TangenX® SIUS® Cassettes For Tangential Flow Filtration

Regulatory Support File

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

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Abbreviations

AAMI	Association for the Advancement of Medical Instrumentation
CFU	Colony Forming Units
cGMP	Current Good Manufacturing Practice
DI	Deionized
ERP	Enterprise Resource Planning
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
kD	Kilodalton
mPES	Modified Polyethersulfone
MWCO	Molecular weight cutoff
NMWL	Nominal Molecular Weight Limit
NWP	Normalized water permeability
PD	Process development
TFF	Tangential Flow Filtration
WFI	Water for injection
UF	Ultrafiltration

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® 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 Repligen Quality Policy

A copy of the Repligen quality policy can be found at https://www.repligen.com/resources/quality.

1.2 Safety notices

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

1.3 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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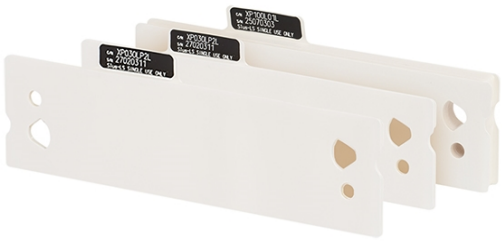
1.4 Product description

TangenX® SIUS® Cassettes are the first truly single-use tangential flow filtration (TFF) cassettes for the biopharmaceutical industry. These single-use cassettes have been designed to offer comparable performance to reusable products at a fraction of their cost. The TangenX® SIUS® Cassette product family is completely interchangeable with existing cassette hardware currently 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 (i.e., enzymes, proteins, oligonucleotides, etc.). It recirculates the retentate across the membrane surface, which minimizes the fouling of the membrane. The TangenX® SIUS® TFF Cassette is a rigid, flat rectangular filter design comprised of multiple layers of permeable membrane and polypropylene screens. Fluid is pumped through the feed channel, which may or may not have a screen, tangentially to the membrane's 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 and ready for processing after equilibration with buffer. The TangenX® SIUS® Cassette is simply installed, conditioned with buffer to neutralize the pH and it is ready for the process. Most importantly, 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 m^2, 0.02 m^2, 0.1 m^2 surface areas, as well as three different channel configurations. The L screen (also referred to as L-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 also available in 0.5 m^2, 1.5 m^2, 2.5 m^2 surface areas, as well as three different channel configurations. 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 10s - 1000s of liters.

The TangenX® SIUS® Cassette family represents the latest development in tangential flow filtration cassette design and performance. They are 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.

1.5 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
- All fluid paths meet USP <88> Biological Reactivity Tests for Class VI Plastics criteria

2. Product information

2.1 Cassette design

TangenX® SIUS® Cassettes are designed and constructed using FDA approved materials that have been validated for use for demanding biopharmaceutical applications. Each cassette is manufactured in a fully validated and documented manufacturing process according to the principles of cGMP and meet specified 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 our TangenX® PRO reusable product line. TangenX® SIUS® PD and TangenX® SIUS® Cassettes are constructed of FDA approved materials.

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: 2.75 inch (7.0 cm)
Height: 0.125 – 0.650 inch (0.3 – 1.65 cm)
Membrane area
0.01 m^2 (0.11 ft^2)
0.02 m^2 (0.22 ft^2)
0.1 m^2 (1.1 ft^2)

Table 3. TangenX® SIUS® PD Cassette hold-up volume

Surface area	Channel type	
	Screen	Open
0.01m^2 (0.11 ft^2)	1.2 mL	5.4 mL
0.02m^2 (0.22 ft^2)	2.1 mL	8.0 mL
0.1m^2 (1.1 ft^2)	8.7 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: 0.5 – 2.5 inch (1.3 – 6.4 cm)
Membrane area
0.5 m^2 (5.4 ft^2)
1.5 m^2 (16.2 ft^2)
2.5 m^2 (26.9 ft^2)

Table 5. TangenX® SIUS® Cassette hold-up volume

Surface area	Channel type	
	Screen	Open
0.5 m^2 (5.41 ft^2)	1.2 mL	5.4 mL
1.5 m^2 (16.2 ft^2)	2.1 mL	8.0 mL
2.5 m^2 (26.9 ft^2)	190 mL	633 mL

2.2 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

2.3 Important information before you begin

Cassettes

- TangenX® SIUS® Cassettes are compatible with all TangenX® Cassette Holders.
- Cassettes may be stacked to increase filtration surface area; however, use only one type of membrane molecular weight cutoff at one time. *Do not install a mixture of cassettes with different pore sizes in the same hardware*.
- Cassettes must be equilibrated with an appropriate buffer (i.e., phosphate buffered saline) to ensure the neutralization of the 0.2 M sodium hydroxide 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 you replace gaskets 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 feed channel type and process fluid dependent.

2.4 TangenX® SIUS® Cassette installation

1. Lift the end plate off the manifold.
2. Rinse the EDPM gaskets with deionized water or WFI. Place a rinsed gasket flat against the bottom manifold; ensure that the holes in the gasket line up with the holes in the manifold.
3. Using scissors carefully open the cassette bag to 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, gaskets, and cassette are completely aligned.

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

If you are using multiple cassettes, continue the same gasket/cassette/gasket pattern, ending with a gasket between the last cassette and the end plate.

5. Place the end plate on top of the last gasket of the cassette or cassette stack.
6. Install the tie-rod spacers (if used) and washers on each bolt leaving a minimum of 18mm (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.
7. 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 values

Holder type	# bolts	Recommended torque range	
		inch-lbs	Nm
TangenX® SIUS® PD	2	120 - 180	14 - 20
TangenX® SIUS®	4	300 - 450	35 - 50
TangenX® SIUS®	2	600 - 900	70 - 100

8. **TangenX® SIUS® PD 2-bolt torque sequence:** Using the calibrated torque wrench with an 11/16-inch hex “deep style” socket, pick a bolt and place the socket over that nut and tighten the nut 1/4 turn. Next move the wrench across to the other bolt and tighten the nut ¼ turn. Alternate back and forth until the torque wrench “clicks” at each nut. Repeat this sequence until the wrench “clicks” without turning the nut. The “click” of the torque wrench indicates that the nut has reached the set point torque value.
9. **TangenX® SIUS® 4-bolt torque sequence:** Using the calibrated torque wrench with a 1 ¼ -inch hex “deep socket”, pick a bolt (B1) and place the socket over that nut and tighten the nut 1/4 turn. Then move the wrench to the next bolt (B2) diagonally across the cover and tighten the nut 1/4 turn. Next move the wrench back across the cover to the other bolt (B3) and tighten the nut 1/4 turn. Then move to the last of the four bolts (B2) and tighten the nut 1/4 turn. Alternate back and forth using this crisscross pattern until the torque wrench “clicks” at each nut. Repeat this sequence until the wrench “clicks” without turning the nut. The “click” of the torque wrench indicates that the nut has reached the set point torque value.
10. Wait 5 - 10 minutes and allow the gaskets to relax before re-torquing. Check each nut’s torque, per Table 6 using the torque wrench at its set point torque value.
11. Re-torque as needed to create a liquid-tight seal, but do not exceed the maximum torque limit for the TangenX® SIUS® holder type used (see Table 6).

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.

2.5 Equilibration of TangenX® SIUS® Cassettes

TangenX® SIUS® Cassettes must be equilibrated with an appropriate buffer (i.e., phosphate buffered saline) to ensure the neutralization of the 0.2 M sodium hydroxide storage agent in the membrane filter. Verify 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.

2.6 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 sodium hydroxide 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.

2.7 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.

2.8 Storage of Unused TangenX® SIUS® Cassettes

Membrane cassettes must remain sealed in their original packaging prior to use to maintain their characteristics, integrity, and 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

2.9 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, it also promotes increased concentration polarization and membrane compaction, which ultimately limits flow. With very low NMWL membranes, lower operating pressure may also reduce the retention of salts and very low molecular weight species.

2.10 Catalog and serial numbering system

Serial number system

Decade Code

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

Year Code

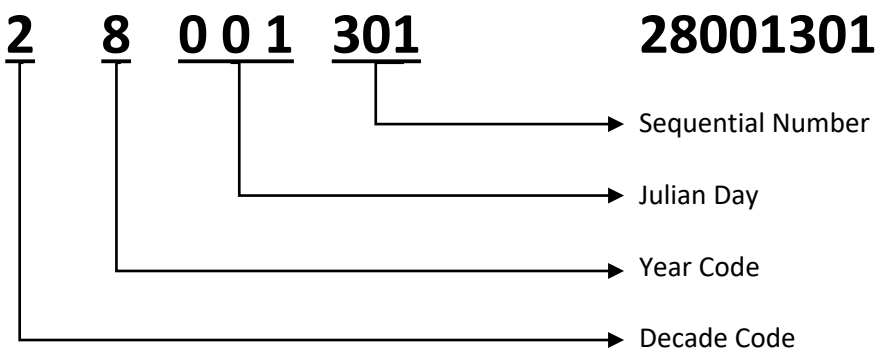
- 1-digit(last digit of current year) 0 thru 9

Julian Day

- 3-digit 001 thru 366

Sequential number

- 3-digit 001 thru 999

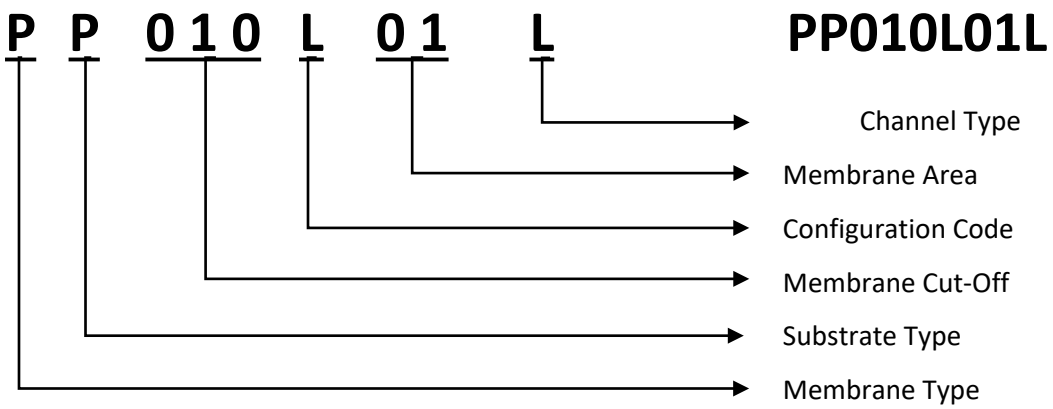


Cassette batch numbers

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 6 different 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. Catalog numbers system

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® SIUS® PD	TangenX®, Pall,	Single use (lab/pilot)	L
TangenX® SIUS® PD	Millipore, Sartorius	Single use (lab/pilot)	M
TangenX® SIUS®		Single use (process)	G
Membrane area:			
0.01 m^2	(0.11 ft^2)	Available: L, M, P1	
0.02 m^2	(0.22 ft^2)	Available: L, M, P2	
0.1 m^2	(1.1 ft^2)	Available: L, M, 01	
0.5 m^2	(5.4 ft^2)	Available: L, M, G	05
1.5 m^2	(16.2 ft^2)	Available: G	15
2.5 m^2	(26.9 ft^2)	Available: G	25
Channel type:			
LP Screen Channel		Medium woven	L
EP Screen Channel		Coarse woven	E
J Open Channel		0.5 mm	J



3. Product performance

3.1 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 micro-porous/UF transition interface. The macro-porous and UF “zones” of this membrane are now 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, 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 comparable chemical resistance 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. At typical working conditions in stirred cells (50 psig), purified water was used to measure the membrane’s 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 cutoff (MWCO) series. When reviewed in conjunction with the MWCO series normalized water permeability (NWP) data in Figure 3, the user can specifically select a membrane that best balances flux and retention for that 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 4. Membranes above 0.1 µm are characterized using latex particles (not a marker with a defined M.W.) and are therefore not included in the Figure plotting molecular weight vs percent retention. Retention of the latex particles is shown in Figure 24.

Figure 3. ProStream and HyStream membrane cutoff (MWCO) vs. normalized water

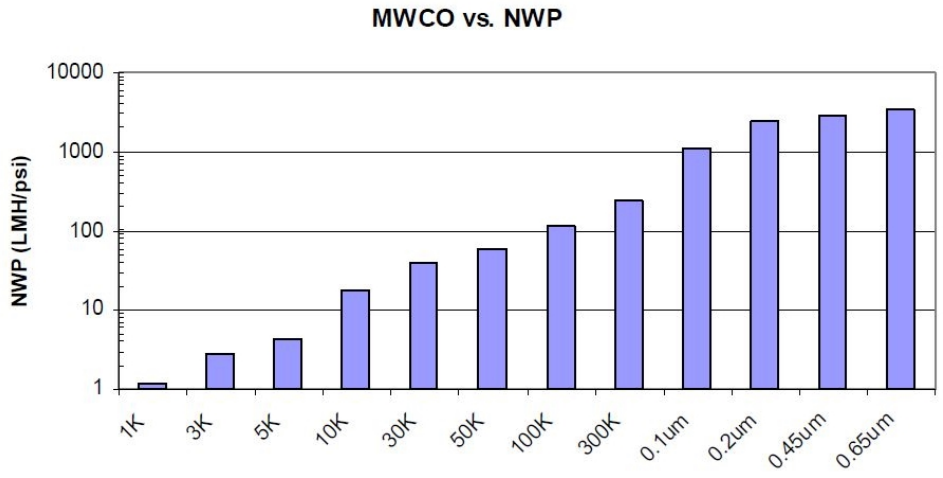
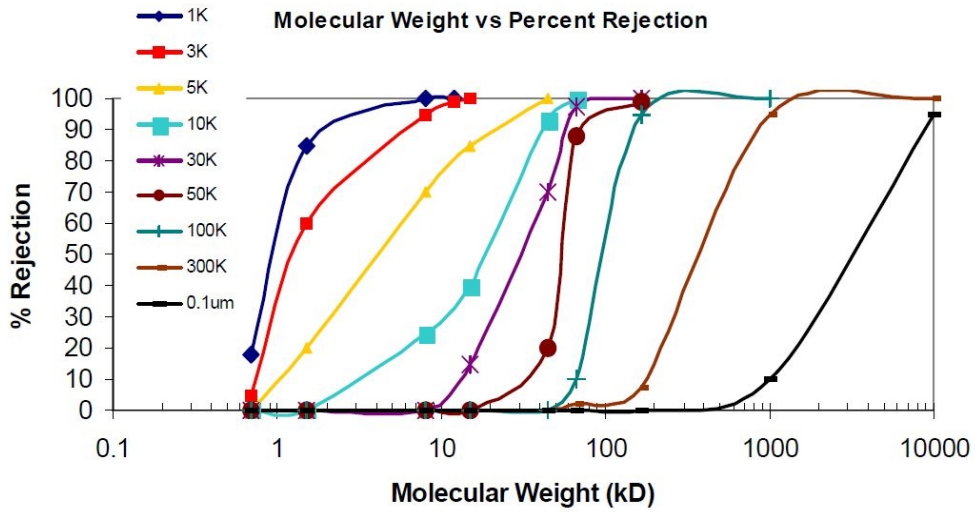


Figure 4. Membrane selectivity performance



3.2 No specific protein binding

The protein binding study was conducted to quantify the level of non-specific protein binding of two different 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 (i.e., charge effect, hydrophobic interaction, etc.). Non-specific protein binding tends to lead to yield loss and membrane fouling; both are undesirable effects.

The approved test procedure provided methods to be followed while evaluating the membranes manufactured at Repligen for non-specific protein binding. This study was applied to 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 cutoff 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 and then recorded. Several different proteins were used as models to test the non-specific binding of the membranes. Each of the proteins 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 are tabulated and 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 different sets of data. The results show the PES membrane control binds the highest amount of protein while the modified PES 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's 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" when compared to unmodified polyethersulfone (PES) membranes.

3.3 Cassette hydraulic performance

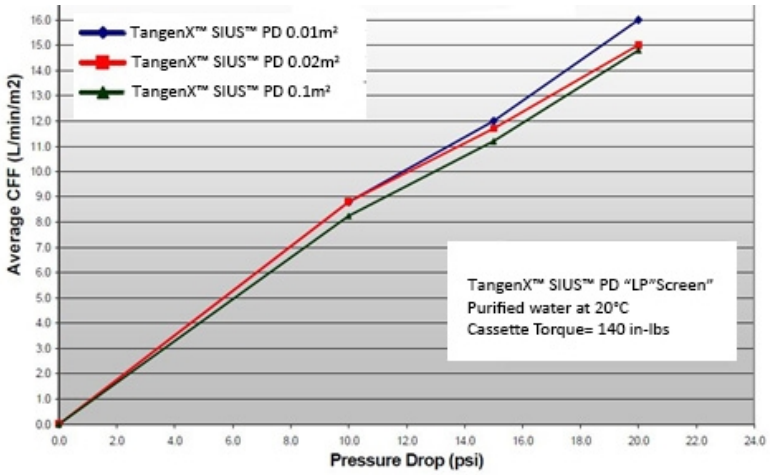
Scale-up performance is critical for successful process development and can be demonstrated by evaluating a TFF cassettes hydraulic performance using purified water. The TangenX® SIUS® Cassettes are manufactured with specific channel geometries and hydrodynamic characteristics. These hydraulic performance characteristics will 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 determined at the less than 0.1m² scale linearly to multiple m² scale

The TangenX® SIUS® PD Cassettes address these factors, as significant developments have been devoted to the channel design. 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 it meets specification. Cassettes are tested for both air integrity and for their hydrodynamic performance. This testing ensures cassette-to-cassette consistency; the result is scalable process development and reproducible manufacturing.

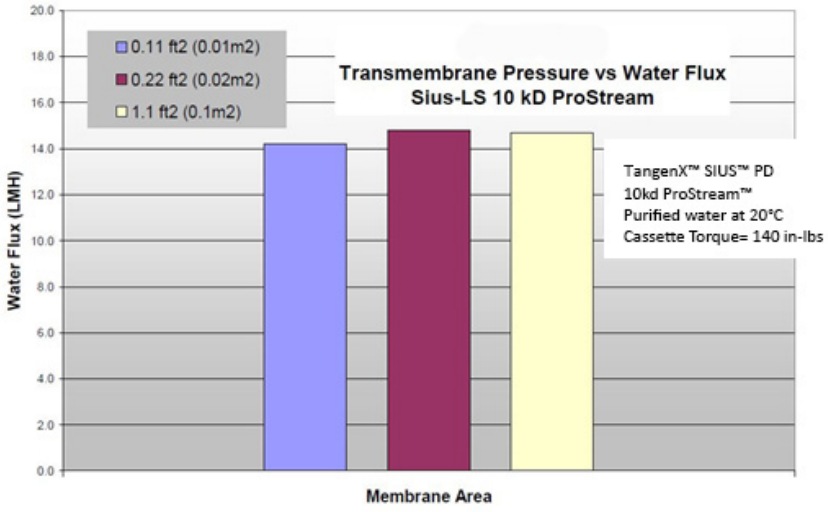
A cassette's hydraulic scalability can be evaluated using purified water under controlled conditions. This data can be used to support scalability of the TangenX® SIUS® PD Cassette product line from 0.01m² to 0.1m². Similar data supports the scale-up between the TangenX® SIUS® PD and TangenX® SIUS® products. Typically, the pressure drop between the feed and the retentate is measured at various cross flow rates. This information can then be generated for each cassette size as well as cassettes stacked together in parallel. Figure 5 shows the pressure drop versus cross flow specification for the TangenX® SIUS® PD Cassettes.

Figure 5. Pressure drop vs. Cross flow 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 or not the clean-in-place (CIP) procedure effectively removes foulants deposited on the membrane's surface during use. Figure 6 shows the transmembrane pressure (TMP) versus water flux for the 10 kD TangenX® SIUS® PD Cassettes through scale up from 0.02m² - 0.5m².

Figure 6. Transmembrane pressure vs. Water flux



A membrane's normalized water permeability (NWP) is dependent on its molecular weight cutoff (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 normalized water permeability (NWP) ranges for a given molecular weight cutoff (MWCO) are shown in Table 9. These values may be used as a guide to determine if a cassette's NWP is within specification.

Table 9. Typical NWP range for TangenX® SIUS® Cassettes

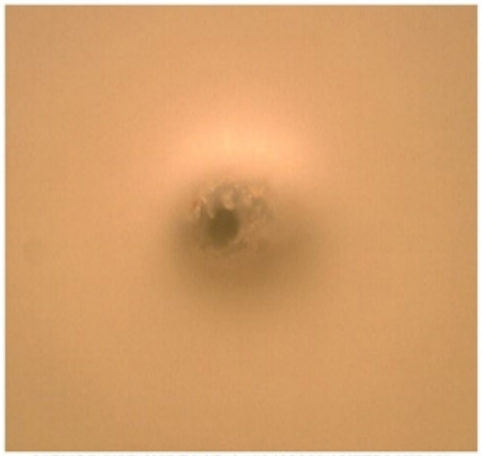
MWCO	Typical NWP range (LMH/psi)
1 kD	0.8 – 1.5
3 kD	1.5 - 3.8
5 kD	2.6 – 5.7
10 kD	8.6 - 20
30 kD	24 - 41
50 kD	34 - 56
100 kD	32 – 91
300 kD	82 – 129
0.1 µm	112 – 225
0.2 µm	138 – 284
0.45 µm	152 -312
0.65 µm	180 - 370

3.4 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 where 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 7. Sensitivity of air integrity test



The result of the integrity test following the defect being added to the cassette is found in Tables 10 and 11 below. 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 cross flow rate. The difference between the two liquid flow rates was not affected and no difference in liquid flow was detected.

Table 10. Cassette integrity test results

Cassette serial #	Cassette status	Results		Results within spec (Y/N)	Difference observed air diffusion (Y/N)	Difference observed flow rate (Y/N)
		Air diffusion rate (ccm)	Liquid flow rate (mL/min)			
17213102	Initial	24	621	Yes	N/A	N/A
	Modified	2196	620	No	Yes	No

Table 11. Cassette integrity specifications

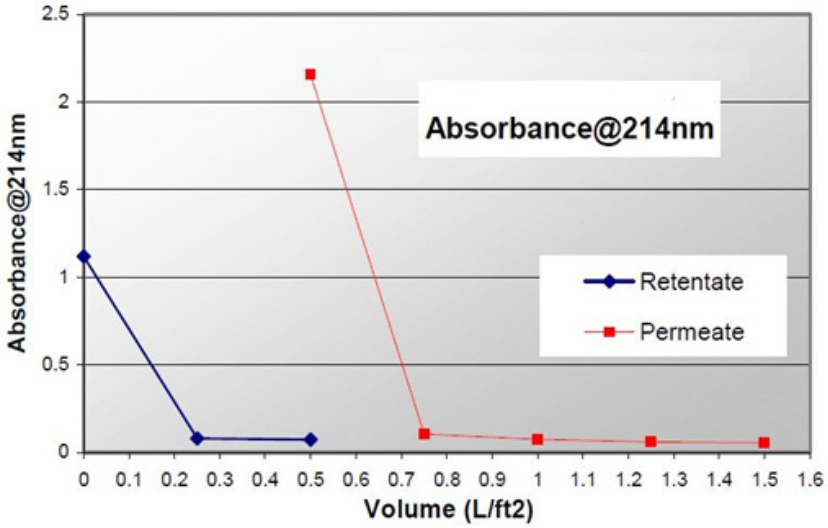
Cassette channel type	Membrane type	Specification
L screen E Screen J Open	Ultrafiltration 1 kD - 5 kD	≤ 323 ccm/m^2 at 1 bar (≤ 30 ccm/ft^2 at 15 psi)
	Ultrafiltration 10 kD - 300 kD	≤ 323 ccm/m^2 at 0.5 bar (≤ 30 ccm/ft^2 at 7.3 psi)
	Microfiltration ≥ 0.1 µM	≤ 323 ccm/m^2 at 0.2 bar (≤ 30 ccm/ft^2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

3.5 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 reduces leachables prior to sanitization with 0.2M sodium hydroxide and final packaging. Membranes used in the cassettes are treated with 20% glycerin and 0.1% sodium azide during the membrane manufacturing process as a storage agent. 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 used at Repligen. The following summary outlines the steps taken to determine the ideal conditions under which to remove the membrane storage solution (leachables) prior to final packaging.

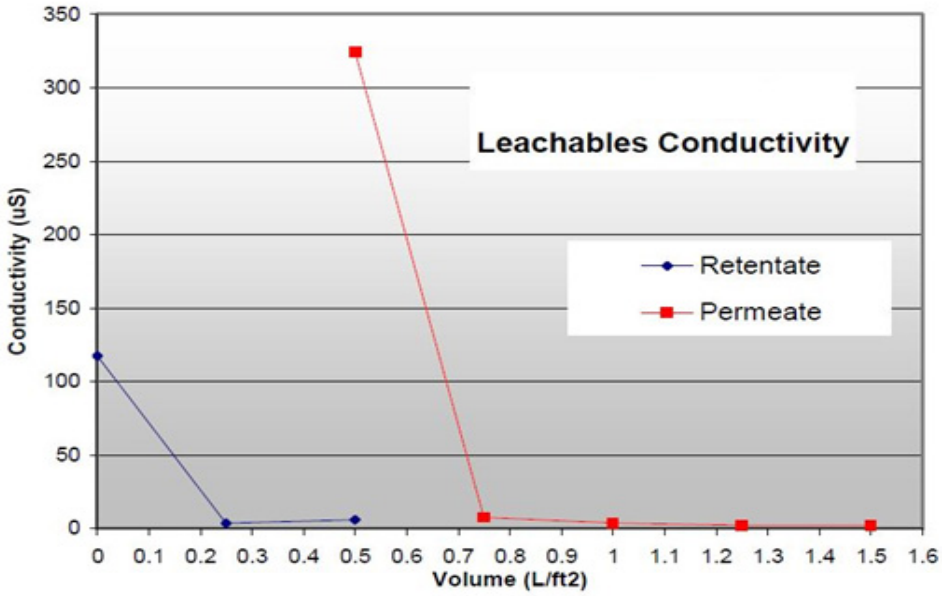
Several different cassettes were manufactured and evaluated for leachables in duplicate; each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. One cassette type was chosen for this study, the TangenX® SIUS® PD 0.1m^2 L-Screen Channel Cassette with 10 kD ProStream membrane. This cassette was chosen as it accurately represents the construction of the entire product line, including the process TangenX® SIUS® Process Scale Cassette. Furthermore, 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. The study of the 10 kD cassettes is indicative of the entire product line, as the materials of construction and process for manufacturing of each are comparable.

Figure 8. Absorbance @ 214nm



A NovaSet™- LS Pilot System was assembled, sanitized with 0.5M sodium hydroxide and then rinsed with DI water. Once the system was rinsed, the cassettes were flushed with purified water and the effluent analyzed for pH, conductivity, and absorbance. The first 0.1m^2 cassette was installed in the hardware and then flushed with 2 liters of DI water. The effluent stream was analyzed, and the results reported in Figures 8 and 9. The data show that the conductivity and UV absorbance at 214nm quickly drop once 0.5 liter of water is flushed through the retentate and then 1.5 liters through the filtrate. The pH of the stream is neutral and the conductivity less than 2µS. The UV absorbance at 214nm falls below 0.05 mAU or approximately 10 parts-per-million (ppm) glycerin, once 1.5 liters of water is flushed through the retentate and the permeate. The following results represent an average of duplicate cassette tests.

Figure 9. Leachables conductivity



The results of the leachables study show that the storage agents are effectively flushed from the cassettes using 2 liters of purified water per square foot of membrane area. The effluent stream was analyzed, and the results reported in Figures 8 and 9. Measurements using a calibrated conductivity meter and UV Spectrophotometer were used to quantify the amount of storage agent removed

during the cassette flush procedure. The successful cassette flushing procedure used included a DI water flush through the retentate and then the filtrate. This flushing procedure will be incorporated into the cassette final release procedure POP-SOP-1033 for all TangenX® SIUS® Cassettes prior to sanitization with 0.2M sodium hydroxide.

3.6 Cassette leachables

Although TangenX® SIUS® Cassettes are shipped ready-for-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 0.2M sodium hydroxide. 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 will quantify the amount of leachables present in a typical TangenX® SIUS® Cassette manufactured by Repligen.

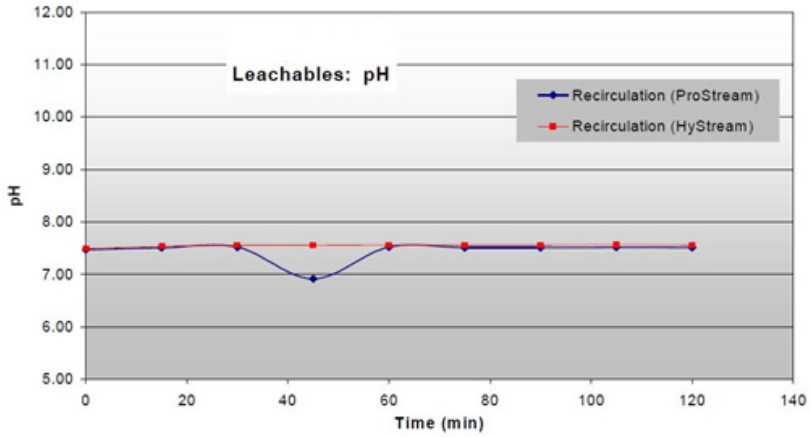
TangenX® SIUS® PD Cassettes with 10 kD ProStream and 10 kD HyStream membrane chemistries were selected for use in this study. The cassettes were manufactured and evaluated in triplicate; each cassette was prepared using current SOPs and reflected the standard cassette manufacturing process at Repligen. The 0.1m^2 TangenX® SIUS® PD Cassette was chosen because it accurately represents the construction of the entire product line including the TangenX® SIUS® Cassette for process scale. Both membrane chemistries were chosen since each represents a product line with unique filtration characteristics. Moreover, 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. Study of the 10 kD cassettes is indicative of the entire product line, as the materials of construction and process for manufacturing for each are comparable.

A NovaSet™- LS Pilot System was assembled, sanitized with 0.5 M sodium hydroxide, and then flushed with DI water. The TangenX® SIUS® PD Cassettes were manufactured and evaluated in triplicate; each cassette prepared using current SOPs and reflect the standard cassette manufacturing process at Repligen. The following SOPs were followed as part of the study:

1. TangenX® SIUS® PD Cassettes will be prepared using POP-SOP-1044.
2. These cassettes will be tested in QC using POP-SOP-1033.

Each cassette was individually installed in the cassette holder and evaluated for leachables. The cassettes were removed from their packaging, installed in the cassette holder, and then equilibrated with 1-liter of PBS buffer (10L/m^2). The PBS buffer was then drained from the system and 1-liter 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 will be referenced in supporting development reports.

Figure 10. Leachables: pH



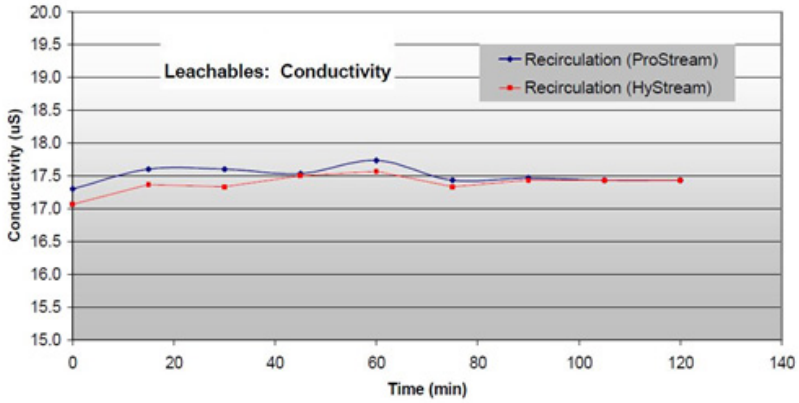
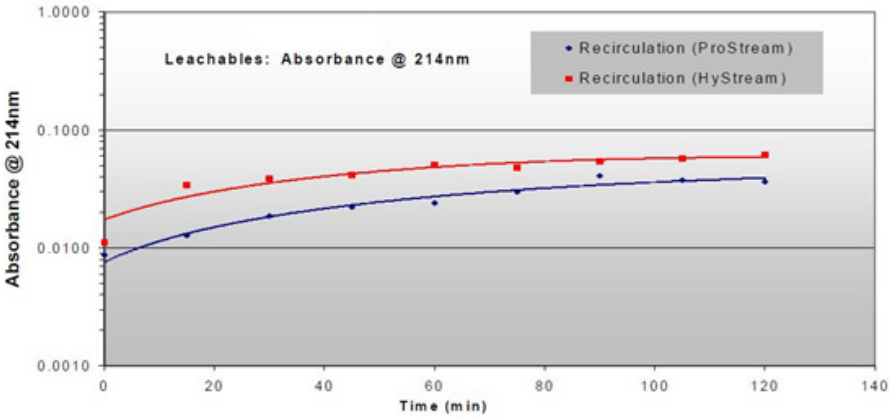


Figure 11. Leachables: conductivity

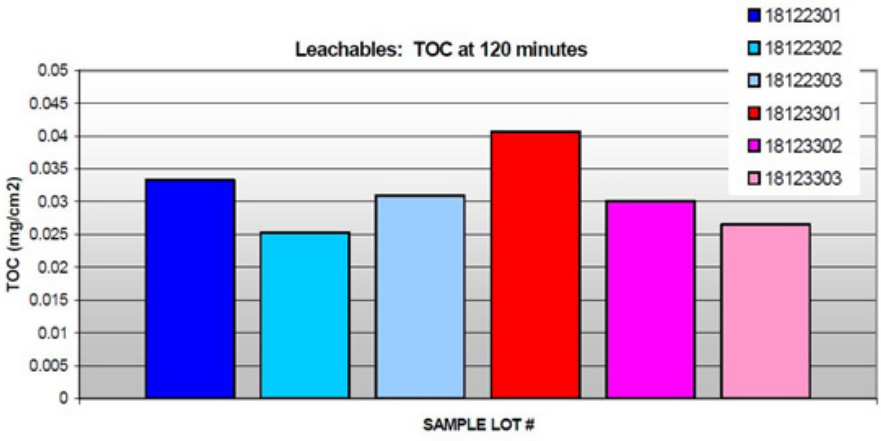
Figure 12. Leachables: absorbance @ 214nm



This 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. The results from the PBS buffer recirculation are reported in Figures 10, 11 and 12. The data show the pH of the buffer is unchanged and remains constant at 7.4. The conductivity increased by 0.2µS before reaching a steady state after approximately 15 minutes. This slight increase in conductivity is likely related to a trace amount of sodium hydroxide 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 TOC value of approximately 0.04mg/cm^2. Figure 13 shows the TOC value of the PBS buffer after 2 hours of recirculation for each of the cassettes tested.

Figure 13. Leachables: TOC at 120 minutes



The results of the leachables study show the 0.2 M sodium hydroxide storage solution was effectively neutralized using 1-liter of PBS buffer per 0.1m^2. Following two hours of recirculating PBS buffer, the stream was analyzed, and the results reported in Figures 10, 11, 12 and 13. Measurements using a calibrated pH probe, conductivity meter, and UV spectrophotometer were used to quantify the amount of residual leachables were within acceptable limits. Additional analysis of the PBS buffer stream demonstrated the level of leachables to be approximately 0.03mg/cm^2.

3.7 Robustness study

The purpose of this study was to evaluate the robustness of the TangenX® SIUS® Single-use TFF Cassettes manufactured by Repligen. To evaluate robustness, the TangenX® SIUS® Cassette samples must demonstrate the ability to operate under stressful conditions. Many different elements contribute to the stress on TFF cassettes. Parameters such as time, temperature, pressure, flow rate, and buffer conditions are several examples. The following steps were used to validate the robustness of the TangenX® SIUS® Single-use Cassettes under very aggressive operating conditions.

Several 0.1 m^2 TangenX® SIUS® PD Cassettes were manufactured and evaluated both individually as well as stacked to 0.5m^2. Each cassette was prepared using approved SOPs and reflects the standard cassette manufacturing process at Repligen. The following steps were taken as part of the study:

1. TangenX® SIUS® PD Cassettes were prepared using POP-SOP-1044.
2. Cassettes tested and released following procedure POP-SOP-133.

Procedure TX1001-POQ-133 provides the methods used to evaluate robustness of TangenX® SIUS® PD Cassettes manufactured at Repligen. The TangenX® SIUS® PD 0.1m^2 L- Screen Channel Cassette with 10 kD ProStream membrane was selected for this study as it accurately represents the entire product line, including the TangenX® SIUS® Cassette. 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. Study of the 10kD cassettes is indicative of the entire product line, as the materials of construction and process for manufacturing of each are comparable.

During the study, each cassette was evaluated at two temperature set points, 4° C and 40° C. Testing was conducted as a single cassette and a stack of five. The cassette(s) was (were) removed from its(their) packaging, installed in the cassette holder, and then equilibrated with PBS buffer. The baseline air integrity, NWP, and pressure drop were measured and recorded. Then 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 - TangenX® SIUS® PD Cassette

Study conditions	0.1m²	0.1m² x 5
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 were able to withstand extended run time, temperature extremes, pressure, and flow rate and pass all release criteria specified in POP-SOP-1033. The TangenX® SIUS® Cassettes have demonstrated their robustness and will withstand at least one process cycle as designed.

3.8 Shelf-life study

3.8.1 Membranes

The following section describes the conclusion of the shelf life study for ultrafiltration and microfiltration membranes manufactured by Repligen after five years. Ultrafiltration and microfiltration membranes are initially cast and then stored for a period of time prior to being incorporated into a cassette product. The time between when the membrane is manufactured and when it is used in a cassette may be up to five years.

Several lots of membranes were cast during the 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 POP-SOP-1027 and POP-SOP-1028.
2. These membranes were sampled and tested following TX1001-POQ-115.

This summary will provide final results for the shelf life study of the ProStream and HyStream membranes manufactured at Repligen. This report will also be used to summarize results of the sampling and testing throughout the study. The membrane storage study procedure TX1001-POQ-115 was applied to both the mPES ProStream (BioFlo) and HyStream (HyFlo) membranes manufactured at Repligen. One membrane of each type was chosen to represent the product line consisting of all MWCO membranes. These membranes were chosen as they correspond to the cassette storage study. Each membrane was tested following the standard QC release procedure POP-SOP-1030.

The storage study consisted of two different conditions, one at ambient temperature and the other at 50° C. The first part of the study was conducted at ambient temperature and had been designed to simulate exposure at a “normal” or median temperature. This type of study spanned (5) five years and was the standard shelf life study. The second part of the study conducted at 50° C had been designed to simulate exposure at the maximum temperature limit of the product. This type of study

was concluded within 1 month and was an accelerated study. 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 (PAQ-SOP-1033). 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 three membranes fail during any one time point, the endpoint of the study would have been reached and the study concluded. A detailed analysis of the membranes that did not meet release criteria would be included in the final report.

This study was reported on several times, once in an interim summary and the final one after five years.

Table 13. Membrane acceptance criteria for shelf life study

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 @ 15psi**
Total number of discs with air diffusion	≤ 6 (of 18 discs)

Table 14. Membrane acceptance test results - Elevated temperature (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 acceptance test 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
	Pass	Pass	Pass	Pass

Conclusions

This study report describes the final results of the shelf life storage study of the ProStream and HyStream membranes manufactured by Repligen after five years. Several membrane batches were manufactured as part of the initial process validation. Each batch was prepared using current SOPs and reflects the standard membrane manufacturing process at Repligen. One batch of each type of ProStream and HyStream membrane was used for this 5-year shelf life study. These membrane types represent the entire line of mPES membranes manufactured by Repligen.

The results show both the ProStream and HyStream membranes meet or exceed all release specifications after both the accelerated study after one month and ambient conditions after five years. The membrane's performance, based on water permeability, rejection, and integrity were not affected after five years' time. The membrane storage study successfully reached its five-year conclusion.

3.8.2 TangenX® SIUS® Cassettes

The following section summarizes the conclusion of the shelf life storage study for the TangenX® SIUS® Cassettes manufactured by Repligen after three years. The cassettes are manufactured, packaged, and stored for a period of time prior to shipment. Once shipped, the cassette may then remain unopened for a period of time before it is put into 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 in triplicate; each cassette was prepared using current SOPs and reflect the standard cassette manufacturing process at Repligen. The following steps were taken as part of the study:

1. Cassettes were prepared using POP-SOP-1044.
2. These cassettes were sampled and studied following the procedure in TX1001-POQ-134.

This summary will provide final results from cassettes manufactured at Repligen for shelf life storage stability. Each cassette was initially evaluated following the standard QC release procedure POP-SOP-1033.

One cassette type with two different membranes was chosen for this study, the TangenX® SIUS® PD 0.1m^2 L-Screen cassette using 10kD ProStream and 10kD HyStream membranes. The 0.1m^2 TangenX® SIUS® PD Cassettes were chosen as they accurately represent the construction of the entire product line, including the 0.5m^2 - 2.5m^2 TangenX® SIUS® Cassettes. Two different membrane

chemistries were chosen to represent each membrane type: 10kD ProStream and 10kD HyStream. Each 10kD membrane represents the entire membrane line manufactured by Repligen. A separate membrane storage study was used to evaluate the effect of aging on various membrane types and pore sizes. See study procedure TX1001-POQ-115 and interim report DR-09-010.

The storage study consisted of two different conditions, one at ambient temperature and the other at 50° C. The first part of the study conducted at ambient temperature has been designed to simulate exposure at a “normal” or median temperature. This type of study was concluded after (3) three years and was the standard storage study. The second part of the study was conducted at 50° C and had been designed to simulate exposure at the maximum temperature limit of the product. This portion of the study was concluded within one month and is considered an accelerated study. A study at lower temperatures, below ambient, was not be conducted.

In the event one cassette had failed during the study, a failure analysis would have been conducted through the deviation procedure (PAQ-SOP-1033). The mode of failure and impact on product quality would then have been assessed. If the cassette were deemed to be an anomaly, the study would continue as planned. The documented failure would have accompanied the final report. If all three cassettes were to have failed during any one time point, the endpoint of the study would have been reached and the study concluded.

Each cassette type at a given time point was evaluated in triplicate for increased accuracy. The acceptance criteria for each of the cassettes are shown below and include standard release testing, normalized water permeability/rejection, and testing for leachables.

Table 16. TangenX® SIUS® Cassette acceptance criteria for shelf-life study

Description	Specifications
Water cross-flow rate Flow rate (liter per minute) Pressure drop (psi)	 0.4 - 0.8 LPM @ Pressure drop 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 POP-SOP-1033.	

Table 17. Cassette acceptance test results - Elevated temperature (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 acceptance test 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 months	Pass	Pass	Pass
1 year	Pass	Pass	Pass
2 years	Pass	Pass	Pass
3 years	Pass	Pass	Pass

Conclusions

The TangenX® SIUS® Cassette storage study has successfully met its three-year conclusion. This study report summarizes the final results of the shelf life storage study of the TangenX® SIUS® Cassettes manufactured by Repligen after three years. Several different cassettes were manufactured and evaluated in triplicate; each cassette was prepared using current SOPs and reflects the standard cassette manufacturing process at Repligen.

The results show the TangenX® SIUS® Cassettes meet or exceed all release specifications after both the accelerated study and ambient conditions after three years. Additionally, the cassette's performance, based on water permeability and rejection, are not affected over three years of time. Finally, the cassette's leachables profile has not increased and endotoxin is within acceptable limits following this time point.

3.9 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	x	x
Hydrocarbons	√	√
Butanol (70%)	√	x
Butyl Acetate (40%)	√	√
Butyl Cellosolve (10%)	√	√
Calcium chloride (5%)	√	√
Chloroform (0.8%)		
Citric Acid (1%)	√	√
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 (6M)	√	√
Hydrochloric Acid (0.1N @ 25 C)	√	√
Hydrochloric Acid (0.1N @ 50 C)	√	√
Hydrochloric Acid (1.0N @ 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)(1M)	√	√
Phosphoric Acid (1N)	x	x
Sodium Azide (1%)	√	√
Sodium Chloride (5%) (50 C)	√	√
Sodium Deoxycholate (5%)	x	x
Sodium Dodecyl Sulfate (0.01M)	√	√
Sodium Hydroxide (0.1N @ 25 C)	√	√
Sodium Hydroxide (0.1N @ 50 C)	√	√
Sodium Hydroxide (0.5N @ 25 C)	√	√
Sodium Hydroxide (0.5N @ 50 C)	√	√
Sodium Hydroxide (1.0N @ 25 C)	√	x
Sodium Hypochlorite (100ppm)	√	√
Sodium Hypochlorite (400ppm)	√	x
Sodium Hypochlorite (1000ppm)	x	x
Sodium Nitrate	√	√
Sulfuric Acid (1N)	√	
Terg-a-zyme (1%)	√	√
Tetrahydrofuran (5%)	x	x
Toluene (1%)	x	x
Tris buffer (pH: 8.2) (1M)	√	
Triton X-100 (0.002M)	√	√
Urea (25%)	√	√
Ultrasil 11 (1%)	√	

√ = Compatible
no significant changes in either rejection or flow rate

x = Not Compatible
significant change noticed

4. Safety information

4.1 USP Class VI

The purpose of UPS 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 the Table 4.1. Sample dimension, sample mass and test regime are identified as well.

Figure 14. USP testing 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 would 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 also 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 the complete USP report that can be provided by Repligen. A summary of the test results is below.

Figure 15. Summary of USP testing results

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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

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.

4.2 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 were described in the study plan M-TANGENX-210301 and results generated are summarized in Study Report 11510.3777. The results generated from this analysis were derived from 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 then 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 sodium chloride, 0.5 M sodium hydroxide, 0.1 M phosphoric acid, 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 entire duration of the extraction. Once the sample time point was 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 as 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 the study report 11510.3777 and summarized in Tables 3 – 15.

GC/MS was performed on selected component extracts according to the conditions as 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 the study report 11510.3777 and summarized in Tables 17 – 29.

Headspace GC/MS was performed on selected component extracts according to the conditions as 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 the study report 11510.3777 and summarized in Tables 31 – 43.

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

TOC, pH, and Non-Volatile Residue analysis were performed on selected component extracts according to the conditions as described in the study plan. Results for total organic carbon, pH, and non-volatile residue are provided in Tables 20 - 22 below. 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 summary

Sample description	Extraction solvent	Results			
		1 day		21 day	
		µg /ml	µg /cm²	µg/mL	µg/cm²
Cassette	WFI	286	25.5	1.11E+03	99.3
	0.1 M H_3PO_4	286	25.5	1.21E+03	108
	0.5 N NaOH	278	24.8	1.24E+03	110
	5 M NaCl	3.41	0.304	9.26	0.826

Table 21. pH results summary

Sample description	Extraction solvent	Results	
		1 day	21 day
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 summary

Sample description	Extraction solvent	Results			
		1 day		21 day	
		µg /ml	µg /cm²	µg/mL	µg/cm²
Cassette assembly	WFI	748	66.8	2.88+03	257
	50% EtOH	1.29E+03	115	3.57E+03	319

4.2.1 Acceptance criteria

The extractables testing is compliant when the study has reached its 21-day conclusion. Information gathered will be presented in a report format and reviewed to ensure study protocols were followed. Failure to follow protocols as written will require a deviation to be written in order to justify 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 protocol
- Instrument control test results must be valid

4.3 Endotoxin

TangenX® SIUS® Cassettes produced by Repligen are flushed, packaged, and stored in 0.2 M sodium hydroxide 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 following 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 had been recirculated through a 0.1m² 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 for this study, the TangenX® SIUS® PD 0.1m² L- Screen Channel Cassette with 10 kD ProStream and 10 kD HyStream membrane. The 0.1m² 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 0.5M sodium hydroxide and then flushed with DI water. The first line of Table 4.3.1 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-liter of phosphate buffered saline. The buffer was drained from the system and then 1-liter 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. Results of endotoxin count study (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. Results of endotoxin count study (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**

4.4 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 for the production of 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. The reasons are 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 bars for at least 6 hours.
- The stearic acid does not come from high risk countries.

4.5 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 and endotoxin count 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
- These 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, the TangenX® PRO PD 0.1m^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. During the study, each cassette type/membrane combination was evaluated in triplicate. The cassettes were installed in the TangenX® PRO PD Cassette holder and evaluated for particulate and endotoxin 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 number of particles present in the units tested does not exceed 12 per milliliter equal to or greater than 10 µm; and does not exceed 2 per milliliter 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 sodium hydroxide, 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 cassettes were then evaluated in triplicate, the data was tabulated and summarized below. The raw data from each set of cassettes may be found in the completed development report.

Table 25. Results of particulate count study

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

The first experiment conducted was for the filtration system alone, with no membranes installed. The system was assembled, sanitized with 0.5 M sodium hydroxide, and then flushed with DI water. The effluent streams were analyzed, and the results reported in Table 23. The data show the system contributes to a portion of the particles found in the test samples but did not contribute to a failure.

Once a baseline was generated for the filtration system, the experiments for a set of three cassettes containing ProStream membrane began. One 0.1m^2 cassette was installed in the hardware and then flushed with 100mL of water for injection. The effluent streams were analyzed, and the results reported in Table 23. The data show the 100 mL flush contains a minimal number of particles found

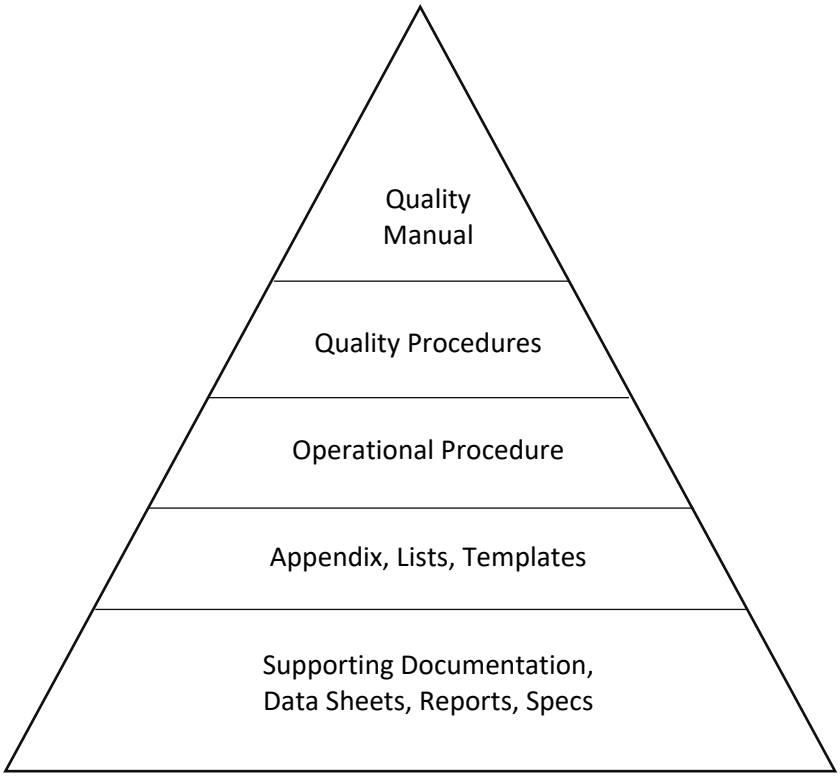
in the cassettes manufactured at Repligen. The sample complied with the test and the average number of particles present in the units tested did not exceed 12 per milliliter equal to or greater than 10 µm; and did not exceed 2 per milliliter equal to or greater than 25 µm.

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.

5. Documentation system

The pyramids below show the document systems, which are applicable within the scope of the cassette validation. The first pyramid illustrates the general document system put in place at Repligen through the Quality Manual and Quality Systems Procedures. These two pyramids address validation and qualification documents.

Figure 16. General document pyramid



Quality Manual
A document that defines in a general manner the Quality Management System applied to the different Repligen processes.

Quality Procedures
A document outlining specific work processes and how the requirements of the ICH Q7 standard are being met.

Operational Procedure
Step by step directions on how a task should be done.

Appendix
Documents used to further clarify or show examples of information described in the procedures and work instructions.

Lists
List of material elements or others (e.g., equipment, nomenclature).

Templates
Electronic documents used to create quality system documentation.

Supporting Documentation
Information used to support the requirements, production or results of a given process.

Data Sheets
Documents used to make a record of completing all or part of the process described in procedures and work instructions.

Records
Completed forms or information generated as a result of the process described in a document and retained as within a procedure.

Specifications
Raw material, intermediates or finished products requirements for the production and release of product.

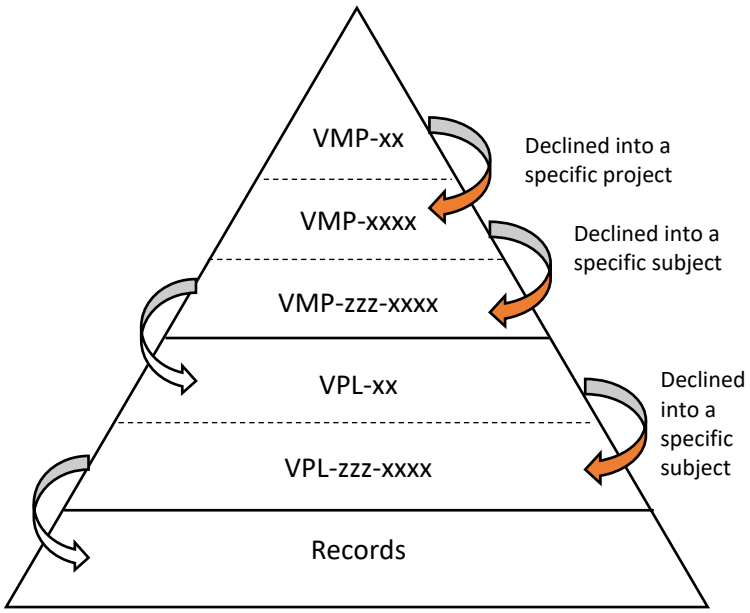


Figure 17. Validation document pyramid

VMP-xx / VMP-xxxx / VMP-zzz-xxxx Validation Master Plans

These validation documents clearly describe the validation project by presenting a global and synthetic view. They must allow identification of the systems that are to be validated, the necessary

resources are to be defined, the schedules are to be generally defined, and all activities related to the validation is to be defined.

The general Validation Master Plan is identified as VMP-xx or if specific to a given project will be identified as VMP-xxxx project (with xxxx indicating the project identification number). The VMP-xxxx specific to project xxxx allows the scope of the validation process to be presented. The VMP-xxxx may refer to VMP-zzz-xxxx Master Validation Plans specific to a rather specific validation subject (examples VMP-zzz-xxxx: cleaning process validation, manufacturing process validation, methods validation).

VPL-xx / VPL-zzz-xxxx Validation Plans
A general Validation Plan is identified as VPL-xx or if specific to a given project will be identified as VPL-zzz-xxxx. A Validation Plan describes the specific tasks and operations. It may refer to the PAQ-xx, but in particular to the POP-xx. The forms that allow the tasks and operations performed, to be tracked are shown in the Validation Plan appendices. At the conclusion of the validation operations, a final report synthesis the conditions of the operations and draws a conclusion on the validation. The report must be approved by the Quality Assurance Department.

Change Control
Management of the changes are performed according to approved procedures and using change control request forms. The nature and significance of each change may be different (for example: critical, major, minor), and it is the responsibility of Repligen to justify the classification of these changes.

As described in the change control procedure, changes having a real or supposed incidence on the quality of the product, the process, the production, or control equipment, and or the specifications must be documented and, if necessary, give rise to:

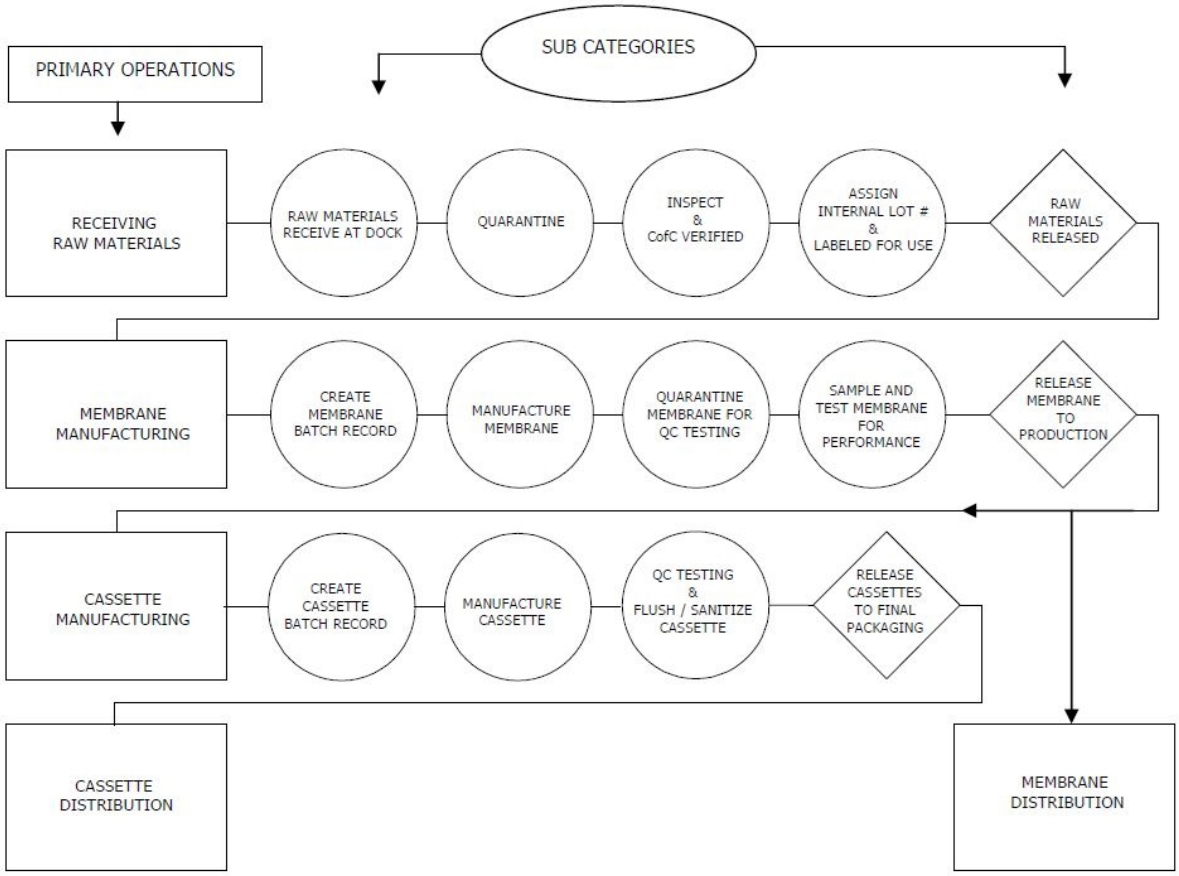
- Tests and trials to evaluate their consequences
- Validation of the change if it is significant

Changes may lead to revision of a document and its revision status.

6. Product manufacturing

Tangential flow filtration membranes and cassettes manufactured at Repligen are produced in a facility whose Quality Management System is approved by an accredited registering body to the ISO® 9001 2015 Quality System Standard. The following process flow chart depicts the primary operations and their sub-categories. Specified raw materials are initially received and quarantined prior to inspection for conformity. Once inspected the raw materials are identified and labeled with a unique internal lot numbering system for easy traceability. Approved raw materials are then used to manufacture the membranes using state of the art equipment and techniques. Membrane lots are tracked using a batch recording system and then tested for permeability and selectivity. Membranes meeting specification are then transferred to a holding area where they will be fabricated into cassettes. Cassettes are manufactured using approved standard operating procedures and then individually tested for both air integrity and hydrodynamic performance. Cassettes meeting specification are released to final packaging where they await distribution.

Figure 18. Process flow chart



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's 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 must have had to have been 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 (LP)
- Extra Low Pressure Screen Channel (EP)
- 0.5 mm Open Channel (J)

Within the framework of the validation, QC methods, the utilities, equipment, and personnel must have been 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 results for the process validation of the ultrafiltration and microfiltration membranes produced at Repligen were carried out as specified. The validation included approved procedures for the casting solution preparation, membrane casting procedure, 20% glycerin solution/0.05% sodium azide procedure, membrane QC testing procedure, and their 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 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 SOP. 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. Each membrane batch was manufactured in accordance with approved standard operating procedures.
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 results for the process validation of the tangential flow filtration cassette produced at Repligen were 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 their 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® 0.5m^2 L-Screen Channel, with two different 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 grouping 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 below in Figure 21 and 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 and VPL-PRO-102-TX1001 applies for 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. The defined validation team, while following documented procedures, manufactured three consecutive lots for each cassette type and membrane, as defined in Section 8.

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 under this validation plan was found to be in conformance with the defined expectations.

Figure 21. TangenX® SIUS® PD Cassette process validation summary table

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 table

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 were 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 each membrane's water permeability and protein rejection. This information is then used to accept or reject the membranes manufactured at Repligen. 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 each cassette's air diffusion and cross flow rate. 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 including acceptance specifications for the methods were met during the method development and validation cycle 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 are listed in Figure 23, as taken from document I/F.SRG-POP.1033. Figure 24 presents the release specifications for both membrane chemistries, as taken from document I/F.MSG-POP.1030.

- Cassette QC release specifications: I/F.SRG-POP.1033
  - See Figure 23
- Membrane QC release specifications: I/F.MSG-POP.1030
  - See Figure 24

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

Membrane Specification Guide
Identification: IF.MSG-POP.1030
Revision: R20

Normalized Water Permeability					Selectivity																		Integrity
					1.3KD		1.5 KD		12KD		12-18KD		45 KD		67 KD		167 KD		2000 KD		0.16 um		0.3 um		0.5 um		0.8 um		Total # of discs positive with air diffusion
					Vitamin B-12		Bacitracin		Cytochrome-C		C15		C60		BSA		IgG		Blue Dextran		Latex Bead		Latex Bead		Latex Bead		
Part Number (4)	MWCO (Daltons)	WATER FLUX (LMH@Psi)	Deviation (%)	Pressure (psi)	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	Flux (LMH)	%Rej.	1K -300K @ 15psi 0.1um - 0.65um @ 3psi ≤ 5 (or ≤ 5 Discs)
PPN65	0.65K	0.30 - 0.60	≥25%	50	12 - 24	25 - 55	5 - 14	≥85																					
PP001 XP001	1K	0.80 - 1.50	≤15%	50	24 - 48	≤45	13 - 24	≥70																					≤5 (or ≤5 Discs)
PPH01	H01	1.30 - 3.20	≤15%	50			20 - 50	60 - 80	40 - 80	≥95%																			≤5 (or ≤5 Discs)
PP003 XP003	3K	1.65 - 3.85	≤15%	50			30 - 60	50 - 70	42 - 90	≥95%																			≤5 (or ≤5 Discs)
PP005 XP005	5K	2.75 - 6.00	≤15%	50			80 - 120	≤40%			90 - 150	≥65																	≤5 (or ≤5 Discs)
PP010 XP010	10K	9.50 - 22.0	≤15%	50							140 - 250	30 - 60	75 - 110	≥85															≤5 (or ≤5 Discs)
PP030 XP030	30K	30.0 - 50.0	≤15%	50									80 - 110	60 - 80	120 - 170	≥95													≤5 (or ≤5 Discs)
PP050 XP050 PP100 XP100	50K 100K	45.0 - 75.0 5.00 - 140	≤15% ≤15%	50 10									95 - 150	30 - 60	140 - 200 305 - 550	≥75 ≤20	50 - 90	≥90											≤5 (or ≤5 Discs)
PP300 XP300	300K	190 - 300	≤15%	10													≥530	≤15	65 - 110	≥90									≤5 (or ≤5 Discs)
PPM10 XPM10 PPM20 XPM20	0.1um 0.2um	750 - 1500 1720 - 3550	≤15% ≤15%	10 (5) 5 (5)															650-1300	≤20	TBD TBD	≥80% ≤20%	TBD	≥80%					≤5 (or ≤5 Discs)
PPM45 XPM45	0.45um	> 2175	≤15%	3 (5)																			TBD	≤20	TBD	≥80%			≤5 (or ≤5 Discs)
PPME5 XPME5	0.65um	> 2550	≤15%	3 (5)																					TBD	≤20%	TBD	≥80%	≤5 (or ≤5 Discs)
Test Solution Concentration					0.25 g/l 20% Ethanol w / DI Water		1.0 g/l		0.25 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		1.0 g/l		
Test Solution Buffer (2)					DI Water		PBS pH 7.4		PBS pH 7.4		DI Water		DI Water		PBS pH 7.4		PBS pH 7.4		DI Water		DI Water		DI Water		DI Water		DI Water		
Spectrophotometer Measurement Technique	Mode / Wavelength				Absorbance @350nm		Absorbance @252nm		Absorbance @410nm		Absorbance @214nm		Absorbance @214nm		Absorbance @280nm		Absorbance @280nm		Absorbance @550nm		Absorbance @410nm		Absorbance @410nm		Absorbance @410nm		Absorbance @410nm		
	Optical Path				10mm Cuvette		10mm Cuvette		10mm Cuvette		Nano Drop Pedestal		Nano Drop Pedestal		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		

NOTES:
(1.) NWP test measurements are performed at the specified applied air pressure. Latex bead solutions are tested with no applied air pressure; head pressure is approximately 5.5 in H2O (140 mm H2O)
(2.) Prepare each test solution in the specified test solution buffer. For PBS buffer, use 1 Pkg of PBS (OPS1009 to 1 Liter of DI water. For 20% Ethanol use 200ml of EAP 4001 diluted to 1 Liter with DI water.
(3.) All test solutions should be stored at 4C and discarded after 5 Days.
(4.) Part Number Suffix: S1 = 12.5" Width, S2 = 18.0" Width
(5.) Test solutions should be maintained between 15° - 25° C

IF.MSG-POP.1030

9.5 Certificate of Compliance

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
Phone: 508-845-6400
Fax: 508-845-3030

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:

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

TangenX™ SIUS™ Cassette

BATCH # 99999999 ■ SINGLE-USE ONLY

USE BY: 21-FEB-2024
MEMBRANE: HyStream (Low Fouling mPES)
MWCO: 300 kD
CHANNEL: LP Screen Channel
AREA: 0.5 m² (5.4 ft²)
SERIAL # 31052999
CATALOG # XP300G05L

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.

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. Certifications that the components used in the production of the filters are BSE/TSE free.
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

Multiple lots of 0.1m2 cassettes were extracted in one (1) liter of phosphate buffered saline and tested for:

1. Endotoxin testing following references USP 30, NF 25, 2007 <85> bacterial endotoxin test, guidance on validation of the limulus amebocyte lysate (LAL) test as an end product. These levels were < 0.005 EU/ml as determined by the LAL test method.
2. Total Organic Carbon following references USP 31, NF 26, 2008. <643> Total Organic Carbon. The mean value was found to be 0.0302 mg/cm2 as determined by the TOC test method.

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 11137-1. 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

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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