 L/min	2.0 to 4.0 L/min	YES
		15 ccm			≤ 150 ccm	YES
	18310306	2.57 L/min	23	2.40 L/min	2.0 to 4.0 L/min	YES
		23 ccm			≤ 150 ccm	YES
HyStream 10 kD	18310301	2.72 L/min	22	2.60 L/min	2.0 to 4.0 L/min	YES
		80 ccm			≤ 150 ccm	YES
	18310302	2.54 L/min	22	2.43 L/min	2.0 to 4.0 L/min	YES
		70 ccm			≤ 150 ccm	YES
	18310303	2.61 L/min	22	2.49 L/min	2.0 to 4.0 L/min	YES
		20 ccm			≤ 150 ccm	YES

9. Release Testing

9.1 Analytical Method Validation

Selective analytical methods for the quantitative evaluation of membrane and membrane-based products are necessary for the QC release of these devices. Analytical method qualification includes all the procedures that demonstrate that a particular method used for quantitative measurement of samples in a given matrix is reliable and reproducible for the intended use. The fundamental parameters for qualification include specificity, linearity, accuracy, precision, and robustness.

Method validation involved documenting that the performance characteristics of the methods were suitable and reliable for the intended applications. The acceptability of analytical data corresponds directly to the criteria used to qualify the method. Specific, detailed descriptions of the analytical methods were written in the form of a standard operating procedure for both membrane and cassette QC testing. Each step in these methods was investigated to determine the extent to which environmental, matrix, or procedural variables can affect the estimation of material in the matrix.

In the case of sensitive quantitative procedures such as these, appropriate steps were taken to ensure the lack of matrix effects throughout the application of the method. These analytical methods were validated for the intended use of membrane characterization and cassette release. All experiments used to make claims or draw conclusions about the validity of the method are presented in a method qualification report. In-process test methods include both membrane and cassette QC methods.

9.2 Membrane QC Method Validation

The purpose of the membrane QC testing method validation was to validate the membrane QC testing procedure. This procedure refers to ultrafiltration and microfiltration membranes manufactured by Repligen. Membranes are initially manufactured and then tested for performance prior to being incorporated into a cassette product. A report summarizing the verification of specificity, linearity, accuracy, precision, and robustness of the membrane QC test procedure was written. Minimum requirements, including acceptance specifications for the methods were set during the method development and validation cycle. The acceptance criteria are found in each of the data sheets found in the body of the report.

The principles followed for the membrane QC method validation were based on cGMP guidelines and helped Repligen ensure the test method was acceptable for use. The membrane QC procedure is used to verify water permeability and protein rejection for each membrane. This information is then used to accept or reject the membrane. At the conclusion of the validation, it was proven that membrane QC method meets requirements set by Repligen for specificity, linearity, accuracy, precision, and robustness. Minimum requirements, which were essentially acceptance specifications for the methods, were met during the method development and validation cycle and the QC membrane test procedure considered validated.

9.3 Cassette QC Method Validation

The purpose of the cassette QC testing method validation was to validate the cassette QC testing procedure. This procedure refers to ultrafiltration and microfiltration cassettes manufactured by Repligen. The cassettes are initially manufactured and then tested for performance prior to being released as final product. A written report summarizes the verification of specificity, linearity, accuracy, precision, and robustness of the cassette QC test procedure. Minimum requirements, including acceptance specifications for the methods, were set during the method development and validation cycle. The acceptance criteria are found in each of the data sheets found in the body of the report. The procedure used for the method validation was described in the validation protocol listed the steps that were followed during the validation.

The principles followed for the validation were based on cGMP guidelines and helped Repligen ensure the cassette QC test method was acceptable for use. The cassette QC procedure was used to verify air diffusion and cross flow rate for each cassette. This information is then used to accept or reject the cassettes manufactured at Repligen. At the conclusion of the validation, it was proven that cassette QC method meets requirements set by Repligen for specificity, linearity, accuracy, precision, and robustness. Minimum requirements, which were essentially acceptance specifications for the methods, were met during the method development and validation cycle and the QC membrane test procedure considered validated.

9.4 Release Specifications

A complete set of TangenX SIUS PD Cassette and TangenX SIUS Cassette release specifications is listed in Figure 23, as taken from document FORM-0491. Figure 24 presents the release specifications for both membrane chemistries, as taken from document FORM-0467.

Figure 23. Cassette QC Release Specifications

CONFIG ID #	PRESSURE DROP		AIR INTEGRITY	
	PRESSURE PSI	FLOW RATE[2] LITER/MINUTE	FLOW RATE CCM	AIR PRESSURE
LOW PRESSURE SCREEN CHANNEL : CHANNEL ID = L				
LP1 / MP1	10 ±0.5	0.040 TO 0.080	≤ 3	SEE NOTE 4
LP2 / MP2		0.080 TO 0.160	≤ 6	SEE NOTE 4
L01 / M01		0.40 TO 0.80	≤ 30	SEE NOTE 4
G02		0.80 TO 1.60	≤ 60	SEE NOTE 4
G05		2.00 TO 4.00	≤ 150	SEE NOTE 4
G15		6.00 TO 12.00	≤ 450	SEE NOTE 4
G25	5 ±0.5	5.00 TO 10.00	≤ 750	SEE NOTE 4
EXTRA LOW PRESSURE SCREEN CHANNEL : CHANNEL ID = E				
LP1 / MP1	5 ±0.5	0.06 TO 0.12	≤ 3	SEE NOTE 4
LP2 / MP2		0.12 TO 0.24	≤ 6	SEE NOTE 4
L01 / M01		0.60 TO 1.20	≤ 30	SEE NOTE 4
G05		3.00 TO 6.00	≤ 150	SEE NOTE 4
G15	2.5 ±0.5	4.50 TO 9.00	≤ 450	SEE NOTE 4
G25		7.50 TO 15.00	≤ 750	SEE NOTE 4
0.5mm OPEN CHANNEL : CHANNEL ID = J				
LP1 / MP1	≤ 1.0	0.11 TO 0.13	≤ 3	SEE NOTE 4
LP2 / MP2		0.23 TO 0.25	≤ 6	SEE NOTE 4
L01 / M01		1.14 TO 1.26	≤ 30	SEE NOTE 4
G05	≤ 1.0	5.70 TO 6.30	≤ 150	SEE NOTE 4
G15	≤ 0.5	8.55 TO 9.45	≤ 450	SEE NOTE 4
G25	≤ 0.2	5.70 TO 6.30	≤ 750	SEE NOTE 4

NOTES

1) SPECIFICATIONS APPLY FOR PROSTREAM AND HYSTREAM MEMBRANES.

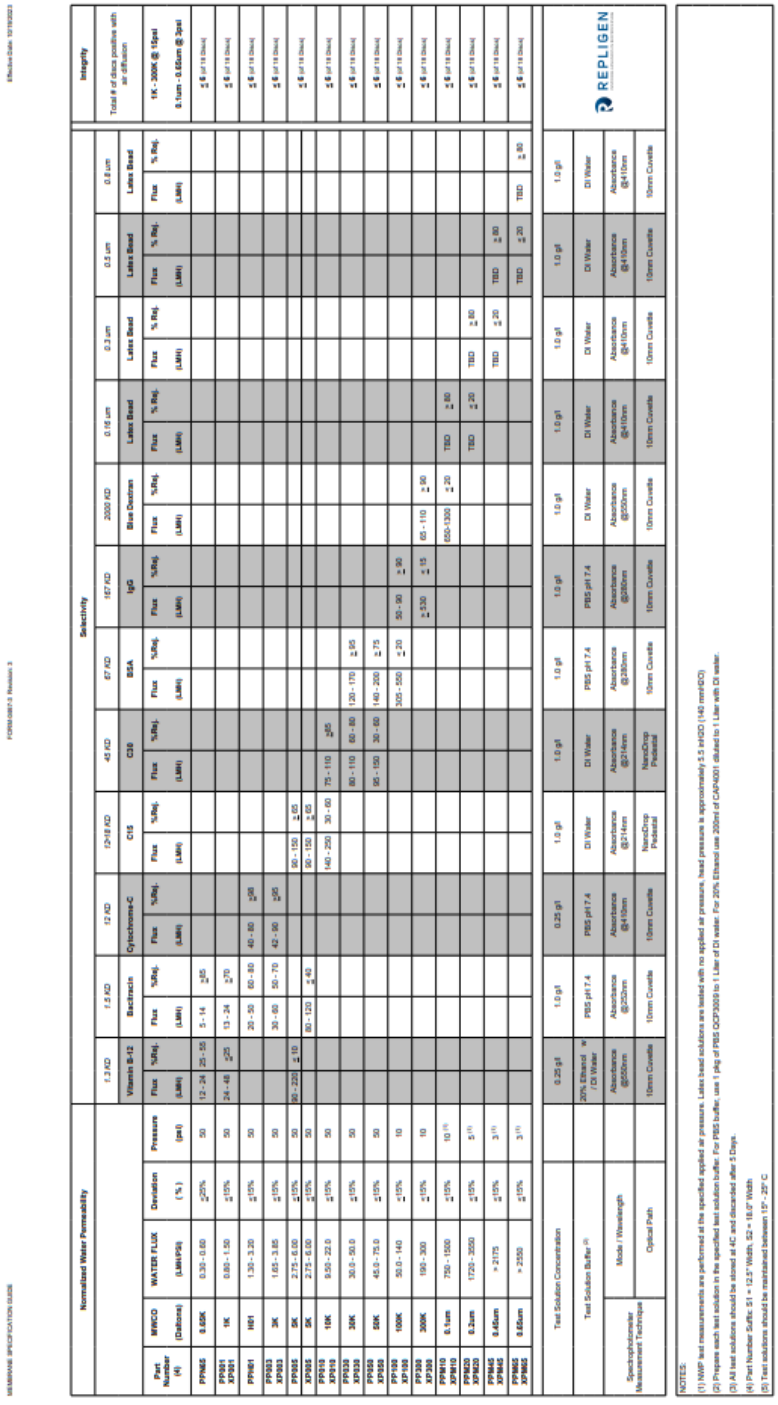
2) CHECK TEMPERATURE OF SODIUM HYDROXIDE SOLUTION AND USE THE APPROPRIATE FLOW RATE VALUES FOR THAT TEMPERATURE (PAGES 1-17).

3) CHECK THAT GASKETS ARE IN GOOD CONDITION. IF NOT, REPLACE WITH NEW FROM INVENTORY AND STORE IN NaOH WHEN NOT IN USE.

4) CASSETTE AIR INTEGRITY SET PRESSURES ARE DEFINED BELOW BASED ON MEMBRANE CUT OFF (MWCO). MASS FLOW RATE LIMITS SHOWN IN THE TABLES APPLY AT EACH PRESSURE.
- USE 15 psi for 1kD, 3kD, 5kD
- USE 7.3 psi for 10kD, 30kD, 50kD, 100kD, 300kD
- USE 3 psi for 0.1um, 0.2um, 0.45um, 0.65um

USE THE APPROPRIATE AIR PRESSURE FOR NON-STANDARD MWCO's BASED ON WHERE THE MWCO FALLS AMONGST THE RANGES SHOWN ABOVE.

Figure 24. Membrane QC Release Specifications



9.5 Certificate of Conformance

Figure 25 shows an example of the standard Quality Assurance Certificate provided with each cassette manufactured by Repligen. A specific product part number, serial number, and description will be included on the label attached in the upper left corner of the certificate.

Figure 25. QA Certificate of Conformance

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

Repligen Corporation
111 Locke Drive
Marlborough, MA 01752

Quality Assurance Certificate

This is to certify that the TangenX® SIUS® Cassettes as indicated by the affixed label complies with the following descriptions and specifications:

Product Quality – TangenX® SIUS® Cassettes

This product has been manufactured in a fully validated and documented manufacturing process under an ISO 9001:2015 quality management system.

This product has been manufactured and tested in accordance with standard operating procedures and meets all release criteria. Repligen Corporation certifies that this product will perform according to published specifications providing it is used according to the manufacturer's recommendations.

Each membrane lot is visually inspected prior to incorporation into a cassette. Before assembly, the membrane used in each cassette is tested for conformance with flow rate, retention, and other physical specifications.

Each cassette has been flushed with D.I. water, sanitized with 0.2M NaOH, and individually tested to ensure conformance to the following performance specifications:

1. Hydraulic performance - a measure of the cross flow rate at a specified pressure drop.
2. Integrity - a measure of the rate of air diffusion through the cassette at a specified pressure differential.

The results of these tests were found to meet or exceed the minimum requirements set by our Quality Assurance Department.

REPLIGEN Marlborough, Massachusetts USA www.repligen.com/tangenx

TangenX® SIUS® Cassette

BATCH # 99999999 ■ SINGLE-USE ONLY
USE BY: 06-OCT-2027
MEMBRANE: HyStream (Low Fouling mPES)
MWCO: 30 kD
CHANNEL: LP Screen Channel
AREA: 2.5 m² (26.9 ft²)
SERIAL #
34280002
CATALOG #
XP030G25L

USP Safety Information

All component materials meet:

1. Current requirements for USP Class VI biological test for plastics.
2. The test article(s) meets the test requirements as defined in the USP guidelines: USP 30, NF 25, 2007, <788> Particulate Matter in Injections.
3. EMA/410/01 Rev.3
   Note: Trace amounts of animal derived material originating from tallow exist, but the processing conditions meet the requirements described in section 6.4 of the Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3).
4. Certifications that the components used in the production of the filters are free of melamine.

All components materials used in cassettes manufactured by Repligen have been independently tested for USP safety and were shown to be safe according to:

1. L929 MEM Elution per USP <87>
2. Class VI per USP <88>
3. Hemolysis — Indirect with Rabbit Blood

All finished component materials were tested under GLP conditions for extractable substances using:

- Total Organic Carbon
- Oxidizable Substances
- Reverse Phase HPLC
- GCMS
- Non-Volatile Residue
- Infrared Spectrophotometry

A population of randomly selected TangenX® SIUS® cassettes were tested for:

1. Endotoxin testing following references the limulus amebocyte lysate (LAL) test as an end product. Acceptance criteria specified as < 0.25 EU/ml as determined by the LAL test method.
2. Bioburden testing for Aerobic, Anaerobic, and Yeast & Mold by membrane filtration following references ANSI.AAMI/ISO 11737-1. Acceptance criteria specified as <10 CFU/100 ml as determined by the test method.

Signature Required:
Reviewed and approved for accuracy and completeness.
Paul Wallace, Director of Quality
Signature and Title

SAMPLE

Document Number: QS-2995 Revision: 3 Effective Date: 10/8/2024 Legacy Document #: QADOC012
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10. List of Study Reports

- TX1001-POQ-117-R Protein Binding Study Report
- TX1001-POQ-135-R TangenX SIUS Cassette Leachables Study Report
- TX1001-POQ-133-R TangenX SIUS Cassette Robustness Study Report
- TX1001-POQ-125-R Membrane QC Testing Method Validation Report
- TX1001-POQ-126-R TangenX Water Systems Report
- TX1001-POQ-132-R Cassette QC Testing Method Validation Report
- VPL-PRO-101-TX1001-R Membrane Validation Report
- VPL-PRO-103-TX1001-R TangenX SIUS Cassette Process Validation Report
- DR-07-005 Cassette Particulate and Endotoxin Count Study Report
- DR-09-010 Membrane Storage Study Interim Report
- DR-09-012 TangenX SIUS Cassette Storage Study Interim Report

11. References

- Agalloco, J. (1995), 'Validation: an unconventional review and reinvention', PDA J Pharm Sci Technol., vol. 49, no. 4, pp. 175-179.
- FDA (1987), Guideline on general principles of Process Validation, US Food and Drug Administration, Maryland, USA.
- ISO (1994), ISO 8402:1994: Quality management and quality assurance -- Vocabulary, International Organization for Standardization, Geneva, Switzerland.

12. Index

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