TangenX® PRO Cassettes for Tangential Flow Filtration

Regulatory Support File

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Abbreviations

µg	microgram
µm	micron
µS	microSiemens
BSA	bovine serum albumin
BSE	bovine spongiform encephalopathy
C	Celcius
ccm	cubic centimeter per minute
CIP	clean-in-place
cm	centimeter
cm^2	centimeter squared
CMC	Chemistry, Manufacturing & Controls
DI	deionized
EPDM	Ethylene Propylene Diene Monomer
ERP	Enterprise Resource Planning
EU	endotoxin units
FDA	Food and Drug Administration
ft^2	Feet squared (square feet)
FTIR	Fourier Transform Infrared Spectroscopy
g	gram
GC/MS	gas chromatography/mass spectrometry
GMP	Good Manufacturing Practice
HDPE	high density polyethelene
HPLC	High-performance liquid chromatography
HPLC/DAD	High-performance liquid chromatography/photodiode-array detection
ICP/MS	Inductively Coupled Plasma/Mass Spectrometry
in	inch
in-lb	inch-pounds
IQ	Installation Qualification
ISO	International Organization for Standardization
kD	kilodalton
L	liter
LMH	liter per square meter per hour
M	molar
m^2	meter squared (square meter)
mg	milligram
mL	milliliter
mm	millimeter
mPES	modified polyethersulfone
MW	molecular weight

MWCO	molecular weight cut-off
NaOH	sodium hydroxide
nm	nanometer
N-m	Newton-meter
NMWL	Nominal Molecular Weight Limit
NVR	non-volatile residue
NWP	normalized water permeability
OQ	Operation Qualification
PP	polypropylene
ppm	parts per million
psi	pounds per square inch
psig	pounds per square inch gauge
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
RSF	Regulatory Support File
SOP	Standard Operating Procedure
TFF	tangential flow filtration
TMP	transmembrane pressure
TOC	total organic carbon
TSE	transmissible spongiform encephalopathy
UF	ultrafiltration
USP	United States Pharmacopeia
VMP	Validation Master Plan
VPL	General Validation Plan
WFI	water for injection

Table 1. Current Names of TangenX Products

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

Note: *TangenX TFF Cassettes are now a product line of Repligen. The previous and current product names are listed in the table above.*

1. Introduction

The Regulatory Support File (RSF) for TangenX PRO 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.

2. Quality Documentation

2.1 Quality Policy

2.1.1 Repligen Corporation – A Higher Standard

Repligen Corporation has over 50 years of experience providing products that meet the quality required in bioprocessing applications. We can satisfy the quality needs of customers with particular application requirements. Full compliance with regulatory requirements and meeting customer needs are the driving forces for the Repligen higher standard of quality.

2.1.2 Complying with Quality Regulations

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

- TangenX PRO 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 PRO 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.

3. Product Description

The TangenX PRO Cassette for tangential flow filtration (TFF) is a membrane device that is used to concentrate, diafilter, and fractionate a wide range of macromolecules (i.e., enzymes, proteins, oligonucleotides). TangenX PRO Cassettes recirculate the retentate across the membrane surface, minimizing the fouling of the membrane and permitting longer membrane use and resulting in higher product yields. The TangenX PRO TFF Cassettes consist of a rigid, flat, rectangular design with multiple layers of permeable membrane and polypropylene screens. The fluid is pumped through the screens tangentially to the membrane surface. Pressure generated by the pumping process is used to drive the filtration operation. Typical membrane surface area for the TangenX PRO Cassettes ranges from 100 cm^2 to 60 m^2 depending on the application.

Figure 1. TangenX PRO PD Cassettes

TangenX PRO PD Cassettes for pilot applications are available in a wide range of membrane pore sizes from 0.65 kD – 300 kD (ProStream) and 5 kD – 0.65 µm (HyStream) modified polyethersulfone (mPES) membrane formats. TangenX PRO PD Cassettes are available in 0.01 m^2, 0.02 m^2, and 0.1 m^2 surface areas and four channel configurations. High channel (H Screen) is ideal for dilute streams where high flux and lower recirculation rates are desired. Low channel (L Screen) is ideal for medium viscosity streams

where a lower pressure drop is desired. Suspended channel (S channel) is used for streams of high viscosity or those containing particulates. E screen is ideal for high concentration, high viscosity streams.

Figure 2. TangenX PRO Cassettes

TangenX PRO Cassettes for process applications are available in a wide range of membrane pore sizes from 0.65 kD – 300 kD (ProStream), and 5 kD – 0.65µm (HyStream) mPES membrane types. TangenX PRO Cassettes are available in 0.5 m^2, 1.5 m^2, and 2.5 m^2 surface areas and four channel configurations. High channel (H Screen) is ideal for dilute streams where high flux and lower recirculation rates are desired. Low channel (L Screen) is ideal for medium viscosity streams where a lower pressure drop is desired. Suspended channel (S channel) is used for streams of high viscosity or those containing particulates. E screen is ideal for high concentration, high viscosity streams. TangenX PRO Devices are designed for processing volumes from tens to thouands of liters.

TangenX PRO Cassettes 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.

3.1 Cassette Design

TangenX PRO Cassettes are designed and constructed using FDA approved materials and are validated for use in demanding biopharmaceutical applications. Cassettes are manufactured in a fully validated and documented manufacturing process according to the principles of cGMP and meet specified release criteria. TangenX PRO PD Cassettes and TangenX PRO TFF Cassettes are designed for optimal performance and long life.

Table 2. Materials of Construction

Component	Material
Membrane	Modified Polyethersulfone (mPES)
Membrane Support	Polypropylene (PP)
Channel Configurations	
H Screen Channel – Feed/Retentate Channel – Permeate Channel	Fine Woven PP Screen Medium Woven PP Screen
L Screen Channel – Feed/Retentate Channel – Permeate Channel	Medium Woven PP Screen Medium Woven PP Screen
E Screen Channel – Feed/Retentate Channel – Permeate Channel	Coarse Woven PP Screen Coarse Woven PP Screen
S Screen (Suspended) Channel – Feed/Retentate Channel – Permeate Channel	High Density Polyethylene (HDPE) Spacer with Fine Woven PP Screen Medium Woven PP Screen
Encapsulant	Polyurethane

3.2 Physical Dimensions

3.2.1 TangenX PRO PD Cassette

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)

Size (approximate):

- Length: 8.1 in (20.6 cm)
- Width: 2.2 in (5.6 cm)
- Height: The height of the cassette varies with the channel type.
  - 0.08 – 0.1 in (0.21 – 0.27 cm) for 0.01 m2 cassette
  - 0.10 in – 0.13 in (0.26 – 0.34 cm) for 0.02 m^2 cassette
  - 0.55 – 0.72 in (0.14 – 0.18 cm) for 0.1 m^2 cassette

Table 3. Feed Channel Holdup Volume

Surface Area	Channel Type			
	H Screen	L Screen	E Screen	S Screen
0.01 m^2 (0.11 ft^2)	1.0 mL	1.2 mL	2.2 mL	5.4 mL
0.02 m^2 (0.22 ft^2)	1.7 mL	2.1 mL	3.8 mL	8.0 mL
0.1 m^2 (1.1 ft^2)	7.0 mL	8.7 mL	15.7 mL	28.7 mL

Table 4. Permeate Channel Holdup Volume

Surface Area	Channel Type			
	H Screen	L Screen	E Screen	S Screen
0.01 m^2 (0.11 ft^2)	1.2 mL	1.2 mL	1.2mL	1.2 mL
0.02 m^2 (0.22 ft^2)	2.1 mL	2.1 mL	2.1 mL	2.1 mL
0.1 m^2 (1.1 ft^2)	8.7 mL	8.7 mL	8.7 mL	8.7 mL

3.2.2 TangenX PRO Cassette

Size (approximate):

- Length: 8.1 in (20.6 cm)
- Width: 6.7 in (17.0 cm)
- Height: 0.5 to 4 in (1.3 to 10.2 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. Feed Channel Holdup Volume

Surface Area	Channel Type			
	H Screen	L Screen	E Screen	S Screen
0.5 m^2 (5.41 ft^2)	30 mL	38 mL	68 mL	136 mL
1.5 m^2 (16.2 ft^2)	91 mL	114 mL	205 mL	385 mL
2.5 m^2 (26.9 ft^2)	152 mL	190 mL	N/A	633 mL

Table 6. Permeate Channel Holdup Volume

Surface Area	Channel Type			
	H Screen	L Screen	E Screen	S Screen
0.5 m^2 (5.41 ft^2)	38 mL	38 mL	38 mL	38 mL
1.5 m^2 (16.2 ft^2)	114 mL	114 mL	114 mL	114 mL
2.5m^2 (26.9 ft^2)	190 mL	190 mL	N/A	190 mL

3.3 Package Contents

TangenX PRO PD Cassette Package Contents

Package includes the following:

- One (1) TangenX PRO PD TFF Cassette in one of three available sizes:
  - 0.01 m^2 cassette
  - 0.02 m^2 cassette
  - 0.1 m^2 cassette
- Two (2) Gaskets (silicone for 0.1 m^2; EDPM for 0.01 and 0.02 m^2)
- Certificate of Conformance
- Operating Instructions

TangenX PRO Cassette Package Contents

Package includes the following:

- One (1) TangenX PRO TFF Cassette in one of the following sizes:
  - 0.5 m^2 cassette
  - 1.5 m^2 cassette
  - 2.5 m^2 cassette
- Two (2) Silicone gaskets
- Certificate of Conformance
- Operating Instructions

3.4 Important Information Before You Begin

Cassettes

- Cassettes may be stacked to increase filtration surface area; however, only one type of membrane molecular weight cutoff should be used at one time. *Do not install a mixture of cassettes with different pore sizes in the same hardware*.
- Cassettes must be flushed with deionized (DI) water, water for injection (WFI) or buffer to ensure removal of storage agents and preservatives from the membrane filter. It is critical to use the highest quality water possible to avoid fouling the membrane or introducing contaminants into the system that could affect membrane performance and product recovery.

Gaskets

- Gaskets lose their resiliency over time. Repligen recommends that you replace gaskets a minimum of every six months. Repligen supplies two gaskets per cassette. Installation of the first cassette requires two gaskets; stacking each additional cassette requires one more gasket. Extra gaskets should be saved to replace worn or damaged gaskets.

Pump

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

4. Membrane Cassette Installation

1. Lift the end plate from the manifold.
2. Rinse the 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.

Note: *Each cassette is stored in an aqueous solution containing 15 – 20% glycerin and 0.1% sodium azide, pH 7 – 10. Follow standard safety procedures for handling aqueous glycerin/sodium azide, including the use of gloves, safety goggles, and lab coat.*

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. 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 18 mm (0.75 in) 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 below using a calibrated manual torque wrench.

Table 7. Recommended Torque Values

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

8. **TangenX PRO PD 2-bolt torque sequence:** Using the calibrated torque wrench with an 11/16-inch hex deep socket, place the socket over one nut and tighten the nut 1/4 turn. Move the wrench across to the other bolt and tighten the nut 1/4 turn. Alternate back and forth until the torque wrench clicks at each nut. The click of the torque wrench indicates that the nut has reached the set point torque value.
9. **TangenX PRO 4-bolt torque sequence:** Using the calibrated torque wrench with a 1¼ inch hex deep socket, place the socket over one nut (B1) and tighten the nut 1/4 turn. Then move the wrench to diagonally to the next nut (B2) and tighten the nut 1/4 turn. Move the wrench straight across the cover to the third bolt (B3) and tighten the nut 1/4 turn. Then move to the last bolt (B4) and tighten the nut 1/4 turn. Continue using this crisscross pattern until the torque wrench clicks at each nut. The click of the torque wrench indicates that the nut has reached the set point torque value.

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

10. Wait 5 – 10 minutes and allow the gaskets to relax before confirming torques are within range by checking for the click of the torque wrench on each bolt.
11. Retorque as needed to create a liquid-tight seal, but do not exceed the maximum torque limits.

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

4.1 First-time Use of Membrane Cassettes

Cassettes should be flushed with DI water, WFI, and/or buffer to ensure removal of storage and preservative agents from the membrane filter and to minimize any possible interaction with your application. For some applications, further sanitization may be required.

4.2 Cleaning of Membrane Cassettes

Cassettes can be reused if cleaned and stored properly. To clean, flush each cassette (or cassette stack) with a recommended cleaning solution. Use 2 liters of cleaning solution per 1 m^2 of membrane area. Upon completion of the cleaning cycle, flush each cassette (or stack) with DI water, WFI, or buffer prior to placing the cassette (or stack) into storage conditions.

4.3 Storage of Membrane Cassettes

Membrane cassettes must be stored wet to maintain their characteristics and integrity and prevent microbial growth. Below are critical factors to remember when storing:

- Cassettes stored greater than 2 – 4 weeks should be removed from the holder.
- Cassettes left in the holder should be flushed with fresh storage agent about every 2 weeks. The recommended storage agent is 0.1 M – 0.2 M NaOH.
- Recommended pH ranges:
  - pH 2 – 13, long term storage (longer than 1 week)
  - pH 1 – 14, short term cleaning (less than 24 hours)
- Recommended storage temperature:
  - 4°C – 15°C (optimal)
  - 25°C (maximum)

- Do not freeze cassettes

4.4 Membrane Operating Characteristics

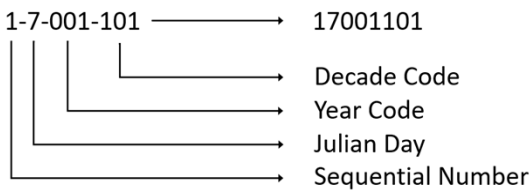
Take care to use the membrane at the lowest pressure possible while still producing consistent permeate flow. Higher operating pressures initially improve flow rate but also promote increased concentration polarization and membrane compaction, which ultimately limit flow. With very low NMWL membranes, lower operating pressure may also reduce the retention of salts and very low molecular weight species.

5. Catalog and Serial Number System

Table 8. Serial Number System

Code Description		Numerical Designation
Decade Codes	2000 – 2009	1
	2010 – 2019	2
	2020 – 2029	3
Year Code (last digit of year)		0 – 9
Julian Day		1 – 366
Sequential Number (three-digit)		001 – 999

Figure 3. Serial Number Example



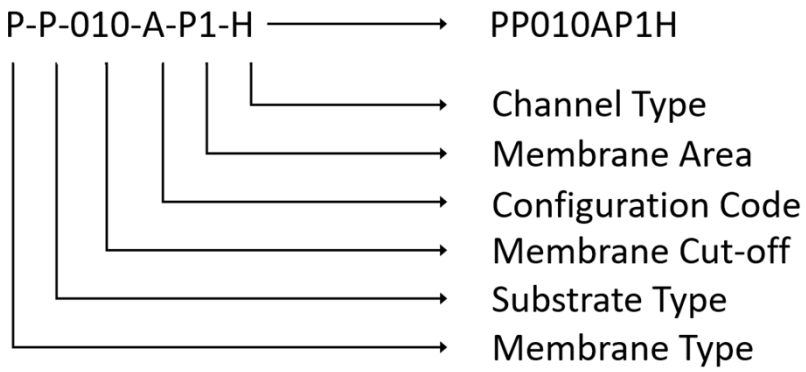
5.1 Cassette Batch Numbers

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 (Enterprise Resource Planning) system. A batch is defined as a group of consecutively serialized cassettes manufactured on the same day, built from up to 6 raw material lots and generated from the same ERP process order. Batch traceability is maintained on the batch record and in the ERP system.

Table 9. Catalog Number System

Code Description		Alphanumeric Designation
Membrane Type	ProStream (mPES, low protein binding) HyStream (mPES, ultra-hydrophilic and low protein binding)	P X
Substrate Type	Polypropylene	P
Membrane cut-off	0.65 kD 1 kD 3 kD 5 kD 10 kD 30 kD 50 kD 100 kD 300 kD 0.1 µm 0.2 µm 0.45 µm 0.65 µm	N65 001 003 005 010 030 050 100 300 M10 M20 M45 M65
Configuration Code	TangenX PRO PD (Pall, reusable) TangenX PRO PD (Millipore, Sartorius, reusable) TangenX PRO (process)	A W B
Membrane Area	0.01 m^2 (0.11 ft^2); PD (A, W) 0.02 m^2 (0.22 ft^2); PD (A, W) 0.1 m^2 (1.1 ft^2); PD (A, W)) 0.5 m^2 (5.4 ft^2); Process (B) 1.5 m^2 (16.2 ft^2); Process (B) 2.5 m^2 (26.9 ft^2); Process (B)	P1 P2 01 05 15 25
Channel Type	H Screen Channel; PD (A), fine woven L Screen Channel; PD (A), medium woven E Screen Channel; PD (A), course woven S Channel (Suspended Screen); Process (B), fine woven with HDPE spacers	H L E S

Figure 4. Catalog Number Example



6. Product Performance

6.1 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, ultrafiltration (UF) membranes are made in multi-step manufacturing processes that often include a post-casting surface modification. The TangenX mPES membranes were developed using state-of-the-art technology including two unique features that deliver significant user benefits:

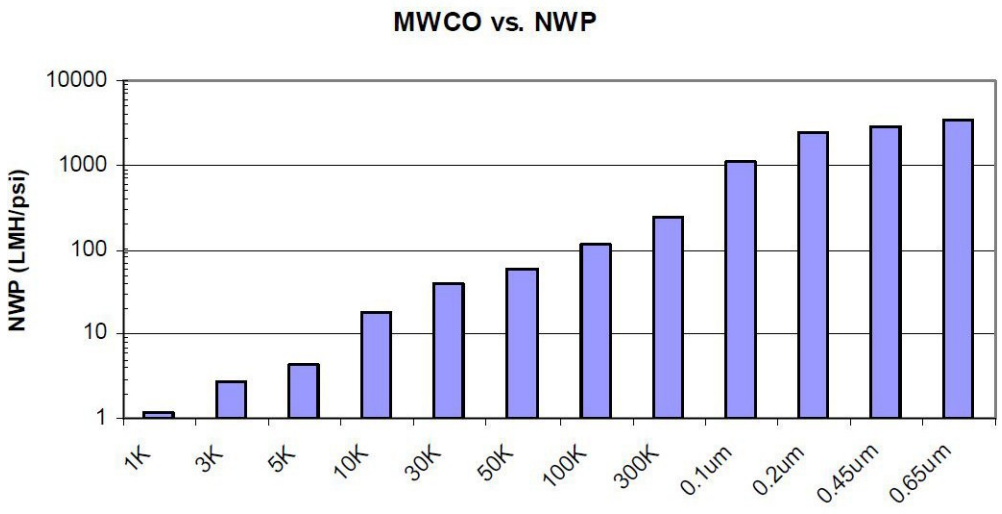
1. Manufactured in a single-cast, uniquely controllable process.
    - Reduced numbers of manufacturing steps equal lower cost and excellent consistency and reliability.
    - Balanced flux and selectivity. This highly controllable manufacturing process enables tight control of the micro-porous/UF transition interface. The macro-porous and UF zones of this membrane are 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.
    - Achieved by the addition of a second polymer into the pre-casting membrane solution, ensuring total and consistent surface modification that delivers.
    - Very low protein binding due to the neutral charge of the membrane.
    - Excellent chemical resistance.

The result is application-focused membranes 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.

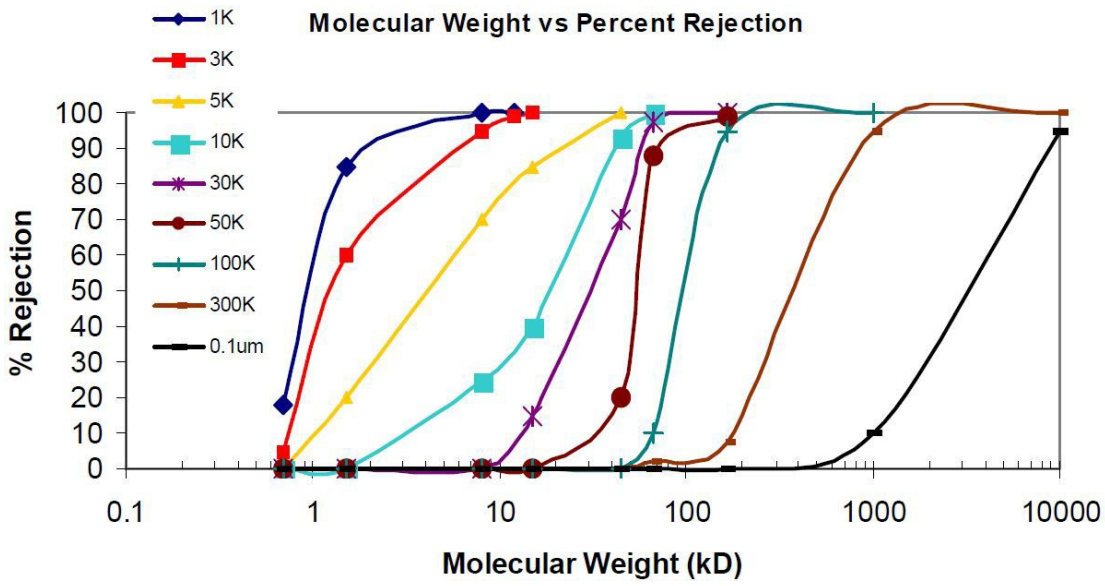
Water flux data (Normalized Water Permeability, NWP) was generated using membrane cut to 44.5 mm discs in stirred cells at 50 psig and purified water at 20°C. TangenX ProStream and HyStream mPES membranes demonstrate comparable water permeability.

Figure 5. ProStream and HyStream Membrane Cutoff (MWCO) vs. Normalized Water Permeability



Many membranes are formulated for either retention or flux. The TangenX ProStream membrane was designed and balanced or both. The following data 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 5, a membrane that best balances flux and retention can be chosen for a specific application.

Figure 6. Membrane Selectivity Performance



Under specific test conditions using stirred cells, purified proteins and molecular weight markers were used to challenge the membranes. TangenX mPES membranes demonstrate excellent selectivity (Figure 6) Membranes above 0.1 µm are characterized using latex particles (not a marker with a defined MW) and are therefore not included. Retention of the latex particles is shown in Figure 35.

6.2 Non-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 (e.g., charge effect, hydrophobic interaction). Non-specific protein binding tends to lead to yield loss and membrane fouling.

The approved test procedure provides methods for 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 MWCO membranes were chosen as they retained each of the proteins tested. The amount of protein bound to the membrane was measured by absorbance at 280 nm and recorded. Several proteins were used as models to test the non-specific binding of the membranes. Proteins differed 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 10. 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 10 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 modified PES binds significantly less protein. Lower protein binding is a desirable attribute of these membranes as lower binding leads to higher product recovery.

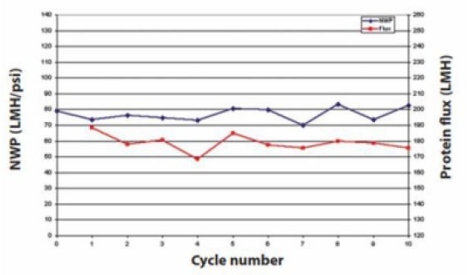
Additionally, lower protein binding reduces the chances of a secondary boundary layer forming on the membrane surface, reducing productivity. Based on the information gathered, it may be claimed that the modified PES membranes manufactured by Repligen are considered low protein binding when compared to unmodified PES membranes.

6.3 Membrane Cleaning Cycles

The unique process for modification of the TangenX membranes also provides excellent chemical resistance. To be of practical use, chemical resistance must be measured both in terms of maintenance of selectivity post-cleaning and regeneration of water flux. To demonstrate this, both 50 kD ProStream and HyStream membranes were challenged with 1 mg/mL Bovine Serum Albumin using 13 cm^2 stirred cells. The membrane clean water flux was initially evaluated and recorded. Next, the membranes were used to concentrate 1 mg/mL BSA, and cleaned using 0.5 N NaOH, 200 ppm bleach at (40°C) for 35 minutes. After cleaning, the water flux was measured again and compared to the clean water flux where recovery of greater than 90% was established as a target. Following this cycle, the membranes were challenged and cleaned nine more times, for a total of ten cycles.

Figure 7 shows that, after 10 cycles (6 hours of exposure in the cleaning solution), the ProStream membranes consistently demonstrate greater than 90 percent water flux recovery. Additionally, the data show that the protein flux remains consistent from cycle to cycle. Figure 8 shows that the rejection of the membrane is greater than or equal to the initial rejection of a new membrane. In conclusion, the ProStream membrane has good chemical resistance to aggressive cleaning processes and can be consistently recovered without a significant change in flux or selectivity.

Figure 7. ProStream Membrane Regeneration (Flux)



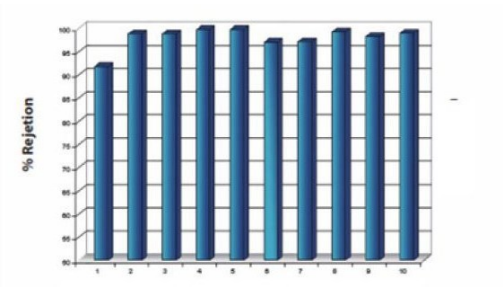
Note: *Membrane: 50 kD ProStream mPES membrane disc in a 13 cm^2 stirred cell*

Note: *NWP was generated using purified water at 25°C.*

Note: *Protein flux data was generated using purified bovine serum albumin (1 mg/mL) in PBS pH 7.4.*

Note: *Membranes were cleaned using 0.5 M NaOH, 200 ppm bleach at 40°C for 35 minutes per cycle.*

Figure 8. ProStream Membrane Rejection



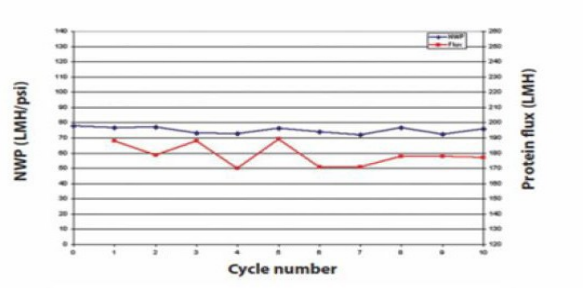
Note: *Membrane: 50 kD ProStream mPES membrane disc in a 13 cm² stirred cell.*

Note: *Protein rejection data was generated using purified bovine serum albumin (1 mg/mL) in PBS pH 7.4.*

Note: *Membranes were cleaned using 0.5 M NaOH, 200 ppm bleach at 40°C for 35 minutes per cycle.*

Figure 9 shows that, after 10 cycles (6 hours of exposure in the cleaning solution), the HyStream membranes consistently demonstrate greater than 90 percent water flux recovery. Additionally, the data show that the protein flux remains consistent from cycle to cycle. Figure 10 shows that the rejection of the membrane is greater than or equal to the initial rejection of a new membrane. In conclusion, the HyStream membrane has good chemical resistance to aggressive cleaning processes and can be consistently recovered without a significant change in flux or selectivity.

Figure 9. HyStream Membrane Regeneration (Flux)



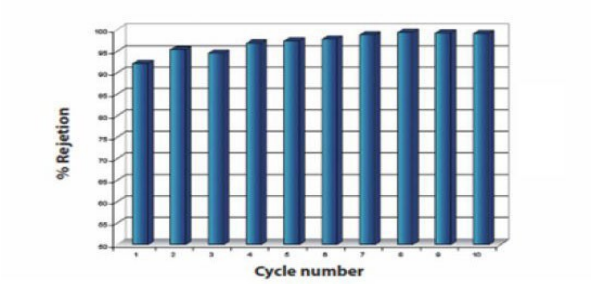
Note: *Membrane: 50 kD HyStream mPES membrane disc in a 13 cm² stirred cell.*

Note: *NWP was generated using purified water at 25°C.*

Note: *Protein flux data was generated using purified bovine serum albumin (1 mg/mL) in PBS pH 7.4.*

Note: *Membranes were cleaned using 0.5 M NaOH, 200 ppm bleach at 40°C for 35 minutes per cycle.*

Figure 10. HyStream Membrane Rejection



Note: *Membrane: 50 kD HyStream mPES membrane disc in a 13 cm² stirred cell.*

Note: Protein rejection data was generated using purified bovine serum albumin (1 mg/mL) in PBS pH 7.4.

Note: *Membranes were cleaned using 0.5 M NaOH, 200 ppm bleach at 40°C for 35 minutes per cycle.*

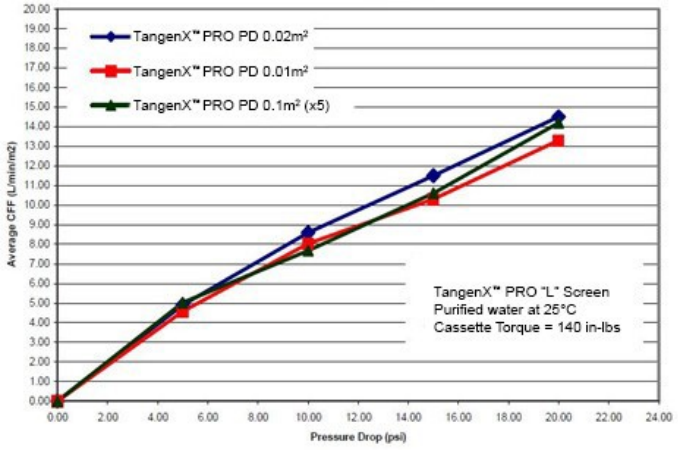
6.4 Cassette Hydraulic Performance

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

- The effect of channel type on the process flux and selectivity profile.
- Scalability: the performance determined at less than 0.1 m^2 scaled linearly to larger filter areas.

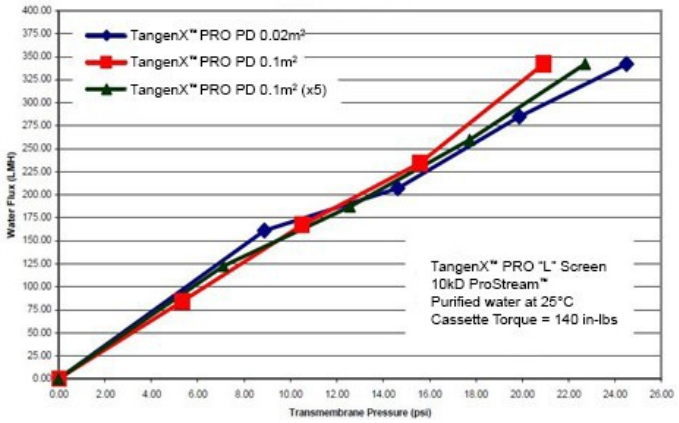
The TangenX PRO Cassette addresses these concerns, as significant development has been devoted to the channel design. Optimized channel geometry, with enhanced rigidity ensures hydraulic performance is maintained when scaling up through the TangenX PRO PD Cassette and TangenX PRO Cassette family, resulting in optimal and reproducible scaling performance. Additionally, each cassette undergoes rigorous QA release testing to verify they meet specification. Cassettes are tested for both air integrity and hydrodynamic performance. This testing ensures cassette-to-cassette consistency. The result is scalable process development and reproducible manufacturing.

Figure 11. Pressure Drop vs. Crossflow Flux: TangenX PRO PD L Screen



The hydraulic scalability of a cassette can be evaluated using purified water under controlled conditions. These data can be used to support scalability of the TangenX PRO PD TFF Cassette product line from 0.01 m^2 to 0.5 m^2 (5 x 0.1 m^2). Similar data support the scale-up between the TangenX PRO PD TFF Cassette and TangenX PRO Cassette products. Typically, the pressure drop between the feed and the retentate is measured at various crossflow rates. This information can then be generated for each cassette size as well as cassettes stacked together in parallel. Figure 11 shows the pressure drop versus crossflow specification for the TangenX PRO PD Cassettes.

Figure 12. Transmembrane Pressure (TMP) vs. Water Flux: TangenX PRO PD Cassette, 10 kD



The hydraulic scalability of a cassette can also be evaluated using purified water to measure NWP. Most importantly, NWP is used to characterize cassettes before use and following post-use cleaning. The NWP recovery demonstrates the effectiveness of the clean in place (CIP) procedure for removing foulants deposited on the membrane surface. Figure 12 shows the transmembrane pressure (TMP) versus water flux for the 10 kD TangenX PRO PD Cassettes through scale-up from 0.02 m^2 to 0.5 m^2 (5 x 0.1 m^2).

Table 11. Typical NWP Ranges for TangenX PRO Cassettes

MWCO	Typical NWP Range (LMH/psi)
0.65 kD	0.4 – 0.6
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

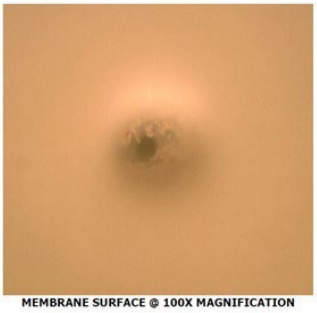
NWP is dependent on MWCO; therefore, there is a range of permeability rates for each cassette of a given MWCO. Table 11 shows typical water permeability rates for the TangenX PRO TFF Cassette with the L Screen channel. It is important to note that external influences such as manifolds, piping, and valves create restrictions and can affect the measured NWP. Therefore, it is important to measure the initial NWP of your cassette in its designated system.

6.5 Cassette Integrity

The purpose of the cassette integrity testing is to provide a non-destructive method to verify the integrity of a TFF cassette. Each cassette manufactured by Repligen undergoes strict release testing, including an air integrity test. Release testing 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, the upstream side of a 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 the table below. 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 13. Sensitivity of Air Integrity Test



The result of the integrity test following the defect being added to the cassette is found in the tables below. The pinhole defect in the membrane allowed air to pass through the membrane and the flow was measured. The airflow for the modified sample was

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

Table 12. Cassette Integrity Specifications

Cassette Channel Type	Member Type	Specification
H Screen – Fine Screen L Screen – Medium Screen E Screen – Coarse Screen S Channel – Suspended Screen	Ultrafiltration 0.65 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)

Table 13. Cassette Integrity Test Results

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

6.6 Cassette Leachables

The following study was conducted to evaluate the leachables of TFF cassettes manufactured by Repligen. These cassettes are packaged in 20% glycerin and 0.1% sodium azide prior to shipment. Prior to use, the storage solution must be flushed from the cassette. This storage solution would be considered leachables by the end user if not sufficiently removed following the recommended rinse and sanitization procedures provided by Repligen. The following report outlines steps that were taken to determine the ideal conditions under which to remove the storage solution (leachables).

Several different cassettes were manufactured and evaluated in triplicate. Each cassette was prepared using current standard operating procedures (SOP), reflecting the standard cassette manufacturing process at Repligen. One cassette type with two membrane chemistries was chosen for this study: the TangenX PRO PD 0.1 m^2 L Screen Cassette using 10 kD ProStream and 10 kD HyStream membranes. These configurations are considered representative for the entire product line.

The first set of experiments conducted was for the filtration system alone, with no membranes installed. Measurements using a calibrated pH probe, conductivity meter, and reversed phase HPLC were used to quantify the amount of storage agent removed. The recommended cassette flushing procedure includes an initial DI water flush, a sanitization in 0.5 M NaOH, and a second DI water flush. Once the flushing procedure was complete, a minimal volume of DI water was recirculated for two hours and analyzed over time to quantify residual leachables extracting from the cassette into the DI water.

The effluent stream was analyzed, and the results reported in Figure 14 and Figure 15. The data show that the system is effectively flushed following approximately 3 to 4 liters of water through the retentate and then through the permeate. The pH of the stream after flushing is neutral and the conductivity <2 µS. These are considered baseline conditions and will be referenced during subsequent experiments.

Figure 14. Hardware Baseline: Flush Volume vs. Effluent Stream pH

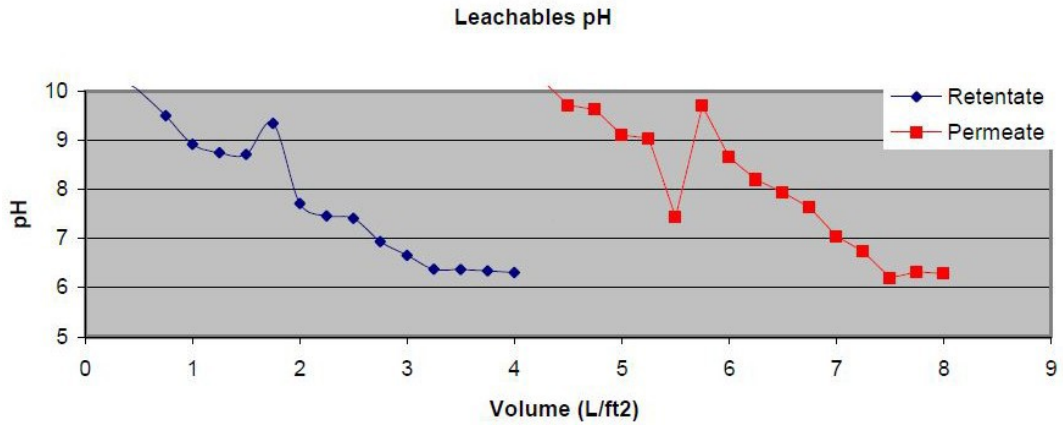
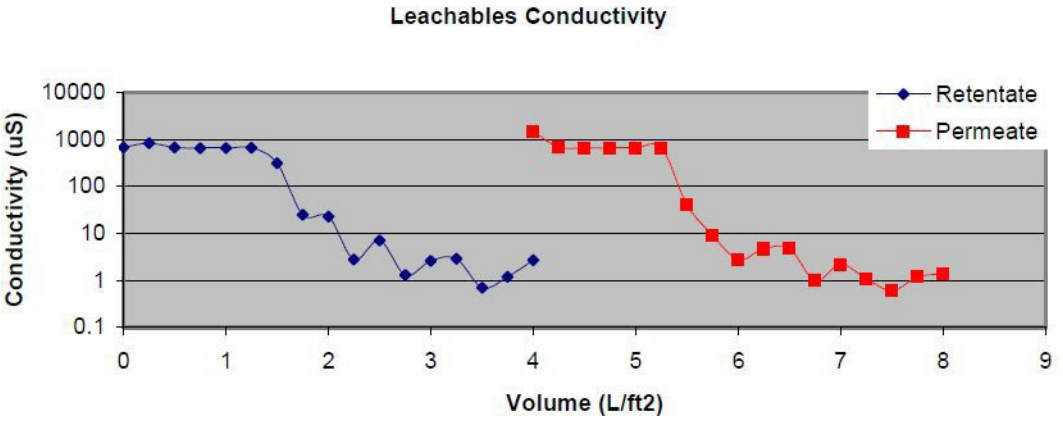


Figure 15. Hardware Baseline: Flush Volume (L) vs. Effluent Stream Conductivity



Once a baseline was generated for the filtration system, the experiments for a set of three cassettes containing ProStream and HyStream membranes began. One 0.1 m^2 cassette was installed in the hardware and flushed with DI water. The effluent stream was analyzed, and the results reported in Figure 16, Figure 17, and Figure 18 The data show that the pH and conductivity quickly drop once approximately 1 to 2 liters of water are flushed through the retentate and then through the permeate. The pH of the stream is neutral and the conductivity <2 µS. The glycerin concentration drops below 10 ppm after 4 liters of water is flushed through the retentate and the permeate.

Figure 16. ProStream and HyStream Flush #1: Flush Volume vs. Effluent Stream pH

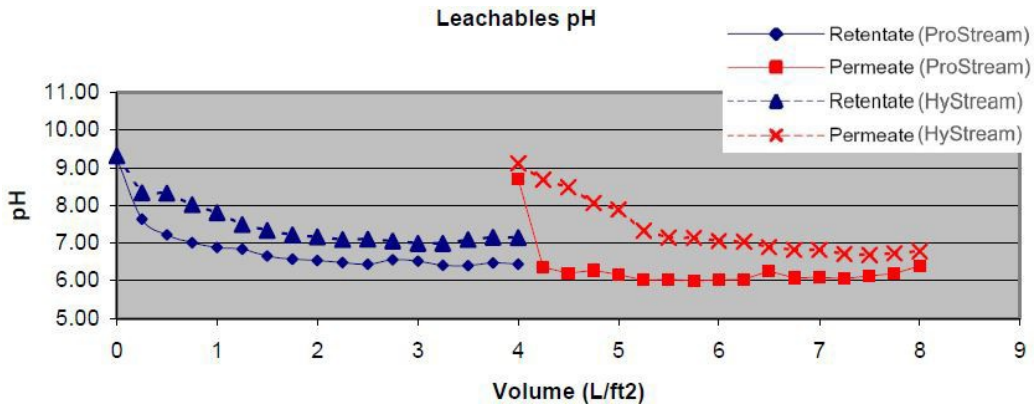


Figure 17. ProStream and HyStream Flush #1: Flush Volume vs. Effluent Stream Conductivity

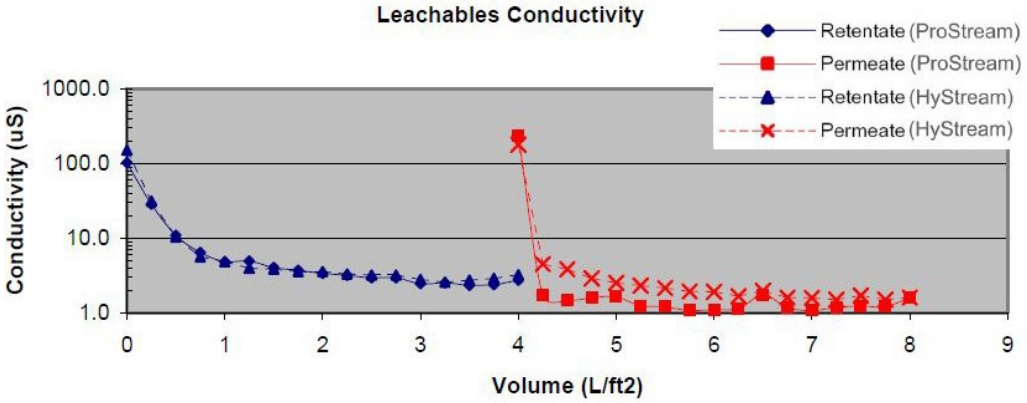
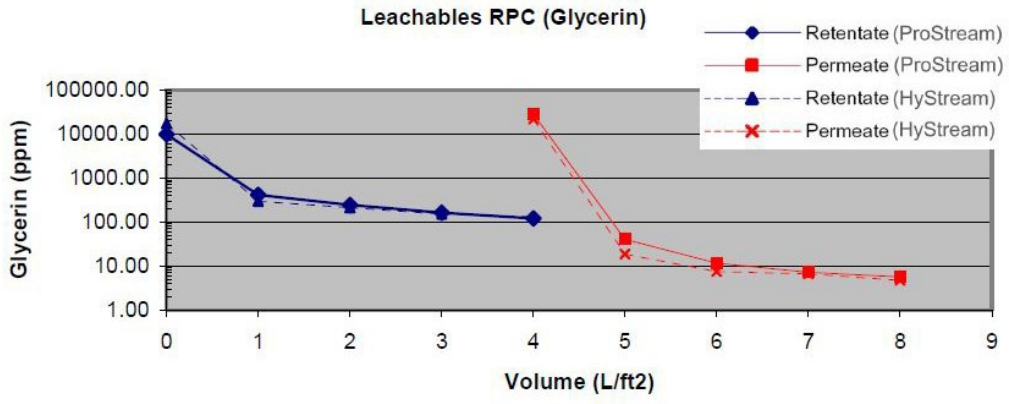


Figure 18. ProStream and HyStream Flush #1: Flush Volume vs. Effluent Stream Glycerin Concentration



Approximately 500 mL of 0.5 M sodium hydroxide was then recirculated thought the cassette for 30 minutes with all valves open. The cassette was then flushed a second time with DI water and the effluent stream was analyzed. The following results represent an average of each cassette type in triplicate. The results from Flush #2 are reported in Figure 19, Figure 20, and Figure 21. The data show the pH and conductivity drop following 3 – 4 liters of water through the retentate and then through the permeate. The pH of the stream is neutral and the conductivity <2 µS. The glycerin concentration drops below 1 ppm once 1 – 2 liters of water is flushed through the retentate and the permeate. This procedure was repeated, and the results below represent an average of each cassette type in triplicate.

Figure 19. ProStream and HyStream Flush #2: Flush Volume vs. Effluent Stream pH

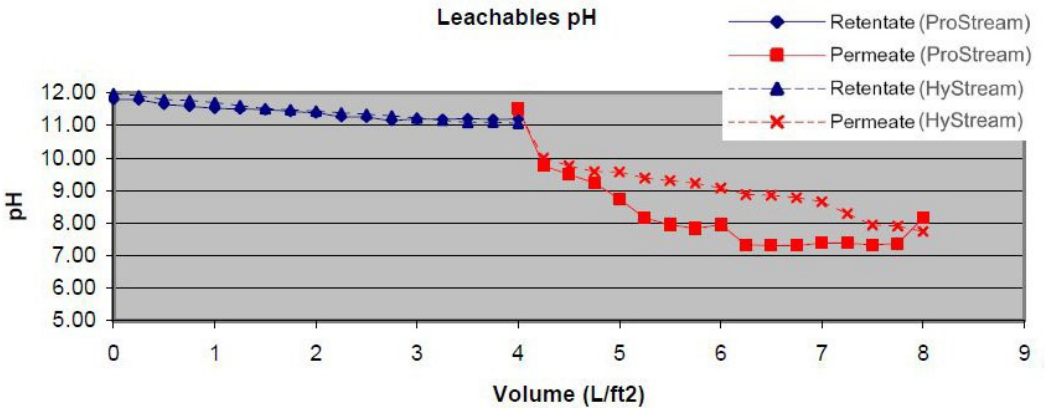


Figure 20. ProStream and HyStream Flush #2: Flush Volume vs. Effluent Stream Conductivity

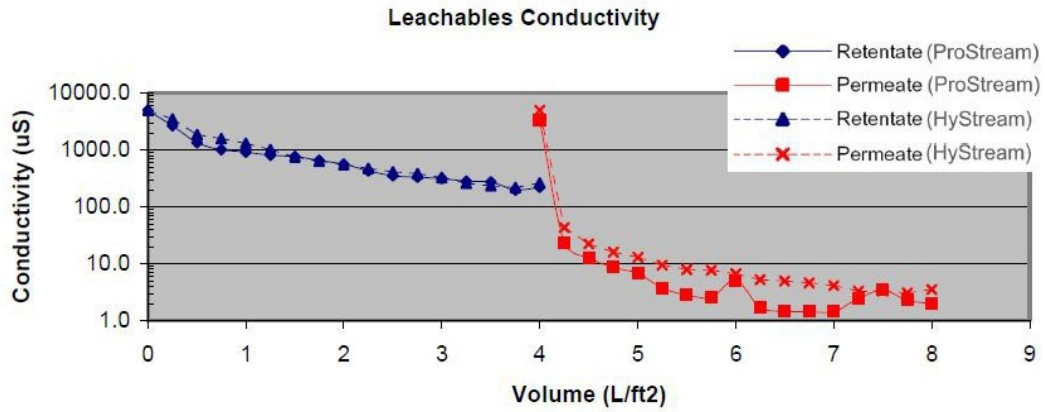
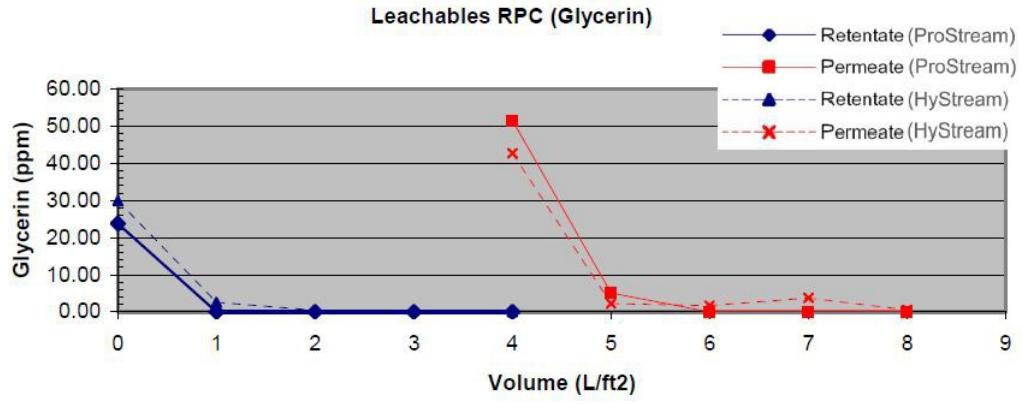


Figure 21. ProStream and HyStream Flush #2: Flush Volume vs. Effluent Stream Glycerin Concentration



The results from the DI water recirculation are reported in Figure 22, Figure 23, and Figure 24.The data show the pH and conductivity plateau after approximately 15 minutes. The pH of the stream reaches an approximate pH of 9 and then remains stable. A slight pH shift such as this is to be expected due to the lack of buffer capacity of DI water. Likewise, the conductivity of the stream reaches an approximate value of 10 µS before it stabilizes. The mass of glycerin in the stream reaches a maximum value of 0.2 mg/m^2, representing the worst case of leaching into DI water. This procedure was repeated two more times for the second and third cassette in the series. The results represent an average of the three cassettes.

Figure 22. ProStream and HyStream: Recirculation Time vs. Effluent Stream pH

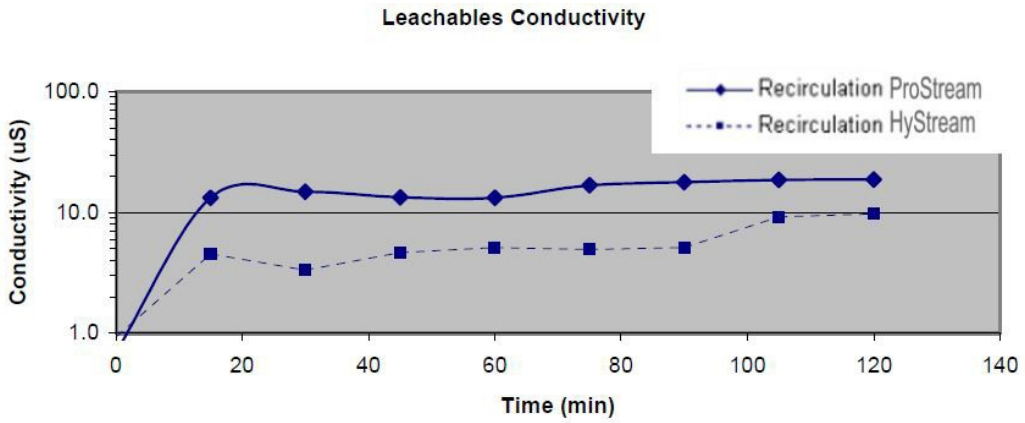


Figure 23. ProStream and HyStream: Recirculation Time vs. Effluent Stream Glycerin Mass

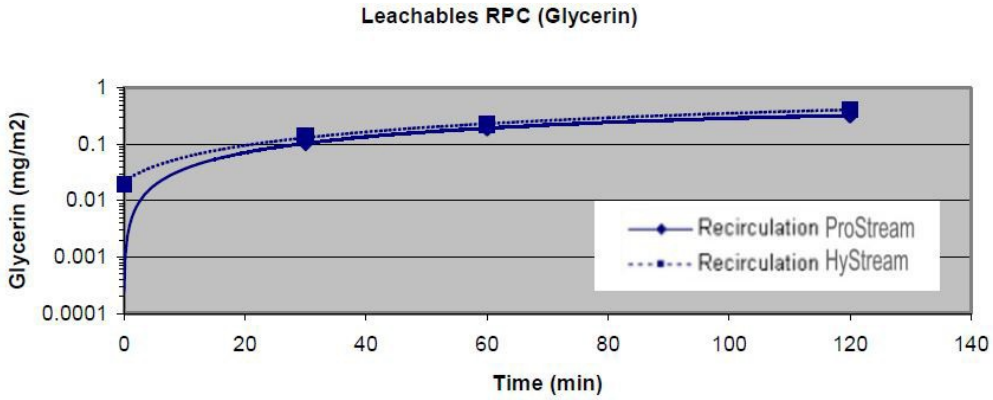
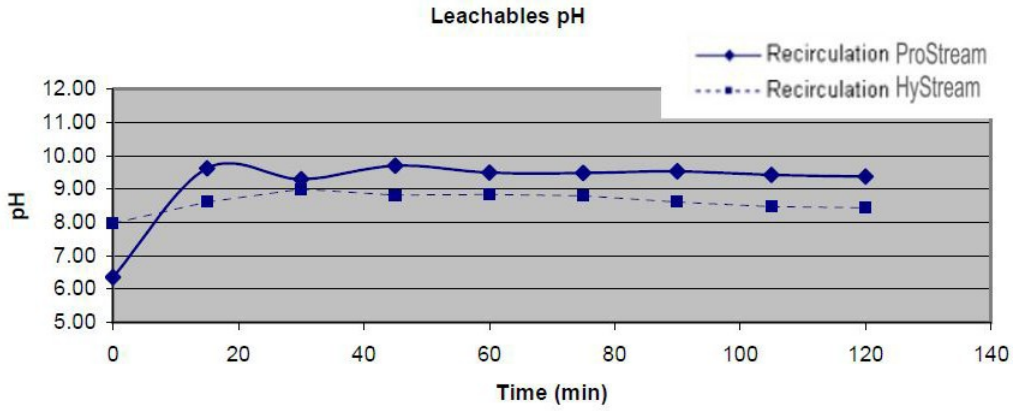


Figure 24. ProStream and HyStream: Recirculation Time vs. Effluent Stream Conductivity



The leachables study showed that storage agents are effectively flushed from the cassettes using the validated method. The cassette flush procedure includes an initial DI water flush, a sanitization, followed by the second DI water flush. Measurements made using a calibrated pH probe, conductivity meter, and reversed phase HPLC quantified the amount of storage agent removed during the cassette flush procedure. It was shown that the recommended cassette flushing procedure was effective for removing the cassette storage agents and minimizing leachables.

7. Shelf-life Studies

Two shelf life studies were conducted by Repligen, the first for membranes and the second for TangenX PRO Cassettes. The following sections summarize the conclusion of both studies.

7.1 Membranes

A shelf life study for ultrafiltration and microfiltration membranes manufactured by Repligen was performed. 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 SOP and reflected the standard membrane manufacturing process at Repligen. The following steps were taken as part of the study:

- Membranes were prepared using SOP-0448, SOP-0564, and SOP-0565
- These membranes were sampled and tested following TX1001-POQ-115

The membrane storage study procedure TX1001-POQ-115 was applied to both the modified PES 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 SOP-0463.

The storage study was undertaken at both ambient temperature and 50°C. The ambient temperature study was designed to simulate exposure to a normal or median temperature. This study spanned five(5) years and was the standard shelf life study. The second part of the study, conducted at 50°C, was designed to simulate exposure at the maximum temperature limit. This accelerated study concluded after one (1) month. A study at lower temperatures (below ambient) was not conducted.

Each membrane sample sheet, at a given time point, was evaluated in triplicate. In the event one membrane failed during the study, a failure analysis would have been conducted through the deviation procedure (SOP-0847). The mode of failure and impact on product quality would have 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.

Table 14. Membrane Shelf Life Study Acceptance Criteria

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

Table 15. 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 16. 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

Conclusions

Several membrane batches were manufactured as part of the initial process validation. Each batch was prepared using current SOP and reflect 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 following both the accelerated study after one month and ambient conditions after five years. The membrane performance, based on water permeability, rejection, and integrity were not affected after five years. The membrane storage study successfully reached its five-year conclusion.

7.2 TangenX PRO Cassettes

A shelf life storage study for the TangenX PRO Tangential Flow Filtration Cassettes manufactured by Repligen was performed. The cassettes are manufactured, packaged, and stored for a period of time prior to shipment. Once shipped, the cassette may then remain unopened for another period of time before it is put into use. The maximum projected duration for the TangenX PRO Cassette shelf life is up to five years. Several cassettes were manufactured and evaluated in triplicate. Each cassette was prepared using current SOP and reflect the standard cassette manufacturing process at Repligen. The following steps were taken as part of the study:

- Cassettes were prepared using SOP-0475
- These cassettes were sampled and studied following the procedure in TX1001-POQ-116

One cassette type with two membrane chemistries was chosen for this study: the TangenX PRO PD 0.1 m^2 L Screen Cassette using 10 kD ProStream and 10 kD HyStream membranes. These configurations are considered representative for the entire product line. The storage study was performed under three temperature conditions: ambient, 37°C, and 5 °C. The ambient study was designed to simulate exposure to a normal or median temperature. This was spanned five (5) years and was a standard storage study. The studies conducted at 37°C and 50°C were designed to simulate exposure at higher temperatures. This portion of the study was

concluded within 3 months and was considered an accelerated study. A study at lower temperatures (below ambient) was not conducted.

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

Table 17. TangenX PRO Cassette Shelf Life Study Acceptance Criteria

Descriptions		Specifications
Water Crossflow Rate	Flow Rate Pressure drop	0.5 – 0.8 @ Pressure drop 10 ± 0.5 psi (0.7 ± 0.03 bar)
Air Diffusion Rate	Rate (ccm)	≤30 @ 7.3 ± 0.5 psi
Visual Inspection	Lot Number	Matches data sheet
Particulates	Count	≤10
Standard Release Testing	Per TangenX PRO Cassette Storage Study Procedure (TX1001-POQ-116) and Release Testing (SOP-0482)	

Table 18. Results: Cassette Storage Study @ 37°C (3 months)

Cassette Type	Membrane Type/MWCO	Results			Number of Samples Tested
		Time Initial	1 Month	3 Months	
TangenX PRO PD	ProStream/10 kD	Pass	Pass	Pass	6
0.1 m^2 L Screen Channel	HyStream/10 kD	Pass	Pass	Pass	6

Table 19. Results: Cassette Storage Study @ Ambient (5 years)

Cassette Type	Membrane Type/MWCO	Results								Number of Samples Tested
		Time Initial	Months		Years					
			3	6	1	2	3	4	5	
TangenX PRO PD	ProStream/10 kD	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	6
0.1 m^2 L Screen Channel	HyStream/10 kD	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	6

Conclusions

Several cassettes were manufactured and evaluated in triplicate; each cassette was prepared using current SOPs and reflect the standard cassette manufacturing process at Repligen. The results show the TangenX PRO TFF Cassettes meet or exceed all release specifications after both the accelerated study and ambient conditions after five years. The TangenX PRO TFF Cassette storage study successfully reached its five-year conclusion.

8. Chemical Compatibility

Table 20. ProStream and HyStream Chemical Compatibility

Reagent	ProStream	HyStream
pH Range	1-14	1-14
Acetic Acid (5%)	√	√
Acetic Acid (25%)	√	x
Acetone (≤ 30%)	√	√
Acetonitrile (≤ 15%)	√	x
Alconox® (1%)	√	√
Aliphatic and Aromatic Esters	x	x
Amines	x	x
Ammonium Chloride (1%)	√	√
Ammonium Hydroxide (5%)	x	x
Aromatic and Chlorinated Hydrocarbons	x	x
Butanol (70%)	√	√
Butyl Acetate (40%)	√	x
Butyl Cellosolve™ (10%)	√	√
Calcium chloride (5%)	√	√
Chloroform (0.8%)	√	√
Citric Acid (1%)	√	√
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%)	√	√

Reagent	ProStream	HyStream
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)	√	x
Tergazyme® (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 observed

9. Safety Testing

9.1 USP Class VI Testing

The purpose of UPS Class VI testing is to verify the biological safety of each of the components used in the TangenX PRO Cassette product line. Samples for USP Class VI testing consisted of each of the five components of the TangenX PRO TFF Cassette. Each component used to construct the cassettes is listed in the Figure 25. Sample dimension, sample mass and test regime are identified as well.

Figure 25. 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	HyFlo Membrane	Polyethersulfone	~ 45 grams from 3 lots	25mm (diameter) x 0.2mm (1)	A,B,C
4	BioFlo Membrane	Polyethersulfone	~ 45 grams from 3 lots	25mm (diameter) x 0.2mm (1)	A,B,C
5	Silicone Gasket	Platinum Cured Silicone	~ 45 grams from 3 lots	25mm (diameter) x 1mm (1)	A,B,C
6	Channel Spacer	Polyolefin	~ 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 USP testing required preparation prior to analysis. Each sample needed to be rinsed with WFI, sanitized with 0.5 M NaOH, and 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 testing. The procedure was used to provide a record of the samples to be prepared, as well as the method of preparation. Experimental deviations, ways to rectify them, and their affect on the study were recorded in a laboratory notebook and a copy attached to the final report.

Results and Discussion

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

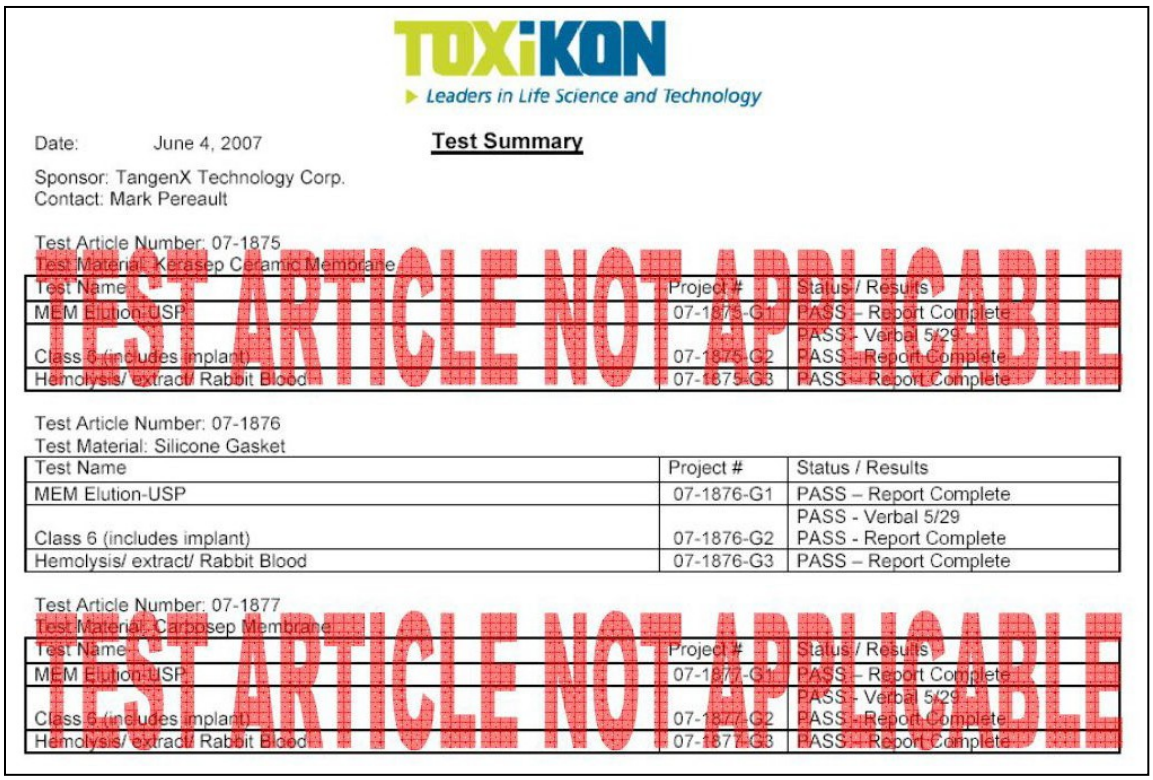
- Current requirements for USP Class VI biological testing for plastics
- The test requirements as defined in the USP guidelines: USP 30, NF 25, 2007, <788> Particulate Matter in Injections

All components materials used in cassettes manufactured by Repligen were 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. The study reports results generated by Toxikon are found in the complete USP report that can be provided by Repligen. A summary of the test results is shown in Figure 26 and Figure 27.

Figure 26. Summary of USP Testing Results (Page 1 of 2)



TOXiKON

Leaders in Life Science and Technology

Date: June 4, 2007

Test Summary

Sponsor: TangenX Technology Corp.
Contact: Mark Pereault

Test Article Number: 07-1875
Test Material: Kerasep Ceramic Membrane

Test Name	Project #	Status / Results
MEM Elution-USP	07-1875-G1	PASS – Report Complete
Class 6 (includes implant)	07-1875-G2	PASS - Verbal 5/29 PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1875-G3	PASS – Report Complete

Test Article Number: 07-1876
Test Material: Silicone Gasket

Test Name	Project #	Status / Results
MEM Elution-USP	07-1876-G1	PASS – Report Complete
Class 6 (includes implant)	07-1876-G2	PASS - Verbal 5/29 PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1876-G3	PASS – Report Complete

Test Article Number: 07-1877
Test Material: Carbosep Membrane

Test Name	Project #	Status / Results
MEM Elution-USP	07-1877-G1	PASS – Report Complete
Class 6 (includes implant)	07-1877-G2	PASS - Verbal 5/29 PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1877-G3	PASS – Report Complete

Figure 27. Summary of USP testing results (Page 2 of 2)

Test Article Number: 07-1885
Test Material: 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 - Verbal 5/29 PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1885-G3	PASS – Report Complete

Test Article Number: 07-1878
Test Material: 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 5/29 PASS - 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 - Verbal 5/29 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 - Verbal 5/29 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 - Verbal 5/29 PASS - Report Complete
Hemolysis/ extract/ Rabbit Blood	07-1882-G3	PASS – Report Complete

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

9.2 Extractables

Samples for extractables testing required preparation prior to analysis. Each sample needed to be rinsed with WFI, sanitized with 0.5M 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 testing.

A controlled extraction study was performed on cassette membrane filtration system components using solvents and extraction techniques across a broad range of polarities. The methodology utilized and results generated are summarized in Study Report 201147-0307-5005. The results generated during this study represent a worst-case scenario, since either the temperature or dissolution properties of the solvents used during this investigation are more aggressive than the solvents used during routine component exposure.

Test samples were initially received immersed in solvent, which was decanted prior to extraction. Samples were rinsed with USP purified water to remove surface contamination as described in the Study Plan. Extraction of the test samples was performed using USP purified water, 25% ethanol in USP purified water, and hexanes in three analytical batches for a 24-hour period. Sub-samples were taken at ~2, 4, 6, 16, and 24 hours and stored in hermetically sealed glass containers for future analysis. An extraction with 5% nitric acid was performed on selected samples for 1 hour.

HPLC/DAD/MS was performed on selected component extracts according to the conditions as described in the Study Plan. Aliquots of the USP purified water, 25% EtOH, and hexanes extracts were analyzed for selected antioxidants. Results of this investigation are

summarized in Figures 1 – 19 of the Study Report. Controls consisting of the three solvents refluxed for 16 hours are also included in these figures. Due to the non-selective nature of the HPLC detectors, tentative identification of the extracted peaks was not possible. The results for these investigations, which include retention times and area responses for unknown peaks are summarized in Tables 3 – 8 of the Study Report. Only extracts containing peaks not observed in the controls are summarized in these tables. No peaks were observed in the hexanes extractions. Figures 1 – 19 depict representative chromatographic profiles for selected extraction solvents acquired at three monitoring wavelengths. Review of the chromatograms indicates the presence of chromatographic peaks at varying intensity. Data is tabulated below in Table 21 and Table 22.

GC/MS was performed on selected component extracts according to the conditions described in the Study Plan. Aliquots of USP purified water, 25% EtOH, and hexanes extracts were assayed after refluxing for 16 hours. Samples were spiked with a known concentration of internal standard that was used in estimating the concentration of tentatively identified analytes and unknowns. For the USP purified water extracts a liquid/liquid solvent partition was performed by adding 2 mL of dichloromethane (DCM) to 5 mL of the sample, vortexing the sample, and transferring the DCM layer to an autosampler vial for analysis. Results are summarized in Tables 9 – 24 of the Study Report. These tables catalog the retention time and estimated concentration of tentatively identified compounds. The identification of compounds was accomplished using the NIST library contained in the GC/MS software. Where possible, the identity of the observed peak was reported, and the estimated concentration determined with the use of an internal standard added to each extraction solvent prior to injection onto the GC/MS system. The final reports are reported in the units of mg/g of material extracted. The identification should be considered tentative until the chromatographic retention time and mass spectra can be compared to authentic reference standards. Data is tabulated below in Table 21 and Table 22.

Total Organic Carbon (TOC) analysis was performed on the USP purified water extracts with quantification of the extracts performed from a five (5) level external calibration curve. Test sample results observed higher than the highest concentration standard was re-assayed following dilution. Results for the USP purified water extracts are summarized in Tables 31 and 32 of the Study Report. Calibration was performed between 0.200 and 20 mg/mL. Two samples were observed to have recovered TOC concentrations above the calibration curve and were re-assayed following dilution. Data is tabulated below in Table 21 and Table 22.

Total Residues following Evaporation (Non-Volatile Residue-NVR) was performed on the USP purified water, 25% EtOH, and Hexanes. Extracts were assayed after refluxing for 16 hours following the procedure outlined in the Study Plan. Results are summarized in Table 33 of the Study Report, with a majority of the final masses recorded as low milligrams except for the HyStream membrane. Data is tabulated below in Table 21 and Table 22.

The test for Oxidizable Substances was performed on the USP purified water extracts sampled from the 16 hour extraction. Results are recorded in Table 34 of the Study Report and include observations and final precipitate mass where applicable. Data is tabulated below in Table 21 and Table 22.

Fourier Transform Infrared (FTIR) Spectroscopy was performed by serial diluting and evaporating the three extraction mediums to dryness, since insufficient residue was available from the NVR extractions. Spectra indicate the absence of significant peaks and are presented as Figures 33 – 52 in the Study Report. Polystyrene calibration film and the extraction solvent method controls from the 16 hour reflux are shown as representative spectra. Data is tabulated below in Table 21 and Table 22.

Table 21. Summary of Results from Extractables Testing (Water Reflux, 16 Hours)

Test	A	B	C	D	E	F	G
Test description	Total residuals following evaporation	FTIR of residue	HPLC/DAD	GC/MS	TOC	ICP/MS	Oxidizable substances
Units	(g)	Wavenumber (cm^{-1})	# of peaks	# of peaks	(ppm)	ppm	Precipitate (g)
Cassette encapsulant	0.00195	2500	2	6	106[a]	N/A	ND
Screen/Channel spacer	0.00053	2300	ND	4	3.62	N/A	ND
BioFlo Membrane	0.00181	2400	ND	2	16.1	N/A	ND
HyFlo Membrane	0.00975	2400	3	2	145[a]	N/A	0.00462
Silicone gasket	0.00208	2300	ND	3	3.95	N/A	ND

[a]Samples reassayed following dilution

Table 22. Summary of Results from Extractables Testing (25% Ethanol Reflux, 16 Hours)

Test	A	B	C	D	E	F	G
Test description	Total residuals following evaporation	FTIR of residue	HPLC/DAD	GC/MS	TOC	ICP/MS	Oxidizable substances
Units	(g)	Wavenumber (cm^{-1})	# of peaks	# of peaks	(ppm)	ppm	Precipitate (g)
Cassette encapsulant	-0.00197	2400	4	4	N/A	N/A	N/A
Screen/Channel spacer	0.00133	ND	ND	1	N/A	N/A	N/A
BioFlo Membrane	0.00542	2400	1	2	N/A	N/A	N/A
HyFlo Membrane	0.01138	ND	6	1	N/A	N/A	N/A
Silicone gasket	0.00229	2300	3	6	N/A	N/A	N/A

Note: *Test article identified as BioFlo is ProStream. Test article identified as HyFlo is HyStream. Summaries of results are from Reports 201147-0307-5005.*

9.3 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 TangenX 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.

9.4 Endotoxin and Particulate

The following study was conducted to quantify the endotoxin and particulate count from an initial flush from the TFF cassettes. The cassettes are packaged in 20% glycerin and 0.1% sodium azide prior to shipment. 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 in which each cassette was flushed with 100 mL of water, displacing the storage solution. The storage solution flush was then evaluated for endotoxin and particulate count by a contract lab. 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 SOP and reflected the standard cassette manufacturing process at Repligen. The following steps were taken as part of the study:

- Cassettes were prepared using approved procedures.
- These cassettes were flushed, and the liquid was analyzed.

The TangenX PRO PD 0.1 m^2 L Screen Cassette using 10 kD ProStream membrane as chosen as it accurately represents the construction of the entire product line, including the 0.5 m^2 TangenX PRO Cassette. During the study, each cassette was evaluated in triplicate. The cassettes were installed in the TangenX PRO PD Cassette Holder, flushed with water, and analyzed for endotoxin and particulate count. The procedure used for the 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. Approved procedural steps were followed during the study. Notes and comments pertaining to the procedure may be found in notebook TAN1001 on pages 53 to 55.

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. The particulate count analysis was conducted under USP 30, NF 25, 2007. <788> Particulate Matter in Injections.

9.5 Endotoxin Results

The results of the endotoxin count study show that the level of endotoxin is considered low and within acceptable limits when compared to industry standards. The control data included only the filtration system with no membrane cassettes installed. The system was assembled, sanitized with 0.5 M sodium hydroxide, and 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 evaluated in triplicate; the data was tabulated and summarized in Figure 28 and Figure 29.

Figure 28. Results of Endotoxin Count Study

SAMPLE NUMBER	REPORTED RESULT (EU/mL)	RESULT W/BASELINE SUBTRACTED (EU/mL)
Control-01	*0.191*	---
CA7331-01	0.269	0.078
CA7331-02	0.450	0.259
CA7331-03	0.743	0.552

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 show that the system contributes a portion of the endotoxin value and was used to correct the sample values.

Once a baseline was generated for the filtration system, the experiments for a set of three cassettes containing ProStream membrane began. One 0.1 m^2 cassette was installed in the hardware and then flushed with 100 mL of water for injection. The data show the 100 mL flush contains endotoxin and may be used to predict endotoxin levels found in the cassettes manufactured at Repligen. The results represent an average of each cassette type in triplicate.

Figure 29. Results of Endotoxin Count Study (Control Subtracted)

SAMPLE NUMBER	FLUSH VOLUME (mL)	CASSETTE MASS (g)	NORMALIZED RESULT (EU/mg)	NORMALIZED RESULT (EU/cm²)
CA7331-01	100	140.33	0.00055	0.037
CA7331-02	100	140.36	0.00185	0.123
CA7331-03	100	140.38	0.00393	0.262
		AVERAGE	**0.00211 EU/mg**	**0.141 EU/cm²**

Once the results were generated, the control result was subtracted from the sample result to generate the final concentration of endotoxin present in the 100 mL sample. The concentration of endotoxin was then multiplied by the same volume and then divided by the mass of the dry filtration cassette to give a normalized result. The relationship between the mass of a TangenX PRO Cassette and its filtration area is approximately 1,000 g per 1.5 m^2. An average value of 0.141 EU/cm^2 can be used to determine the amount of endotoxin in a single filter or a group of stacked filters prior to use.

9.6 Particulate Results

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 mL equal to or greater than 10 µm and does not exceed 2 per mL equal to or greater than 25 µm.

The results of the study are summarized in Table 23. The control data was for the filtration system alone, with no membranes installed, as a baseline. The data show the system contributes to a portion of the particles found in the test samples but did not contribute to a failure. The cassettes were evaluated in triplicate, the data was tabulated, and the data summarized below. The raw data from each set of cassettes may be found in a completed development report.

Table 23. 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 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 mL; equal to or greater than 10 µm and did not exceed 2 per L equal to or greater than 25µm.

In conclusion, the particulate and endotoxin count study showed that the level of endotoxin in this population of cassettes are considered low and are within acceptable limits when utilizing the USP test method <85> for bacterial endotoxins test. Additionally, the results of the particulate count study show 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 utilizes an effective cassette storage solution and minimizes the particulate and endotoxin count prior to shipment of the cassette products. Notes and comments pertaining to the procedure may be found in notebook TAN1001 on pages 53 to 55.

10. Qualification

10.1 Equipment Qualification

Installation Qualification (IQ) and Operation Qualification (OQ) were performed for each piece of critical equipment utilized in the production of the membrane and cassette assembly. The IQ/OQ results were documented in 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.

10.2 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.

10.3 Qualification of Critical Utilities

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

- Water System
- Compressed Air

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

11. 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 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:

- Screen Channel – High Pressure (H) and Low Pressure (L)
- Suspended Screen Channel (S)

Within the framework of the validation, QC methods, utilities, equipment, and personnel must be qualified. Quality Assurance approved the Validation Master Plan 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 and the facility
- Summary of all 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.

11.1 Membrane Process Validation

The process validation of the ultrafiltration and microfiltration membranes produced at Repligen was carried out as specified. The validation included approved procedures for the Casting Solution Preparation, Membrane Casting Procedure, 20% glycerin solution/0.05% Sodium Azide Procedure, Membrane QC Testing Procedure, and the corresponding forms.

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

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. One stage was validation performed on the membrane production process, and the second stage was validation performed on the cassette production process. Once the individual procedures were combined and executed as one validation lot, the membranes manufactured were evaluated for their performance using approved SOP. The test results for each of the validation lots are summarized in Figure 30 and Figure 31.

Each membrane lot met product specifications following approved SOP and was within compliance. The membrane validation is complete and the membrane manufacturing process at Repligen is considered validated.

Figure 30. 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 31. 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

11.2 Cassette Process Validation

The process validation of the tangential flow filtration cassettes produced at Repligen was carried out as specified. The validation included approved procedures for Urethane Part 'A' Mixing Procedure, Die Cutting Procedure, Mold Release Mixing Procedure, Cassette Assembly Procedure, Final Packaging Procedure, Cassette QC Testing Procedure, and the corresponding forms.

The individual procedures were combined and executed as one validation group in which three consecutively serialized cassettes were manufactured. Two cassette types, TangenX PRO PD Cassette 0.1 m^2 L Screen and TangenX PRO Cassette 0.5 m^2 L Screen, each with both ProStream 10 kD and HyStream 10 kD membranes, were validated, representing the entireTangenX PRO 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 (12) cassettes were manufactured during the validation. The cassettes were divided into four (4) groups by product type (TangenX PRO PD Cassette vs. TangenX PRO Cassette) and membrane combinations, as follows:

- 3 each, TangenX PRO PD, 0.1 m^2 with L Screen Channel and ProStream 10 kD membrane
- 3 each, TangenX PRO PD, 0.1 m^2 with L Screen Channel and HyStream 10 kD membrane
- 3 each, TangenX PRO, 0.5 m^2 with L Screen Channel and ProStream 10 kD membrane
- 3 each, TangenX PRO, 0.5 m^2 with L Screen Channel and HyStream 10 kD membrane

Each group contained three consecutively serial numbered cassettes, and 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 Figure 32 and Figure 33.

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.

Figure 32. TangenX PRO PD Cassette Process Validation Summary Table

MEMBRANE	CASSETTE SERIAL #	MEASURED VALUE	TEST SOLUTION TEMP °C	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
ProStream™ 10 kD	17318101	0.646 L/min	20	0.6 to 0.9 L/min	YES
		18 ccm		≤ 30 ccm	YES
	17318102	0.626 L/min	20	0.6 to 0.9 L/min	YES
		19 ccm		≤ 30 ccm	YES
	17318103	0.610 L/min	20	0.6 to 0.9 L/min	YES
		2 ccm		≤ 30 ccm	YES
HyStream™ 10 kD	17316104	0.680 L/min	20	0.6 to 0.9 L/min	YES
		16 ccm		≤ 30 ccm	YES
	17316105	0.620 L/min	20	0.6 to 0.9 L/min	YES
		3 ccm		≤ 30 ccm	YES
	17316106	0.630 L/min	20	0.6 to 0.9 L/min	YES
		19 ccm		≤ 30 ccm	YES

Figure 33. TangenX PRO Cassette Process Validation Summary Table

MEMBRANE	CASSETTE SERIAL #	MEASURED VALUE	TEST SOLUTION TEMP °C	SPECIFICATION	WITHIN SPECIFICATION (YES / NO)
ProStream 10 kD	17313201	3.90 L/min	22	3.14 to 4.71 L/min	YES
		100 ccm		≤ 150 ccm	YES
	17313202	3.72 L/min	22	3.14 to 4.71 L/min	YES
		120 ccm		≤ 150 ccm	YES
	17313203	3.410 L/min	24	3.29 to 4.94 L/min	YES
		60 ccm		≤ 150 ccm	YES
HyStream 10 kD	17316201	4.21 L/min	22	3.14 to 4.71 L/min	YES
		3 ccm		≤ 150 ccm	YES
	17316202	4.11 L/min	27	3.53 to 5.29 L/min	YES
		90 ccm		≤ 150 ccm	YES
	17316203	4.09 L/min	27	3.53 to 5.29 L/min	YES
		8 ccm		≤ 150 ccm	YES

12. Release Testing

12.1 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 method used for quantitative measurement of samples in each matrix is reliable and reproducible. 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 a standard operating procedure for both membrane and cassette QC testing. Each step in these methods was investigated to determine the extent to which environmental, matrix, or procedural variables can affect the estimation of material in the matrix.

Sensitive quantitative procedures such as these require that appropriate steps are 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.

12.2 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.

12.3 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 tested for performance prior to being released. Results of the verification of specificity, linearity, accuracy, precision, and robustness are summarized in a report. Minimum requirements, including acceptance specifications for the methods, were set during the method development and validation cycle. The acceptance criteria are provide in the data sheets included in the report. The procedure, listing the validation steps, was described in the protocol. Notes and comments pertaining to the procedure are in notebook TAN1001 on pages 52 to 53.

The principles followed for the validation were based on cGMP guidelines and helped Repligen ensure the cassette QC test method was acceptable for use. The cassette QC procedure was used to verify air diffusion and crossflow rate for each cassette. This information was used to accept or reject the cassettes. The validation concluded that the cassette method meets requirements set by Repligen for specificity, linearity, accuracy, precision, and robustness. Minimum requirements were met and the QC membrane test procedure is considered validated.

12.4 Release Specifications

A complete set of TangenX PRO PD Cassettes and TangenX PRO Cassettes release specifications is shown in Figure 34. The release specifications for both membrane chemistries are shown in Figure 35.

Figure 34. Cassette QC Release Specifications

High Pressure Screen Channel (HP)

AP1 / WP1	0.06 to 0.09 LPM	@ 15 psi PD	Air Integrity ≤ 3 ccm
AP2 / WP2	0.12 to 0.18 LPM	@ 15 psi PD	Air Integrity ≤ 6 ccm
A01 / W01	0.6 to 0.9 LPM	@ 15 psi PD	Air Integrity ≤ 30 ccm
B05	3.0 to 4.5 LPM	@ 15 psi PD	Air Integrity ≤ 150 ccm
B15	9.0 to 13.5 LPM	@ 15 psi PD	Air Integrity ≤ 450 ccm
B25	12.5 to 20.0 LPM	@ 15 psi PD	Air Integrity ≤ 750 ccm

Low Pressure Screen Channel (LP)

AP1 / WP1	0.06 to 0.09 LPM	@ 10 psi PD	Air Integrity ≤ 3 ccm
AP2 / WP2	0.12 to 0.18 LPM	@ 10 psi PD	Air Integrity ≤ 6 ccm
A01 / W01	0.6 to 0.9 LPM	@ 10 psi PD	Air Integrity ≤ 30 ccm
B05	3.0 to 4.5 LPM	@ 10 psi PD	Air Integrity ≤ 150 ccm
B15	9.0 to 13.5 LPM	@ 10 psi PD	Air Integrity ≤ 450 ccm
B25	12.5 to 20.0 LPM	@ 10 psi PD	Air Integrity ≤ 750 ccm

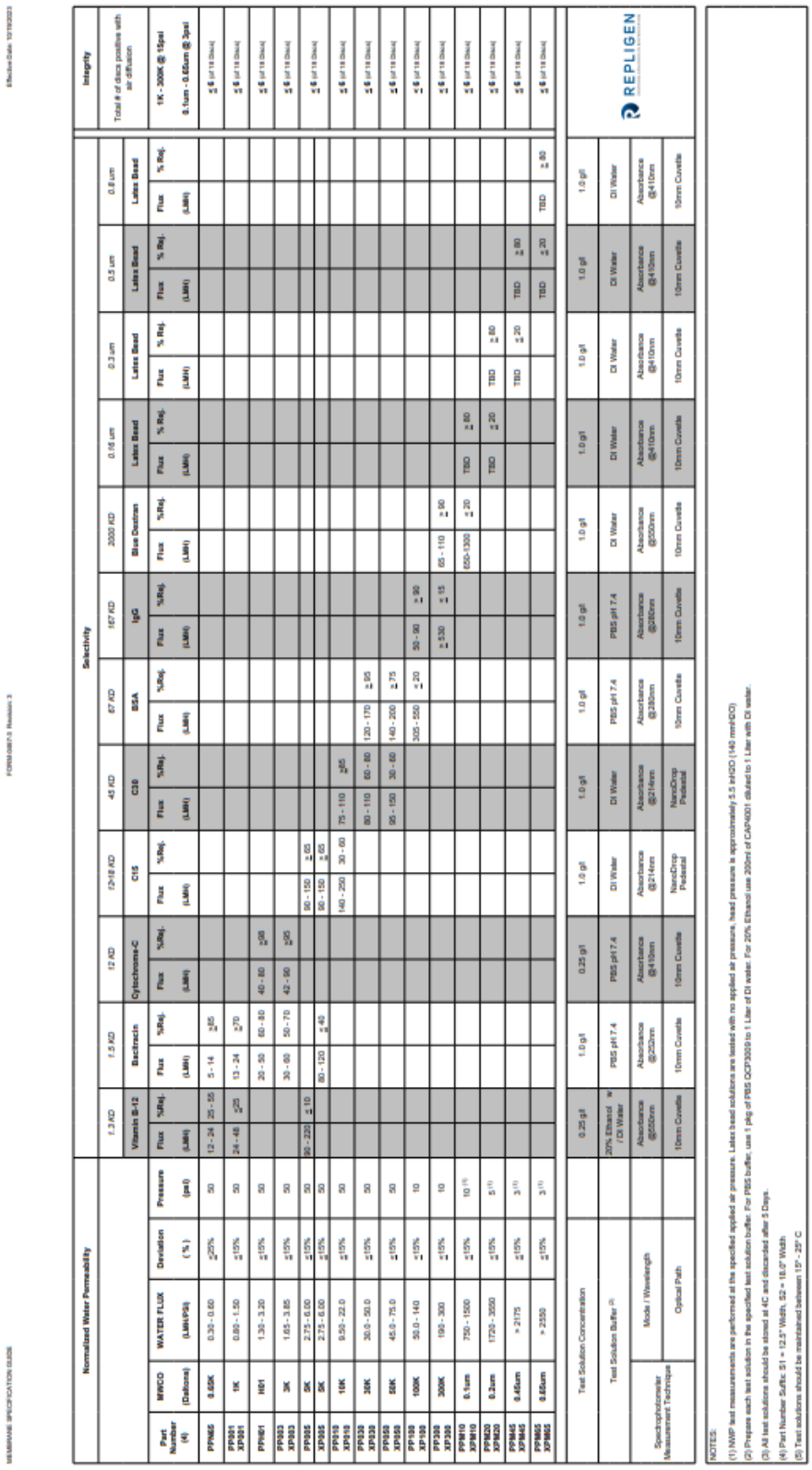
Suspended Screen Channel (S)

AP1 / WP1	0.09 to 0.15 LPM	@ 1.5 psi PD	Air Integrity ≤ 3 ccm
AP2 / WP2	0.18 to 0.30 LPM	@ 1.5 psi PD	Air Integrity ≤ 6 ccm
A01 / W01	0.9 to 1.5 LPM	@ 1.5 psi PD	Air Integrity ≤ 30 ccm
B05	4.5 to 7.5 LPM	@ 1.5 psi PD	Air Integrity ≤ 150 ccm
B15	13.5 to 22.5 LPM	@ 1.5 psi PD	Air Integrity ≤ 450 ccm
B25	22.5 to 37.5	@ 1.5 psi PD	Air Integrity ≤ 750 ccm

CASSETTE RELEASE SPECIFICATION NOTES:

1. AP1, A01, etc., represent the cassette configuration and membrane area codes from Section 2.5
2. Specifications above apply for both ProStream™ and HyStream™ membrane chemistries and all MWCO's
3. Cassettes "wetted" with purified water are tested at the following air pressure:
   - MWCO: 0.65 kD to 5 kD — Air Test Pressure = 1 bar (15 psi)
   - MWCO: 10 kD to 300 kD — Air Test Pressure = 0.5 bar (7.3 psi)
   - Pore Size: ≥ 0.1um — Air Test Pressure = 0.2 bar (3 psi)

Figure 35. Membrane QC Release Specifications



Part Number (4)	MWCO (Daltons)	WATER FLUX (LMH/PSI)	Deviation (%)	Pressure (psi)	Normalized Water Permeability				Selectivity												Integrity
					1.3 KD		1.5 KD		12 KD		218 KD		45 KD		67 KD		167 KD		2000 KD		Total # of discs positive with air diffusion
					Vitamin B-12		Bacitracin		Cytochrome-C		Cl5		C30		BSA		IgG		Blue Dextran		
					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
PPM65	0.65K	0.30 - 0.60	≥25%	50	12 - 24	25 - 55	5 - 14	≥85													≤ 6 (of 19 Discs)
PPM01 XP001	1K	0.80 - 1.50	≤15%	50	24 - 48	≥25	13 - 24	≥70													≤ 6 (of 19 Discs)
PPH01	H01	1.30 - 3.20	≤15%	50			29 - 50	60 - 80	40 - 80	≥88											≤ 6 (of 19 Discs)
PP003 XP003	3K	1.65 - 3.85	≤15%	50			30 - 60	50 - 70	42 - 90	≥85											≤ 6 (of 19 Discs)
PP006	5K	2.75 - 6.00	≤15%	50							90 - 150	≤ 65									≤ 6 (of 19 Discs)
XP005	5K	2.75 - 6.00	≤15%	50			80 - 120	≤ 40			90 - 150	≤ 65									≤ 6 (of 19 Discs)
PP010 XP010	10K	9.50 - 22.0	≤15%	50							140 - 250	30 - 60	75 - 110	≥85							≤ 6 (of 19 Discs)
PP030 XP030	30K	30.0 - 50.0	≤15%	50									80 - 110	60 - 80	120 - 170	≥95					≤ 6 (of 19 Discs)
PP050 XP050	50K	45.0 - 75.0	≤15%	50									95 - 150	30 - 60	140 - 200	≥75					≤ 6 (of 19 Discs)
PP100 XP100	100K	90.0 - 140	≤15%	10											305 - 550	≥20	50 - 90	≥90			≤ 6 (of 19 Discs)
PP300 XP300	300K	190 - 300	≤15%	10													≥ 530	≤15	65 - 110	≥90	≤ 6 (of 19 Discs)
PPM10	0.1um	750 - 1500	≤15%	10 [1]															650-1300	≥20	≤ 6 (of 19 Discs)
PPM20 XPM20	0.2um	1720 - 3050	≤15%	5 [1]																	≤ 6 (of 19 Discs)
PPM45 XPM45	0.45um	> 2175	≤15%	3 [1]																	≤ 6 (of 19 Discs)
PPM65 XPM65	0.65um	> 2550	≤15%	3 [1]																	≤ 6 (of 19 Discs)

Test Solution Concentration			0.25 g/l		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]			20% Ethanol w / 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 @550nm		Absorbance @220nm		Absorbance @410nm		Absorbance @214nm		Absorbance @214nm		Absorbance @280nm		Absorbance @280nm		Absorbance @280nm		Absorbance @550nm		Absorbance @410nm		Absorbance @410nm		Absorbance @410nm	
	Optical Path		10mm Cuvette		10mm Cuvette		10mm Cuvette		NanoDrop Pedestal		NanoDrop Pedestal		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette		10mm Cuvette	

NOTES:
(1) MWP 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 inH2O (140 mmH2O)
(2) Prepare each test solution in the specified test solution buffer. For PBS buffer, use 1 pkg of PBS (QCP30001) to 1 Liter of DI water. For 20% Ethanol use 200ml of CAP40001 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

12.5 Certificate of Conformance

Figure 36 shows an example of the standard Quality Assurance Certificate provided with each cassette manufactured by Repligen. Product part number, serial number, and description are included on the label attached in the upper left corner of the certificate.

Figure 36. QA Certificate of Conformance

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

Repligen Corporation
111 Locke Drive
Marlborough, MA 01752

Quality Assurance Certificate

This is to certify that the TangenX® PRO Cassettes as indicated by the affixed label complies with the following descriptions and specification

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

TangenX® PRO Cassette

BATCH # 99999999 ■ Process Scale RE-USABLE

USE BY: 05-OCT-2029
MEMBRANE: HyStream (Low Fouling mPES)
MWCO: 30 kD
CHANNEL: LP Screen Channel
AREA: 2.5 m² (26.9 ft²)
SERIAL #
34280004
CATALOG #
XP030B25L

Product Quality – TangenX® PRO 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 filter cassette has been individually tested to ensure conformance to the following performance specifications:

1. Hydraulic - 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.

SAMPLE

USP Safety Information

All component materials meet:

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

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

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

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

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

0.1m² cassettes from multiple lots were extracted in 100mL of water for injection and tested for endotoxin 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 < 0.25 EU/ml as determined with the LAL test method.

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

Document Number: QS-2994 Revision: 2
Effective Date: 10/8/2024
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Legacy Document #: QADOC011

13. List of Study Reports

- TX1001-POQ-117-R Protein Binding Study Report
- TX1001-POQ-118-R Cassette Leachables 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-102-TX1001-R Cassette Process Validation Report
- DR-07-005 Cassette Particulate and Endotoxin Count Study Report
- DR-09-010 Membrane Storage Study Interim Report
- DR-09-011 TangenX PRO Cassette Storage Study Interim Report

14. References

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

15. Index

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