PALL Life Sciences

Validation Guide

USTR 2432b(6)

T-Series TFF Cassettes with Omega™ Membrane

For use with Centramate™ and Centrasette™ TFF Systems

*Filtration. Separation. Solution.*SM

Contents

1. Purpose of this Document

This document provides validation support information for Pall's T-Series tangential flow filtration (TFF) cassettes with Omega membrane, and includes summary data to support testing conducted for biological safety, extractables, chemical compatibility, physical and performance attributes, as well as usage conditions (such as temperature limits, chemical limits, cleaning, flushing, integrity testing, and operating methods).

The data contained in this guide is generated under standard conditions as specified. The methods and information contained in this guide are designed to provide the user with guideline for validation ofT-Series TFF cassettes with Omega membrane under actual conditions of use.

Pall Life Sciences offers technical support to customers to develop, troubleshoot, and validate TFF procedures.

1.1 Validating Filtration Processes — General Concepts

TFF membrane cassettes play an important role in purifying, concentrating, and separating biopharmaceutical solutions and products. Typical applications include concentrating human plasma fractions, downstream processing of enzyme and protein solutions, and harvesting mammalian or bacterial cells. Hence, the validation of TFF processes utilizing membrane cassettes is an essential part of ensuring the manufacture of safe and efficacious products.

The U.S. Food and Drug Administration defines validation as “establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes” [Current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals, 21 CFR 210.3]. With respect to a TFF process, validation involves providing assurance that the filtration process operates reproducibly and consistently.

For any given process, a Functional Design Specification must be written based on the requirements of the process and data generated at the pilot scale. For a TFF process utilizing the T-Series cassettes with Omega membrane, this will include developing operational protocols within performance limits outlined in this validation guide and based on the individual cassette operating instructions and the Care and Use manuals supplied with the cassettes.

A process system can then be designed and built to allow direct scale-up to meet specifications established at pilot or bench scale. Because TFF membrane cassettes are incorporated into complex systems, three stages of system validation are followed: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ).

1.2 Installation Qualification (IQ)

Performing the IQ checks that the cassettes selected for the process are the ones supplied and installed in the system, and that specified installation procedures such as torque settings have been adhered to. Additionally, it is confirmed that all required documentation has been received (operating instructions and certificates of conformance) with the cassettes.

1.3 Operational Qualification (OQ)

During OQ, validation personnel test and document the range and operational limits of the filtration process with cassettes in place. OQ does not have to be conducted in the customer process manufacturing area. Validation personnel normally simulate worst-case production conditions for these studies, using water or another surrogate process fluid, to deliberately trigger alarm conditions. As part of the OQ, validation personnel also verify and document procedures such as flushing and sanitizing that are associated with the operation of the membrane cassettes.

1.4 Performance Qualification (PQ)

PQ involves testing the cassette filtration process during production of the final product under actual operating conditions, including installation, sanitizing, conditioning, concentration, diafiltration, product recovery, cleaning, etc. Critical elements of PQ include verification of chemical compatibility and retention characteristics. Since validating a process ensures the process accomplishes what is intended, PQ provides the most meaningful process validation data (which will be confirmed by ongoing performance data collected during system operation) because the data is derived from the process itself, utilizing the intended operating conditions. PQ may not necessarily provide data on the operation of the system at the design limits (alarm conditions), as the process may never reach these limits.

Manufacturers of regulated products must develop and submit protocols, qualification documents, and validation documents for their specific product to be granted approval to manufacture and market their product.

2. Product Specifications

To help prepare IQ documentation, this section provides information on the materials of construction, physical characteristics, and basic performance of T-Series TFF cassettes with Omega membrane.

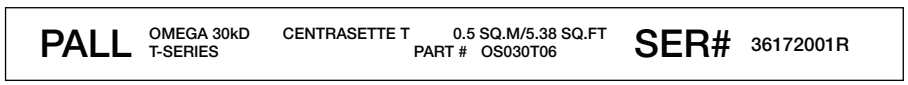
2.1 Packaging

Membrane cassettes are individually packaged in heat-sealed plastic bags with the following information printed on the cassette edge (Figure 1):

- Company name
- Membrane type
- Nominal molecular weight cutoff (NMWC)
- Cassette format
- Membrane area
- Part number
- Serial number

Cassettes are shipped in a box containing two silicone gaskets in a plastic bag, Certificate of Test, T-Series Membrane Care and Use Procedures (USD 2433b) and MSDS documents (where appropriate).

Figure 1
Example of Information Printed on the Side of Cassettes



2.2 Part Numbers

Part numbers give specific information about the cassette. For example, the part number OS030T12 represents an Omega 30 kD membrane Centramate T-Series screen channel cassette with a 0.1 m^2 (1.1 ft^2) area (Figure 2).

The part number for a cassette can be interpreted to identify specific information about the cassette characteristics.

Figure 2
Part Number Code

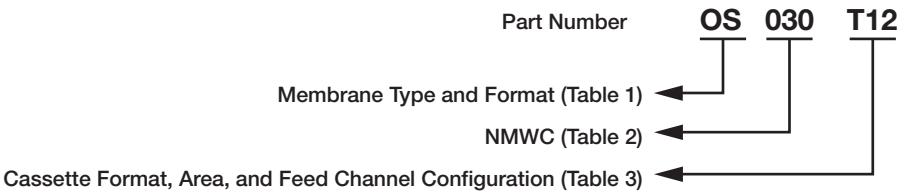


Table 1
Identification Code for Membrane Type

Part Number (Digits 1 – 2)	Membrane Type
OS	Omega

Table 2
Identification Codes for NMWC

Part Number (Digits 3 – 5)	NMWC
001 – 300	1 – 300 kD

Table 3
Identification Codes for Cassette Format and Feed Channel Configuration

Part Number (Digits 6 – 8)	Cassette Format, Feed Channel Configuration	Membrane Area (Nominal)
T02	Centramate Screen Channel	186 cm^2 (0.2 ft^2)
T12	Centramate Screen Channel	0.1 m^2 (1.1 ft^2)
T06	Centrasette Screen Channel	0.5 m^2 (5.4 ft^2)
T26	Centrasette Screen Channel	2.5 m^2 (27 ft^2)

2.3 Serial Numbers

Unique serial numbers enable the tracking of the following cassette information:

- Date of manufacture
- Components used in manufacture
- Water permeability of membrane lot used in construction
- Air integrity (Forward Flow) test results at 2 barg (30 psig)
- Membrane marker retention
- Manufacturing plant location

From the serial number and production records, components can be traced back to their source.

2.4 Materials of Construction

2.4.1 Membrane

Omega membranes are cast from polyethersulfone resins. The membrane is cast on a porous polyolefin substrate that imparts strength and rigidity to the finished membrane product. The nominal thickness is 220 µm, including the backing.

2.4.2 Screens

Screens are constructed of polypropylene with a nominal thickness of 400 to 425 µm.

2.4.3 Encapsulant

The encapsulant is polyurethane with a white pigment — TiO_2.

2.4.4 Permeate Seals

Permeate seals are made from platinum cured silicone, USP Class VI @ 70 °C.

2.4.5 Gaskets

Gaskets are constructed from USP Class VI @ 70 °C medical grade, platinum cured silicone. They have a nominal thickness of 1.6 mm (0.063 in.).

2.5 Dimensions

Pall membrane cassettes are manufactured in a range of formats and membrane areas (Table 4). This allows the ability to directly scale up or down depending on requirements.

Figure 3

Cassette Screen Channel Configuration

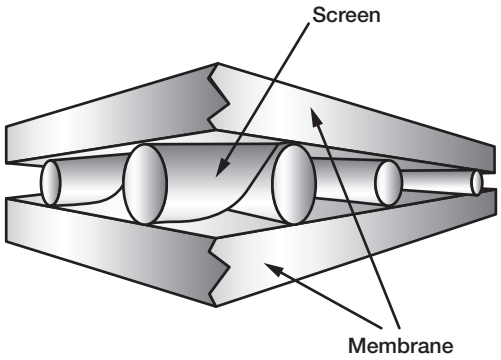


Table 4

Physical Dimensions of T-Series TFF Cassettes with Omega Membrane (Nominal)

Cassette Format	Centramate	Centramate	Centrasette	Centrasette
Part Code	T02	T12	T06	T26
Area	186 cm^2 (0.2 ft^2)	0.1 m^2 (1.1 ft^2)	0.5 m^2 (5.4 ft^2)	2.5 m^2 (27 ft^2)
Weight — kg (lb)*	0.083 (0.183)	0.16 (0.35)	0.47 (1.04)	2.00 (4.41)
Thickness — cm (in.)	0.316 (0.124)	1.6 (0.6)	1.6 (0.6)	7.4 (2.9)
Length — cm (in.)	20 (7.9)	20 (7.9)	20 (7.9)	20 (7.9)
Width — cm (in.)	5.6 (22)	5.6 (2.2)	18 (7.1)	18 (7.1)
Flow Path length — cm (in.) (port center to center)	17 (6.7)	17 (6.7)	17 (6.7)	17 (6.7)
Flow path width — cm (in.)	3.2 (1.3)	3.2 (1.3)	16 (6.3)	16 (6.3)
Port diameter feed — cm (in.)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)
Number of feed ports	1	1	5	5
Port diameter retentate — cm (in.)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)	1.1 (0.4)
Number of retentate ports	1	1	5	5
Port diameter permeate — cm (in.)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)
Number of permeate ports	2	2	8	8

** Approximate weight of a cassette as shipped in a bag (no outer packaging). The cassette is wetted with a storage solution and drained. Weights may vary due to different amounts of storage solution remaining in the cassette.*

2.6 Operating Pressures and Temperatures

Membrane cassettes have operating limits for pressure, temperature, pressure drop (feed pressure minus retentate pressure), and pH (Table 5).

Table 5

Cassette Operating Limits of Pressure, Temperature, and pH for T-Series Cassettes with Omega Membrane (All Formats)

Maximum Recommended Operating Pressure**	Maximum TMP	Temperature Range	pH Range
6 barg (87 psig) @ 23 °C	4 barg (58 psig) @ 55 ºC	-5 to 55 ºC	2 to 14 @ 4 barg (58 psig) @ 55 °C

*** Torque value must be set to the recommended level to avoid leaks.*

2.7 Normalized Water Permeability (NWP) Ranges

NWP is a measure of the membrane's hydraulic resistance. Water quality, temperature, and pressure affect NWP. At a minimum, the water used to measure NWP should be distilled, deionized (DI), 0.2 µm filtered, or preferably, pharmaceutical grade (USP Water for Injection, hereafter called WFI). The presence of biological organisms, organic materials, or minerals in the water may affect NWP.

2.7.1 NWP Specifications for Omega Membranes Used in T-Series Cassettes

The NWP is measured in stirred cells with 43 mm disc membrane samples stamped out from the beginning, middle, and end of each production lot of membrane (Table 6).

Table 6

NWP Specifications For Omega Membranes Used in T-Series Cassettes

Membrane	NWP on Disc Membrane		
NMWC	mL/min/cm² @ 25 °C	LMH/psig @ 25 °C	LMH/barg @ 25 °C
1 kD	> 0.03 at 3.74 barg (55 psig)	> 0.33	> 4.8
5 kD	> 0.15 at 3.74 barg (55 psig)	> 1.6	> 24
10 kD	> 0.65 at 3.74 barg (55 psig)	> 7.1	> 100
30 kD	> 1.5 at 3.74 barg (55 psig)	> 16	> 235
50 kD	> 1.8 at 3.74 barg (55 psig)	> 19	> 280
70 kD	> 1.8 at 3.74 barg (55 psig)	> 19	> 280
100 kD	> 5.0 at 3.74 barg (55 psig)	> 55	> 810
300 kD	> 0.7 at 0.68 barg (10 psig)	> 42	> 620

2.8 Membrane Solute Passage Specifications

Solute passage measurements are not made on finished cassettes because of the invasive nature of the test. However, if needed, a solute passage test can be performed on a cassette to characterize the passage characteristics. The passage data will vary from disc data because of hydrodynamic differences between the formats; for example, pressure drops in the permeate channel. Because the test is invasive, cassettes exposed to a foreign substance would not be reused in many applications (i.e., pharmaceutical processes). To diagnose a specific problem, a cassette can be cut open and pieces of membrane removed for testing. Contact Pall for support if this process is required.

Table 7

Solute Passage Specifications – Ultrafiltration

NMWC and Pore Size	Primary Solute	Passage (%)	Secondary Solute	Passage (%)	Test Pressure barg (psig)
1 kD	Bacitracin	< 35	N/A*	N/A	3.8 (55)
5 kD	PVP K15	25 – 40	N/A	N/A	3.8 (55)
10 kD	PVP K15	50 – 80	N/A	N/A	3.8 (55)
30 kD	BSA	< 5	PVP K30	15 – 35	3.8 (55)
50 kD	BSA	< 25	PVP K30	< 35 – 80	3.8 (55)
70 kD	BSA	< 25 – 80	PVP K30	< 50	3.8 (55)
100 kD	BSA	> 85	IgG	≤ 30	3.8 (55)
300 kD	IgG	< 82	Blue Dextran	< 15	0.68 (10)

** N/A Not Applicable*

2.9 Test Solute Concentrations and Detection Methods

Table 8 lists the concentration of solutes and the detection method used to test membranes for passage.

Table 8

Concentration of Solute and Detection Method Used to Test Membranes

Test Solute	Source*	Concentration (%)	Detection Method	Solvent
PVP K15	ISP Tech.	0.17	Refractometer	DI water
BSA	Sigma	0.2	UV @ 280 Nm	PBS
IgG	Sigma	0.1	UV @ 280 Nm	0.85% salt (w/v) in DI water

** or equivalent*

2.10 Membrane Integrity — Forward Flow Test Values

The membrane integrity test measures air forward flow rates at specified pressures to determine the integrity of membranes. The air forward flow is a measure of air diffusion through the liquid in the membrane pores, air flow through empty pores, plus air leakage around seals. The test identifies gross defects in the cassette membrane or membrane seals. Membrane forward flow integrity test values are given in Table 9 for the different cassette formats.

Use only dry filtered air or nitrogen from cylinders (instrument-quality) when using integrity analyzers incorporating mass flow meters. Fluctuations in house air and nitrogen supplies as well as changes in temperature can cause inconsistent results. Fully wet-out the membrane in a cassette prior to performing the membrane integrity test or high forward flow values may be obtained. The procedures for wetting out cassettes and measuring forward flow are described in the Care and Use Procedures (USD 2433b).

Table 9

Membrane Integrity Test — Forward Flow Limits

Membrane NMWC	Test Pressure	Allowable Air Forward Flow Rate per unit area of membrane (*, **)
< 300 kD	2.0 barg (30 psig)	< 1600 sccm/m^2 (< 150 sccm/ft^2)

** Nitrogen can be used in place of air.*

*** sccm = standard cubic centimeters per minute.*

2.11 Shelf Life of New and Used Cassettes

The expected shelf life of cassettes stored between 4 and 25 °C, away from light, unopened in original packaging, in 0.3 N sodium hydroxide as supplied, is 4 years from date of manufacture. Shelf life studies are ongoing. Users should test the cassette integrity prior to use.

The useful life of cassettes that are properly conditioned, used, cleaned, stored, and maintained is often more than one year. However, it is not possible to specify a shelf life or useful life of a cassette that has been used or removed from the original packaging. The actual useful life for a cassette will depend on the character and complexity of the product to which it is exposed, composition of process fluids, process temperatures, operating pressures, and cleaning regimen. Therefore, customers should validate reuse and the useful life of a cassette in their process. Pall Life Sciences makes no claims of warranty or guarantee of performance related to reuse of cassettes. Consult the Care and Use Procedures (USD 2433b) for recommended storage conditions.

2.12 Compatibility with 0.5 N Sodium Hydroxide + 500 PPM NaOCl, 45 °C

Sodium hydroxide is an effective and commonly used agent in biological applications for cleaning, sanitizing, and depyrogenating membrane cassettes. The addition of the oxidizing agent sodium hypochlorite can improve the effectiveness as a cleaning and sanitizing agent.

To evaluate the chemical resistance of T-Series TFF cassettes with Omega membrane to this mixture, a study was performed in which new Omega 100 kD T-Series Centrasette cassettes were first characterized to determine water permeability, air integrity, and pressure drop. The cassettes were then installed in a holder. A solution of 0.5 N NaOH + 500 PPM NaOCl at 45 °C was circulated through the cassettes for 2 hours after which time the cassettes were flushed with water at room temperature. This process was repeated for 10 cycles or a total of 20 hours contact time. Cassettes were characterized after the fifth and tenth cycle.

2.12.1 Results

After 10 cycles (20-hour exposure time) with 0.5 N NaOH + 500 PPM NaOCl at 45 °C, all cassettes tested were within normal operating parameters for air integrity, pressure drop, and normalized water permeability.

2.13 General Chemical Compatibility

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

Table 10 illustrates the chemical compatibility of T-Series TFF cassettes with Omega membrane at 23 °C (unless otherwise noted) with respect to physical characteristics. The Membrane Chemical Compatibility Chart should be used only as a guide. Cassettes should be tested in the appropriate solvent and product under actual operating conditions and for an appropriate time to determine compatibility for the specific application. Membrane porosity, and consequently both water permeability and retention characteristics, may be affected. Physical changes to the cassette may be permanent or reversible. To determine if changes are permanent, flush and then soak the cassette in water for one to two days and then test the sample again. Changes in water permeability and solute retention may be due to physical changes in the membrane.

Table 10

Membrane Chemical Compatibility Chart

Reagent	Compatible*
Acetic Acid (5%)	4
Alconox® (1%)	4
Citric acid (1%)	4
Ethanol (70%)	4
Formaldehyde (1%)	4
Glycerine (50%)	4
Guanidine HCl (6 M)	4
Hydrochloric acid (0.1 N)	4
Hydrogen peroxide (1%)	4
Phosphoric acid (0.1 N)	4
Sodium dodecyl sulfate (0.01 M)	4
Sodium hydroxide (0.5 N @ 50 °C)	4
Sodium hypochlorite (0.05%)	4
Terg-a-zyme® (1%)	4
Triton® X-100 (0.002 M)	4
Urea (25%)	4

** Data for cassette membrane and components at 20 °C, 24-hour exposure, unless otherwise noted. There may be changes in porosity and/or selectivity of membrane.*

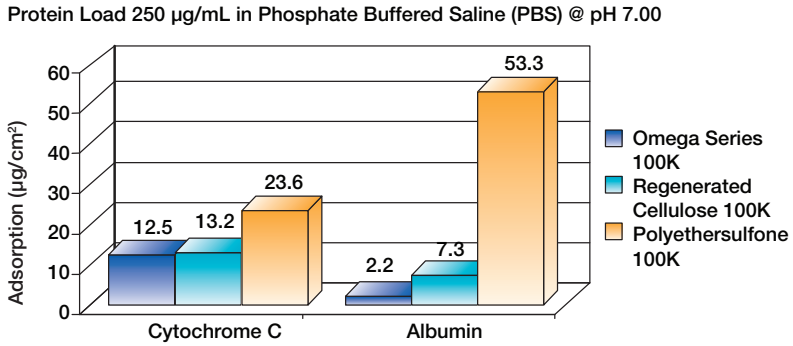
2.14 Protein Binding Characteristics

Omega membranes have low nonspecific adsorption characteristics. The actual amount of protein or other substances that will adsorb (nonspecifically bind) to the membrane is dependent on the specific characteristics of that substance. Adsorption of a molecule to a membrane can vary to a great extent depending on its environment (i.e., the chemical composition of the solution it is in, as well as the number and concentration of other solutes present). Changes in pH, ionic strength, temperature and concentration have a significant effect on binding properties. Buffer salts, detergents, and organic solvents also influence binding. If adsorption is a concern, then a study can be performed on a membrane disc using the actual sample and buffer to determine the level of nonspecific adsorption.

Figure 4 compares the nonspecific adsorption characteristics of three different membrane materials for albumin and cytochrome C in phosphate-buffered saline at pH 7.0. The isoelectric points of albumin and cytochrome C are 4.6 and 10, respectively. Therefore, at pH 7, albumin is negatively charged and cytochrome C is positively charged.

Figure 4

Nonspecific Adsorption Characteristics of Three Membrane Materials



Reference: Strumeyer, Dr. David H, Department of Biochemistry and Microbiology, Rutgers University. Paper presented at 1986 Membrane Conference, Boston, MA.

3. Validation Procedures

This section describes the procedures Pall used to validate specific chemical and physical characteristics of T-Series TFF cassettes with Omega membrane.

3.1 High and Low Temperature Operational Testing at Maximum Recommended Operating Pressure

T-Series Centrasette cassettes with Omega 10 kD membrane (Part No. OS010T26, for high temperature) and Omega 30 kD membrane (Part No. OS010T26, for low temperature) were subjected to low and high operating temperature cycling in water to determine the effects on air forward flow integrity test (IT), pressure drop (DP) and normalized water permeability (NWP) values. Each cassette was preconditioned and then underwent six 3-hour recirculation cycles at the maximum recommended operating pressure. Tests were performed in triplicate. Separate cassettes were used for each test regimen:

Low Temperature: -7 to -5 °C @ a feed pressure of 6 barg (87 psig)

High Temperature: 55 to 60 °C @ a feed pressure of 4 barg (58 psig)

3.1.1 Low Temperature Testing at Feed Pressure of 6 barg (87 psig)

Test Conditions: water at -7 to -5 °C; feed pressure of 6 barg (87 psig); 10 x 2-hour cycles

3.1.1.1 Procedure

1. Characterize the cassettes to establish initial values of IT, DP, and NWP.
2. Install the cassettes in the holder.
3. Open the feed, retentate and permeate valves.
4. Adjust the pump speed, and retentate and permeate valves to obtain a feed pressure of 6 barg (87 psig) and a retentate flow rate of 5 to 10 L/min/m^2 (0.5 to 1 L/min/ft^2).
5. Use a chiller to maintain the temperature -7 to -5 °C. Start the timer when the water temperature is between -7 to -5 °C.
6. Recirculate for 2 hours. Adjust the pressure and retentate flow rate if necessary.
7. After 2 hours, drain the system and remove the cassettes from the holder.
8. Install a cassette in another holder at ambient temperature.
9. Characterize each cassette separately.
10. Repeat steps 2 through 9 nine more times (total of 10 x 2-hour cycles).

3.1.1.2 Results

Two T-Series Centrasette cassettes with Omega 30 kD membrane were subjected to low operating temperature cycling. After circulation in water for 10 x 2-hour cycles at -7 to -5 °C, all cassettes were within normal operating parameters for air integrity, pressure drop, and normalized water permeability.

3.1.2 High Temperature Testing at Feed Pressure of 4 barg (58 psig)

Test Conditions: water at 55 to 60 °C; feed pressure of 4 barg (58 psig); 6 x 3-hour cycles

3.1.2.1 Procedure

1. Characterize the cassettes to establish initial values of IT, DP, and NWP.
2. Install the cassettes in the holder.
3. Open the feed, retentate and permeate valves.

4. Adjust the pump speed, and retentate and permeate valves to obtain a feed pressure of 4 barg (58 psig) and a retentate flow of 5 to 10 L/min/m^2 (0.5 to 1.0 L/min/ft^2).
5. Use a heater to maintain the water temperature at 55 to 60 °C. Start the timer when the water temperature is 55 to 60 °C.
6. Recirculate for 3 hours. Adjust the pressure and retentate flow rate if necessary.
7. After 3 hours, drain the system and remove the cassettes from the holder.
8. Install a cassette in another holder at ambient temperature.
9. Characterize each cassette separately.
10. Repeat steps 2 through 9 five more times (total of 6 x 3-hour cycles).

3.1.2.2 Results

Three (3) Omega 10 kD membrane Centrasette T-Series cassettes were subjected to high operating temperature cycling. After circulation in water for 6 x 3-hour cycles at 55 to 60 °C, all cassettes were within normal operating parameters for air integrity, pressure drop, and normalized water permeability.

3.2 Maximum Pressure Test at Ambient Temperature

To confirm cassette performance at maximum operating pressure, three (3) Omega 10 kD membrane Centrasette T-Series cassettes (Part No. OS010T26) were subjected to 20 x 1-hour cycles at a feed pressure of 6 barg (87 psig) and ambient temperature.

3.2.1 Procedure

1. Install the cassettes in the holder.
2. Characterize the cassettes to establish initial values of IT, DP, and NWP.
3. Set the system to run in recirculation.
4. Adjust the pump to give a retentate flow rate of approximately 5 L/m^2 (0.5 L/ft^2).
5. Adjust the retentate valve and pump speed to give a feed pressure of 6 barg (87 psig) and 75% flow through retentate, 25% through permeate. Maintain conditions for 1 hour.
6. After an hour has elapsed, reduce the feed pressure to 1.7 barg (25 psig) for 5 minutes. This is considered one cycle.
7. Characterize the cassettes for IT, DP, and NWP.
8. Repeat steps 3 to 7. Characterize the cassettes for IT, DP, and NWP after cycles 5, 10, 15, 16, 17, 18, 19, and 20.

3.2.2 Results

After twenty (20) 1-hour cycles at a feed pressure of 6 barg (87 psig) and ambient temperature, all cassettes tested for air integrity, pressure drop, and normalized water permeability were within normal operating parameters. No significant changes in performance were observed between the initial measured values and those measured after the twentieth (20th) cycle.

3.3 Chemical Compatibility

3.3.1 Introduction

Cassettes may be exposed to a number of chemicals, primarily during sanitization and cleaning procedures. To evaluate the effect of commonly used chemicals on cassette performance, cassettes were exposed to chemicals under conditions that simulated typical worst-case use. The criteria used to evaluate the performance of the cassettes after exposure to chemicals were water permeability and membrane integrity test.

3.3.2 Scope

Three different solutions under various conditions were chosen as representative of commonly used cleaning agents. The solutions and exposure conditions were:

1. 1.0 N NaOH at 23 °C for 28 days
2. 0.1 N H_3PO_4 at 45 °C for 28 days
3. 25% EtOH at 23 °C for 28 days

A typical cleaning regimen is less than three hours. Cleaning with acids such as phosphoric acid is used less frequently, for shorter periods (1 to 1.5 hours) and often in addition to a caustic cleaning. Therefore, the exposure time in this study is equivalent to about one cleaning cycle/day (4 to 5 days per week) for one year. Omega 100 kD membrane Centrasette T-Series cassettes (Part No. OS100T06) were chosen for this study. The materials of composition and construction of T-Series cassettes with Omega membrane with different molecular weight cutoffs are the same; hence, they should be similarly affected by the test solutions. The work was performed in duplicate.

3.3.3 Summary of Method

New T-Series Centrasette cassettes with Omega 100 kD membrane were used for each test solution. Cassettes were characterized in water for air integrity, pressure drop, and normalized water permeability.

The cassettes were installed in a holder and the test solution circulated through the cassette for 15 minutes. Cassettes were then removed from the holder and immersed in a plastic bag containing the test solution. The plastic bag was sealed and stored at the required temperature. After a specified time, the cassettes were removed from the plastic bag, installed in a holder, flushed with 0.2 µm filtered DI water and re-characterized according to the test protocol.

Table 11

Compatibility with 1.0 N NaOH (23 °C)

Cassette and Serial Number	NWP @ 10 psig (LMH/psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36036044R	34	35	
36019072R	37		36

Cassette and Serial Number	DP 5 L/min/m² (0.5 L/min/ft²) CFF (psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36036044R	7.0	6.0	
36019072R	7.0		6.0

Table 11 *(continued)*

Compatibility with 1.0 N NaOH (23 °C)

Cassette and Serial Number	Air Forward Flow IT @ 2 barg (30 psig) sccm/ft²		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36036044R	45	9.0	
36019072R	45		16

Table 12

Compatibility with 0.1N H_3PO_4 (45 °C)

Cassette and Serial Number	NWP @ 10 psig (LMH/psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36020074R	26	29	
36038080R	34		36

Cassette and Serial Number	DP 5 L/min/m² (0.5 L/min/ft²) CFF (psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36020074R	5.0	6.0	
36038080R	7.5		4.2

Cassette and Serial Number	Air Forward Flow IT @ 2 barg (30 psig) sccm/ft²		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36020074R	9.0	11	
36038080R	6.7		16

Table 13

Compatibility with 25% Ethanol (23 °C)

Cassette and Serial Number	NWP @ 10 psig (LMH/psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36039049R	33	33	
36020077R	36		38

Cassette and Serial Number	DP 5 L/min/m² (0.5 L/min/ft²) CFF (psig)		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36039049R	8.0	9.1	
36020077R	7.5		7.2

Cassette and Serial Number	Air Forward Flow IT @ 2 barg (30 psig) sccm/ft²		
Omega T06, 100 kD	Initial	After 14 days	After 28 days
36039049R	17	16	
36020077R	26		8.0

3.3.4 Results

Three solutions were chosen as representative of commonly used cleaning agents. The solutions and their exposure conditions were: 1. 1.0 N NaOH at 23 °C for 28 days; 2. 0.1 N H_3PO_4 at 45 °C for 28 days; 3. 25% EtOH at 23 °C for 28 days. After exposure to these chemical solutions at the specified temperature and period, all cassettes tested were within normal operating parameters for IT, DP, and NWP. No significant changes in performance were observed between the initial measured values and those measured after soaking in the test solution for the specified period.

3.4 Cleaning Agent Compatibility — High and Low pH

During the cleaning process, cassettes are often exposed to harsh conditions of extremes of pH (2 to 14) and elevated temperature and pressure in order to remove traces of remaining product and fouling agents. To evaluate the effect of these conditions on T-Series cassettes, studies were performed on Omega 30 kD membrane Centrasette T-Series cassettes (Part No. OS030T06) under simulated test conditions. For this study, cassettes tested for low pH were subjected to 6, three hour cycles in 0.1 N phosphoric acid at 45 °C and a feed pressure of 4 barg (58 psig). Cassettes tested for high pH were subjected to six (6) 3-hour cycles in 0.5 N sodium hydroxide at 55 °C and a feed pressure of 4 barg (58 psig). A summary of the test procedures and results are given below.

3.4.1 Low pH Exposure Study

Test Conditions

0.1 N Phosphoric Acid; 45 °C ±5; feed pressure of 4 barg (58 psig); 6 x 3-hour cycles

3.4.1.1 Procedure

1. Characterize each test cassette individually for IT, DP, and NWP.
2. Prepare four (4) liters of 0.1 N phosphoric acid solution and heat it to 45 ± 5 °C.
3. Install the cassettes in the holder.
4. Open the feed, retentate, and permeate valves.
5. Adjust the retentate valve and pump to achieve a feed pressure of 4 barg (58 psig) at 10 L/min/m^2 (1 L/min/ft^2).
6. Start the timer when the acid solution temperature reaches 45 °C. Do not exceed 50 °C. Adjust torque as necessary.
7. Recirculate for 3 hours. Adjust the pressure/flow rate if necessary.
8. Drain the system. Refill the feed tank with water.
9. Flush with 5 L of DI water at ambient temperature through the retentate to drain (permeate valve partially closed) with a feed pressure of 1 barg (15 psig).
10. Flush with 5 L of DI water at ambient temperature through the permeate to drain (retentate valve closed) with a feed pressure of 1.4 to 2 barg (20 to 30 psig).
11. Remove the cassettes.
12. Reinstall the cassettes individually.
13. Recirculate water; test pH, drain the water and flush with fresh water until pH of 7 is achieved.
14. Characterize each cassette for IT, DP, and NWP.
15. Repeat steps 2 to 14 for 5 additional cycles (Total of 6 x 3-hour cycles).

3.4.1.2 Results

Exposure of Omega 30 kD membrane Centrasette T-Series cassettes to low pH (~2) at elevated temperature (45 °C ± 5) and a high feed pressure of 4 barg (58 psig) shows a reduction in NWP of approximately 25 to 30% over six cycles. The pressure drop increased but is within specifications (2 to 14 pH). Air integrity is unaffected.

Table 14

Low pH, 0.1 N H_3PO_4, Feed Pressure of 4 barg (58 psig), 45 °C

Cassette and Serial Number	NWP @ 10 psig (LMH/psig)		DP 5 L/min/m² (0.5 L/min/ft²) CFF (psig)		Air Forward Flow IT @ 2 barg (30 psig) sccm/ft²	
Omega T26, 10 kD	Initial	After 6 cycles	Initial	After 6 cycles	Initial	After 6 cycles
35068064R	31	24	9.5	14.5	95	68
35068065R	32	23	9	15	88	50

sccm = standard cubic centimeters per minute

3.4.2 High pH Exposure Study

3.4.2.1 Procedure

1. Characterize each test cassette individually for IT, DP, NWP.
2. Prepare four (4) liters of 0.5 N sodium hydroxide solution and heat it to 55 °C ± 5.
3. Install the cassettes in the holder.
4. Open the feed, retentate, and permeate valves.
5. Adjust the retentate valve and pump to achieve a feed pressure of 4 barg (58 psig) at 10 L/min/m² (1 L/min/ft²).
6. Start the time when the 0.5 sodium hydroxide solution temperature is 55 °C or higher.
7. Do not exceed 60 °C. Adjust torque as necessary.
8. Recirculate for three (3) hours. Adjust the pressure/flow rate if necessary.
9. Drain the system. Refill the feed tank with water.
10. Flush with 5 L of DI water at ambient temperature through the retentate to drain (permeate valve partially closed) with a feed pressure of 1 barg (15 psig).
11. Flush with 5 L of DI water at ambient temperature through the permeate to drain (retentate valve closed) with a feed pressure 1.4 to 2 barg (20 to 30 psig).
12. Remove the cassettes.
13. Reinstall the cassettes individually.
14. Recirculate water; test pH, drain water and flush with fresh water until pH of < 9 is achieved.
15. Characterize for IT, DP, and NWP.
16. Repeat steps 2 to 14 for 5 additional cycles (Total of 6 x 3-hour cycles).

3.4.2.2 Results

Exposure of Omega 30 kD membrane Centrasette T-Series cassettes to high pH (~13) at elevated temperature (55 °C ±5) and a feed pressure of 4 barg (58 psig) shows a reduction in NWP of approximately 25 to 30% over six cycles. The pressure drop increases but is within specifications. Air integrity is unaffected.

Table 15

High pH, 0.5 NaOH, Feed Pressure of 4 barg (58 psig), 55 °C

Cassette and Serial Number	NWP @ 10 psig (LMH/psig)		DP @ 5 L/min/m² (0.5 L/min/ft²) CFF (psig)		Air Forward Flow IT @ 2 barg (30 psig) sccm/ft²	
Omega T26, 10 kD	Initial	After 6 cycles	Initial	After 6 cycles	Initial	After 6 cycles
35049040R	36	25	9.2	12	39	20
35049041R	36	23	9.0	12	50	26

3.5 Cassette Flushing Procedure to Remove Storage Agent

A preconditioning procedure was developed to remove storage agents and reduce total organic compound (TOC) levels from new and stored T-Series cassettes prior to use in a TFF process. The procedure includes the following steps:

3.5.1 Install Cassettes

Details of the preconditioning procedure are given in Section 6.1.

1. Flush with DI water to waste (flush out the bulk storage agent).

2. Sanitize with 0.5 N NaOH at 40 to 45 °C.

3. Flush with DI water to waste (flush out the caustic sodium hydroxide).

4 to 6. Water recirculation for 30 minutes (3 cycles).

7. Final water flush to drain.

To evaluate the effectiveness of this flushing/sanitization procedure, Omega 10 kD membrane Centramate T-Series cassettes were preconditioned using this procedure. Samples of the flush or recirculated solution taken from the retentate and permeate at the end of each step were analyzed for TOC. The results are given in Table 16.

Table 16

TOC Results from Cassette Flushing Study

Cassette Serial No.	36017055R		36013056R		36013060R	
Pre-conditioning Step	TOC (PPM) Retentate	Permeate	TOC (PPM) Retentate	Permeate	TOC (PPM) Retentate	Permeate
Step 1	1.0	283	1.59	279	0.8	195
Step 3	0.2	18.3	0.3	19.7	0.33	5.3
Step 4	23.7	21.3	14.3	16.4	12.4	11.9
Step 5	11.2	10.8	15.0	16.9	9.2	9.5
Step 6	7.1	7.6	9.1	10.9	7.0	8.0
Step 7	< 0.1	2.57	< 0.1	1.96	< 0.1	1.96

TOC of flushing water is < 0.1 PPM

3.5.2 Results

Using the preconditioning procedure outlined in Section 6.1, the TOC levels in both retentate and permeate streams were under 1 PPM at the final flush.

3.6 Extractables Tests

3.6.1 Introduction

The purpose of this study was to perform a quantitative analysis and qualitative characterization of materials extracted from Pall Omega membrane T-series cassettes (P/N OS030T06) into Water at 50 ºC after the cassettes had been subjected to a Pall recommended preconditioning protocol.

The repeating layers of membrane and screen spacers that make up a T-Series cassette are encapsulated with a polyurethane sealant. Centramate and Centrasette cassettes are constructed from the same raw materials and by the same procedure. Therefore, the type of extractables determined for one format should be the same for all formats.

3.6.2 Summary of Extraction Conditions

The extraction work was carried out on cassettes manufactured with standard Omega membrane in T Series cassettes which had been preserved in 0.3N NaOH and packaged per SOP. The extractions were carried out on ten cassettes which had been stored at 25 ºC +/- 2 ºC.

OS030T06 cassettes have 5.38 sq ft or 0.5 sq meter nominal membrane effective area.

10 x OS030T06 were tested, preserved, packaged and stored at 25 ºC then subjected to Pall recommended preconditioning sanitizing flush for first time use cassettes and then subjected to 2 x consecutive 16 hr recirculatory extractions.

The volume for each extraction was 5 L, the solvent was Deionized Water at 50 ºC.

The extraction solvent was recirculated through the retentate and permeate channels which were pooled, providing a worse case condition concentrating extracted compounds.

Average Total NVR Extracted over 2 consecutive 16 hr periods is < 145 mgs (5.38 sq ft cassette). NVR decreased over the 2 extraction periods in each case.

For further information regarding extractable quantitative and qualitative characterization please contact your Pall representative .

3.7 Sanitization — Endotoxin Removal — New Cassette Flush Procedure

Endotoxin – a high molecular weight lipopolysaccharide – is associated with the cell wall of gram-negative bacteria. As part of normal functioning and during cell autolysis, bacteria release endotoxin into their environment. Unpurified endotoxin contains lipids, carbohydrates, and protein. Aggregated forms of endotoxin have molecular weights ranging from about 30,000 to 1,000,000 daltons. Active subunits can occur with molecular weights as small as 10,000 daltons. Endotoxin is pyrogenic, that is, it can cause fever in humans and animals, so it is imperative that pharmaceutical products, especially injectables, are endotoxin-free. Endotoxin can be detected by the Limulus Amoebocyte Lysate (LAL) procedure. The lower detection limit is based on the method and sensitivity of the lysate being used. 1 EU (endotoxin unit) = 100 picograms endotoxin

3.7.1 Introduction

Cassettes are not supplied sterile, and Pall does not claim that cassettes are endotoxin-free. Therefore, they should be flushed and sanitized prior to use. To evaluate initial endotoxin levels and the effectiveness of the recommended flushing procedure, the following study was performed.

3.7.2 Summary of Method

A new Omega 10 kD membrane Centramate T-Series cassette (Part No. OS010T12) was tested using the procedure below. Endotoxin level was determined according to the procedure for LAL testing using the ThermoMax 1 assay. The LAL procedure is described in Section 7.3.

The cassette was installed in a Centramate holder and system that had been previously cleaned and tested to be free of endotoxin.

The cassette was then put through a standard preconditioning protocol:

1. Flush with DI water to waste.
2. Sanitize with 0.5 N NaOH at 40 to 45 °C for 60 minutes.
3. Flush with DI water to waste.
4. Water recirculation for 30 minutes (3 cycles).
5. Final water flush to drain.

Details of the preconditioning procedure are given in Section 6.1.

Samples were taken of the endotoxin-free water (±0.01 EU/mL) used for flushing and from the effluent from the cassette at the end of each flush step.

3.7.3 Results and Conclusion

The endotoxin level in water first flushed from new cassettes was measured using the preconditioning procedure outlined above. The endotoxin level in water flushed from the cassette was less than the detectable limit (< 0.005 EU/mL).

3.8 Sanitization — Endotoxin Challenge Procedure

3.8.1 Introduction

Membrane cassettes are typically cleaned and reused many times. There is a risk of contamination with endotoxin from previous samples, or from bacterial growth during storage. Therefore, endotoxin levels should be reduced in the cassette after use and prior to use with a new sample. The following study was performed to show the effectiveness of a typical cleaning procedure with 0.5 N NaOH as recommended in the Care and Use Procedures (USD 2433b). This study also applies to the standard flushing and sanitization protocol recommended for preconditioning of cassettes (Section 6.1.)

3.8.2 Summary of Method

An Omega 30 kD membrane Centramate T-Series cassette (Part No. OS030T12) that had been previously preconditioned using the standard procedure outlined in Section 6.1 was installed in a Centramate holder. The cassette was then challenged by adding three (3) liters of a solution containing ~600 to 800 EU/mL of endotoxin and recirculating the solution for one hour through the cassettes. The TFF system was set for recirculation at a retentate flow rate of ~500 mL/min (5 L/min/m^2) and permeate flow rate of ~75 mL/min (0.75 L/min/m^2) with a flow distribution of 85% through the retentate and 15% through the permeate. Following recirculation with the challenge solution, the cassettes were flushed with water to waste (8 L through retentate, 4 L through permeate). Samples of permeate and retentate (and flushing water as a blank) were taken at the end of the flush.

The system was drained and four (4) liters of 0.5 N NaOH was added to the feed reservoir. The cassette was sanitized by circulating the caustic solution at ~575 mL/min (flow split 75% retentate/25% permeate) for 1 hour at 40 to 45 °C. Following recirculation with the caustic solution, the cassette was flushed with water to waste (8 L through retentate, 4 L through permeate). Samples of permeate and retentate (and flushing water as a blank) were taken at the end of the flush.

The caustic sodium hydroxide recirculation and flush were repeated a second time followed by a final recirculation in 4 L of 0.2 µm filtered DI water for two (2) hours. Samples of the permeate and retentate (and flushing water as a blank) were taken at the end of the recirculation.

The procedure was repeated in triplicate using a new cassette for each test.

3.8.3 Results

Flushing the Centramate cassettes with water with 80 L/m^2 (7 L/ft^2) through the retentate and 40 L/m^2 (4 L/ft^2) through the permeate following the endotoxin challenge, resulted in endotoxin levels measured in the flush water samples below the detectable limit of < 0.005 EU/mL, except for one sample that was at 0.007 EU/mL. The caustic sodium hydroxide recirculation and flush were still required to assure complete cleaning and removal of other foulants and bioburden from the cassette.

3.8.4 Conclusions

The flushing, sanitization and cleaning procedures recommended by Pall Life Sciences effectively reduce endotoxin levels. Using the cleaning procedure described above on a cassette spiked with 3 L of solution containing ~800 EU/mL, reduced the endotoxin level in the final flush to the minimum level of detection (< 0.005 EU/mL).

3.9 Particle Release from T-Series Cassettes

Cassettes are manufactured from components that are considered non-fiber releasing and as such are not tested for particle release in manufacturing. It is further assumed that any particles released by the cassette into the product would be removed further downstream in the process at or before final filtration. A study was performed to evaluate the typical level of particles that may be attributed to the cassette after the cassette has been properly preconditioned.

Criteria was set for maximum particle release using the procedure described below. This criteria is recommended in USP <788> Particle Matter in Injections.

Table 17

Criteria for Maximum Particle Release After Standard Preconditioning Flush

Particle Size Range (µm)	Specification Particles/mL
> 25	< 3
10 – 25	< 25

To evaluate the level of particles that might come from a T-Series cassette with Omega membrane following a standard preconditioning procedure, the following test was performed.

Three (3) Omega 10 kD membrane Centramate T-Series cassettes (Part No. OS010T12) were flushed following the standard procedure given in Section 6.1. After the preconditioning, the system was set to flush to drain. The feed pump was adjusted to give 5 L/m^2 (0.5 L/ft^2) through the retentate at a retentate pressure of 0.5 barg (7 psig). Twenty (20) liters of water was pumped through the cassette. At the end of the flush, 1 L samples of the retentate and permeate were collected and sent for analysis.

Table 18

Particle Count of Flush Solution after Preconditioning

	Particle Analysis* (particles/mL)					
Particle Size Range	1 – 10 µm		10 – 25 µm		> 25 µm	
Test Cassette	OS010T12					
Serial Number	Retentate	Permeate	Retentate	Permeate	Retentate	Permeate
36017053R	< 1	<1	< 1	< 1	<1	< 1
36013059R	< 1	<1	< 1	< 1	<1	< 1
36013061R	< 1	<1	< 1	< 1	<1	< 1

** A sample volume of 1L was drawn down and analyzed*

3.9.1 Results

Following the standard protocol recommended for preconditioning, T-Series Centramate cassettes were flushed with 0.2 µm filtered DI water and samples from the retentate and permeate streams were collected. One-liter (1 L) samples were drawn down on a filter and analyzed. All of the samples analyzed had less than 1 particle/mL in the three particle size ranges evaluated.

4. Quality Assurance (QA)

Membranes and cassettes are produced in conformance with the Pall Corporation manufacturing documentation. Cassette components meet current standards for USP Class VI, 70 °C Biological Reactivity Tests for Plastics.

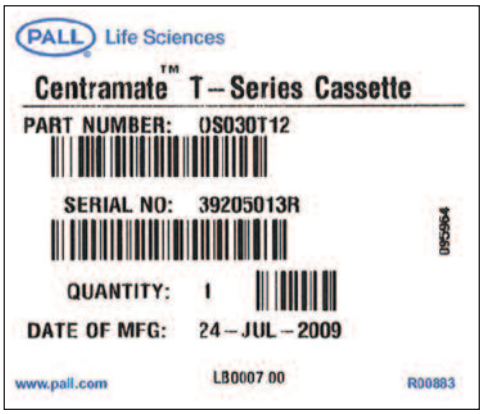
4.1 Quality Control Measures

Raw materials used in production are logged in for traceability and quarantined upon receipt. After inspection by the Quality Control Department, approved raw materials are issued to the warehouse for storage. During manufacturing, multiple samples from the beginning, middle, and end of each lot of membrane are tested for quality. Tests include water permeability and the retention/passage characteristics of selected solute molecules applicable to the specific molecular weight cut-off of the membrane. As required, membrane is removed from inventory, inspected, and sent to the assembly area. Cassettes are produced according to an assembly procedure from approved lots of membrane and other raw materials, which are recorded on a lot control card. The finished cassette is visually inspected, stamped with the cassette identification, and released from assembly to quality control with the accompanying lot card. Quality control inspects each cassette and lot card for completeness. The cassettes are then flushed with a 0.3 N Sodium Hydroxide (NaOH) solution and integrity is tested. Cassettes are then heat sealed in plastic bags.

4.1.1 Labels

Each cassette is sealed in a double plastic bag and packaged in a carton box using foam corners to protect the cassette. Labels affixed to the cradle, box and bag describe the contents (Figure 8). The label identifies the cassette format and contains the part number and the serial number. This information should match the information printed on the side of the cassette (Figure 1).

Figure 8
Example of Box Label



4.1.2 Quality Assurance Certificates

A quality assurance certificate is packaged with each TFF cassette (Figure 9).

Figure 9

Example of a Certificate of Test

Pall Corporation

Certificate of Quality

We hereby certify that

Pall® : **CENTRASETTE T-SERIES**

Part Number: **OS100T26**

Serial Number: **39196019R**

Membrane Lot Number: **H7256F**

was manufactured in a controlled environment.

Materials of Construction

Conformance to Regulatory Requirements
This product may be used in conjunction with current good manufacturing practices as per Title 21 of the **Code of Federal Regulations** (CFR) parts 210 and 211.72.

This product does not contain materials of construction that are considered TSE or BSE risk materials according to current legislation and guidelines (reference European CPMP EMEA/410/01 Rev.2 and **Code of Federal Regulations**, Title 9 Part 94.18).

Bio Safety Data
The fluid path components have met the specifications for biological tests listed in the current revision of the **United States Pharmacopeia** (USP) for Class VI plastics. Contact Pall for further information regarding materials of construction.

Cassette Validation
During validation, cassettes were tested using recommended care and use procedures to establish suitibility with respect to the following characteristics:
- Residual TOC
- Bacterial Endotoxin Levels
- Nonvolatile Extractable Residue

Product Quality

Membrane Quality
Representative membrane samples from this manufacturing lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:
- Water Permeability
- Solute Marker Passage

Integrity
This product successfully passed a final integrity test based on an air diffusion / forward flow test that ensures membrane and seal integrity.

Recommended Storage Conditions
This Pall cassette can be expected to perform within specifications if stored and handled in a manner consistent with the parameters below:
- The cassette is stored unopened in the original packaging at 4-25ºC, and in a dry environment.
- The cassette is protected from direct sunlight, radiation or weather conditions.
- Care is taken to avoid physical damage while handling.
- Thermal shock is avoided.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability in an ISO 9001:2000 certified facility. **These products are not supplied sterile.** Users should test the membrane integrity prior to use. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

15/July/2009

5. Biosafety Evaluation and Test Procedures

5.1 Introduction

The purpose of the biological evaluations and tests was to evaluate the biological suitability of the materials of construction of the T-Series cassettes with Omega membrane. Tests were performed by an outside contract laboratory in order to evaluate the suitability of the materials of construction of the T-Series cassettes with Omega membrane in terms of biological safety. Tests performed included the Biological Reactivity Tests, *In Vivo*, for Plastics (hereafter called the Biological Reactivity Tests), as described in the United States Pharmacopoeia, Chapter <88>; as well as the Hemolysis Test, and the L929 MEM- Cytotoxicity Test (hereafter called the cytotoxicity test).

In addition, a test was also performed to measure the level of oxidizable substances and endotoxin found in a cassette after an appropriate cleaning and flushing procedure had been performed.

5.2 Summary of Test Procedures

The Biological Reactivity Tests described in the United States Pharmacopoeia include injection of extracts of plastic materials, as well as implantation of the material itself into animal tissue. Four extracting media are listed which simulate parenteral solutions and body fluids. These include: (1) sodium chloride injection, (2) 1-in-20 solution of alcohol in sodium chloride injection, (3) polyethylene glycol 400, and (4) vegetable oil (sesame or cottonseed oil). Extracts are prepared at one of three standard conditions: 50 °C for 72 hours, 70 °C for 24 hours, or 121 °C for one hour. Since T-Series cassettes with Omega membrane have a recommended operating temperature limit of 55 °C, cassette components were extracted at 70 °C to provide for the most stringent test condition not resulting in physical changes in the plastic itself.

An acute systemic injection test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium chloride injection and 1-in-20 solution of alcohol in sodium chloride injection extracts were injected intravenously. Vegetable oil extract and polyethylene glycol 400 extract were injected intraperitoneally.

An intracutaneous test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. The four specified extracts were used.

Implantation was also performed in order to subject the materials of construction to the most stringent conditions included in the United States Pharmacopoeia. Each of the components of the filter cassette was implanted separately.

The hemolysis test and cytotoxicity test were conducted to determine the potential toxicity resulting from contact of the materials of construction with blood or tissue. The hemolysis test determines the degree of red blood cell lysis caused by contact of the test material. Using cell culture techniques, the cytotoxicity test determines the lysis of cells and the inhibition of cell growth caused by extracts of the test materials.

Endotoxin and oxidizable substance assays were performed in duplicate to show the level of these substances in retentate and permeate from Omega 10 kD membrane Centramate T-Series cassettes after a recommended flushing procedure. Endotoxin in the retentate and permeate from cassettes that had been flushed with 250 L/m^2 (23 L/ft^2) of water per membrane area was measured using the ThermoMax Chromogenic Assay procedure (Section 7.3). Oxidizable substances were measured in the same flushing solution using a colorimetric assay (potassium permanganate in a sulfuric acid acidified test solution).

5.2.1 Results

All T-Series cassettes with Omega membrane components passed the requirements of the Biological Reactivity Tests, and thus meet the requirements in USP Class VI-70 °C Plastics. Additionally, test samples meet the requirements of the hemolysis test and cytotoxicity test. A bacterial endotoxin level of 0.018 EU/mL was eluted from the test article after cleaning and flushing. No endotoxin was measured in the permeate. No oxidizable substances were detected in the permeate.

The tests were conducted by:

STS division of Ethox International
7500 W. Henrietta Road
Rush, NY 14543

Toxicon
225 Wildwood Ave
Woburn MA 01801

Results from the following tests are included in the appendices.

- USP Biological Test for Plastics, *in vivo*
- L-929-MEM Cytotoxicity Test
- Hemolysis Test-Direct Contact with Rabbit Blood
- Endotoxin Levels and Total Oxidizable Substances

5.3 Materials of Construction Conformance Summary

- Polyethersulfone — Meets FDA 21 CFR, part 177.2440, USP Class VI-70 °C Plastics.
- Polyolefin membrane support — Meets 21 CFR, part 176.170, 177.1520, 177.2800, USP Class VI-70 °C Plastics.
- Polypropylene screen — Meets 21 CFR, part 177.1630, USP Class VI-70 °C Plastics.
- Polyurethane encapsulant — Meets 21 CFR, part 175.103, 175.300, 177.2600, USP Class VI-70 °C Plastics.
- Silicone permeate seals — Meets 21 CFR, part 175.103, 175.300, 177.2600, USP Class VI-70 °C Plastics.
- Silicone gaskets — Platinum cured, medical grade, Meets 21 CFR, part 177.2600, USP Class VI-70 °C Plastics.
- Glycerine — CP/USP grade, plant origin, (added as humectant; removed with flushing).
- Sodium azide — 0.05 to 0.1% sodium hydroxide 0.3 azide (added as a bacteriostat, removed with flushing).

6. Method Details

6.1 Procedure for Flushing Cassettes (Preconditioning)

This procedure is recommended for flushing and preconditioning cassettes before use with product. It has been found effective to significantly remove storage agents and extractables while keeping water usage to under 150 L/m^2 (14 L/ft^2).

6.1.1 Initial Flush

System set-up: Direct the retentate and permeate lines to waste

Volume: 25 L/m^2 (2 L/ft^2) of 0.2 µm filtered DI water

Temperature: Ambient

1. Fill the reservoir with the required volume of DI water.
2. Open the retentate and permeate valves 100%.
3. Flush 0.2 µm filtered DI water at a flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) through the retentate to waste.
4. Adjust the pump flow rate and the retentate valve to achieve a flow distribution of 75% flow through the retentate and 25% flow through the permeate.
5. Adjust the pressures to ~1.4 barg (20 psig) feed, 0.8 barg (4 psig) retentate, and 0 barg (0 psig) permeate.
6. Drain the system after flushing.

6.1.2 Caustic Sodium Hydroxide Recirculation: 0.1 to 0.5 N

System set-up: Direct the retentate and permeate lines to feed reservoir

Volume: 8 L/m^2 (0.7 L/ft^2)

Temperature: 40 to 45 °C

1. Fill the reservoir with the required volume of caustic sodium hydroxide solution. Set up the system for recirculation of the retentate and permeate.
2. Adjust the pump to circulate at a flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) through the retentate (permeate valve open).
3. Run for 60 minutes.
4. Stop the pump. Drain the system.

6.1.3 Post Caustic Sodium Hydroxide Cycle Flush

System set-up: Direct the retentate and permeate lines to waste

Volume: 35 L/m^2 (3 L/ft^2)

Temperature: Ambient

1. Fill the reservoir with the required volume of DI water.
2. Open the retentate and permeate valves 100%.
3. Flush 0.2 µm filtered DI water at a flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) through the retentate to waste.
4. Adjust the pump flow rate and the retentate valve to achieve a flow distribution of ~75% flow through the retentate and 25% flow through the permeate.
5. Stop the pump after appropriate flush volume.

6.1.4 Water Recirculation (3X)

System set-up: Direct the retentate and permeate lines to the feed reservoir

Volume: 8 L/m^2 (0.7 L/ft^2)

Temperature: Ambient

Time: 30 minutes/cycle (3 cycles)

1. Fill the reservoir with the required volume of DI water.
2. Open the retentate and permeate valves 100%.
3. Flush 0.2 µm filtered DI water at a flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) through the retentate to waste.
4. Adjust the pump flow rate and the retentate valve to achieve a flow distribution of ~75% flow through the retentate and 25% flow through the permeate.
5. Stop the pump after 30 minutes.
6. Drain the system.
7. Repeat steps 1 to 6 two additional times.

6.1.5 Final Flush

System set-up: Direct the retentate and permeate lines to waste

Volume: 40 L/m^2 (4 L/ft^2)

1. Fill the reservoir with the required volume of DI water.
2. Open the retentate and permeate valves 100%.
3. Flush 0.2 µm filtered DI water at a flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) through the retentate to waste.
4. At the end of the flush, sample the retentate and permeate and check TOC and pH.

6.2 Procedure for Determination of Extractables from T-Series Cassettes with Omega Membrane

6.2.1 Equipment

- Centrasette holder and gauges
- Peristaltic or diaphragm pump with inert contact surfaces
- Inert (PTFE or FEP) tubing and adapters to sanitary flange connections
- Silicone tubing
- Borosilicate glassware
- Timer/stopwatch
- TOC meter (using CO_2-specific detection)
- pH meter
- Rotary flash evaporator
- Vacuum source with trap
- Muffle furnace
- Drying oven
- Desiccators
- Ceramic crucibles with covers
- Analytical balance
- UV-visible spectrophotometer
- Infrared spectrophotometer
- Particle-free sample bottles (250 or 500 mL)

6.2.2 Preparation of Apparatus and Materials

1. Thoroughly clean the glassware and the liquid contact surfaces of the pumps, tubing, and holder. Where appropriate, a TOC measurement of a DI water rinse is used to assure cleanliness. Clean the porcelain crucibles by heating in a furnace at 500 °C or higher for approximately 30 minutes and allow cooling in a desiccator.
2. Prepare sanitizing solution of 0.1 N NaOH/200 PPM sodium hypochlorite by dissolving 16.8 grams of NaOH in 3 liters of DI water, adding 16 mL of 5.25% sodium hypochlorite (commercial bleach), and bringing the total volume to 4 liters. Filter the solution through a 0.2 µm filter.
3. Prepare 6 liters of 25% ethanol extraction solution in filtered DI water.

6.2.3 Extraction Procedure

The following extraction procedure will be run in either DI water at 45 to 50 °C or 25% ethanol at 20 to 25 °C. A sample blank must be run first. The blank consists of using a flushing gasket in place of a cassette. All other procedural steps are followed for both solvents.

Test will be performed in triplicate.

All glassware must be chromerged with clean chromic acid before use.

1. Prepare 6 L of solution (water or 25% ethanol).
2. Collect two 1 L samples for extraction (blanks) from the 6 L.
3. Record actual volume of feed solution being used.
4. Pour the remaining solution (4 L) into the glass feed container.
5. Adjust the flow rate to ~ 2 L/min to achieve a flow distribution of 90% permeate flow and 10% retentate flow.
6. For water, bring the temperature to 50 °C. For the 25% ethanol solution, the temperature should be between 20 to 25 °C.
7. Recirculate the feed solution for 16 hours. Maintain the temperature throughout the circulation.
8. After 16 hours, drain the system into a feed reservoir and record the final volume.
9. Take two 1L samples from the feed reservoir for analysis.
10. Remove the feed reservoir from the system.
11. Repeat steps 1 to 10 for second extraction with same solvent.
12. Remove the cassette and store.
13. Flush the system with DI water for at least 1 hour before running next extraction cycle. Give the samples for analysis for FTIR, gas chromatography/mass spectroscopy (GC/MS), NVR, and high performance liquid chromatography (HPLC).

6.3 Procedure for Determination of NVR

1. Take an aliquot of the extraction solution (e.g., 1000 mL) and evaporate on a rotary evaporator using a clean 1000 mL, glass, and round-bottom flask. Adjust and maintain the temperature of the water bath to 80 °C. Evaporate the aliquot to less than 25 mL.
2. Weigh crucibles to the nearest 0.0002 g. Repeat until constant weight is obtained (± 0.0002 g). Store in a desiccator. See Section 6.
3. Quantitatively transfer the concentrated extract to the tared crucible contained in the desiccator. If residue remains in the round-bottom flask, add a few drops of fresh DI water, swirl, and add to crucible. If more than a few drops are needed, note the volume used.

4. Carefully place the crucibles in an oven (circulating air type) maintained at 60 to 80 °C. Evaporate the extract to dryness. Remove the crucibles from the oven after evaporation of the water, return to a desiccator, cover, and allow to cool to room temperature.
5. Weigh the crucibles to the nearest 0.0001 g and record the weight.
6. Calculate the NVR for the volume evaporated as follows: NVRV (mg) = CR (mg) - CC (mg).

Where NVRV = NVR for volume evaporated in mg, CR = constant weight of crucible and residue, CC = constant weight of crucible

7. Calculate the total NVR for both the control and each sample.

NVRT (mg) = NVRV (mg) x VI/VE

Where NVRT (mg) = total NVR in mg, VI = initial solvent volume used for extraction, VE = volume of solvent taken from final volume for evaporation

8. Calculate the net NVR for each sample as follows.

Net NVR (mg) = NVRS - NVRC.

Where NVRS = total NVR of the sample in mg, NVRC = total NVR of the control in mg

6.3.1 Determining Chemical Compatibility of Common Cleaning Agents

The following protocol was used to determine the chemical compatibility of T-Series cassettes with Omega membrane in the following solutions:

- 1.0 N NaOH at 23 °C for 28 days
- 0.1 N H_3PO_4 at 45 °C for 14 days
- 25% EtOH at 23 °C for 28 days

Compatibility Study Procedure

1. Characterize cassettes for IT, DP, and NWP.
2. Prepare the test solution.
3. Install the cassettes in holder. Set system up for recirculation of retentate and permeate.
4. Open the feed retentate and permeate valves 100%.
5. Adjust the pump flow rate and the retentate valve to achieve a feed pressure of ~1.4 barg (20 psig) and a retentate pressure of ~0.7 to 1 barg (10 to 15 psig).
6. Recirculate for 15 minutes.
7. Remove the cassette from the holder and put in a plastic bag and seal it.
8. Store the cassettes at specified temperature and time period.
9. Remove cassettes after 14 or 28 days.
10. Install the cassette in a holder/TFF system.
11. Fill the reservoir with water.
12. Flush the cassette with 0.2 µm filtered DI water for at least 20 minutes.
13. Characterize the cassettes for IT, DP, and NWP.

7. Detection Methods

7.1 Detection Methods for Cleaning Agents and Storage Agents

7.1.1 Sodium Hydroxide

Residual sodium hydroxide can be determined by measuring the pH of effluent from the cassette and comparing the pH to that of the influent. When the two match, the residual sodium hydroxide has been removed. The pH is a direct measure of the hydroxyl ion concentration and can be used to calculate residual hydroxyl ion.

7.1.2 Glycerine

The following steps describe the size exclusion chromatography method for determination of glycerine concentration. The sensitivity of the method is 1 PPM.

1. Prepare 100 PPM, 10 PPM, and 1 PPM glycerine standards.
2. Inject 50 µL of the glycerine standards on a size exclusion chromatography column (TOSO Haas, G3000PWXL) connected to an HPLC system (Hewlett Packard, Model #1050) using a refractive index detector (mobile phase: H_2O). Identify the glycerine peak and calculate the peak area for each standard. Prepare a standard curve of peak area versus glycerine concentration.
3. Inject 50 µL of each of the retentate and permeate samples collected. Identify the glycerine peak found in each of the retentate and permeate samples. Measure the peak area and determine glycerine concentrations from the standard curve.

7.2 Endotoxin Assay Procedure — ThermoMax Chromogenic Assay

This section describes the test procedure for detecting endotoxin in solution using Limulus Amoebocyte Lysate reagent incorporated into the ThermoMax Chromogenic (Endochrome[u] K) Assay.

7.2.1 Introduction

Recent advances in computer technology have made kinetic quantitation the method of choice for simultaneous measurement of endotoxin in multiple samples. This procedure provides a standard method for the use of the Molecular Devices ThermoMax microtiter plate reader with endotoxin standards in detecting and quantitating endotoxin in control and unknown samples.

7.2.2 Detection Limit

The detection limit is based on the sensitivity of the lysate being used. Sensitivity of 0.005 EU/mL can be achieved. This method is at least an order of magnitude more sensitive than the gel-clot procedure.

7.2.3 Materials

- Vortex mixer
- Falcon dilution tubes (No. 2057 or equivalent)
- Falcon 96 well microtiter plate (No. 3072)
- 1 and 5 mL pipettes
- 100 mL pipette with tips
- Eppendorf repeater pipette with 0.5 and 5 mL Combitips[u] tips
- 37 °C heat block
- Chromogenic Kit (Endochrome K)
- WFI
- Endosafe[u] Control Standard Endotoxin (1000 EU/mL)
- Microplate reader with Incubator: Molecular Devices, ThermoMax reader and SoftMax[u] Pro 3.0 software

7.2.4 Reagents

Control Standard Endotoxin (CSE)

500 ng in 5 mL = 1000 EU/mL

Using a pipette, reconstitute with 5.0 mL of WFI directly into the vial. Close the vial, cover with aluminum foil, and invert in vortex. Tape the vial into position. Vortex the control sample for five minutes.

7.2.5 Endochrome[11] Assay

Reagent Collect LAL powder into the bottom of its vial by tapping it on a firm surface. Unseal and release the vacuum by slowly lifting the stopper. Using a pipette and the 3.2 mL of LAL water provided with the LAL powder, rehydrate the LAL in its vial just before use. Gently swirl the vial until the LAL dissolves into a colorless solution. Discard the reagent if the seal is broken or color/opacity is present after rehydration. Store hydrated LAL reagent on ice while on the bench. Unused LAL may be frozen and thawed once.

Dilution of Control Endotoxin for Standard Curve Label dilution tubes: 500, 50, 5, 0.5, 0.05 EU/mL (including 0.005 EU/mL for Endochrome assay). Prepare dilution of endotoxin as described in Table 19. Vortex each dilution for one minute.

Table 19

Dilution of Control Endotoxin for Standard Curve

To prepare >	Take (X) mL of solution containing specified EU/mL	Dilute with (X) mL of WFI	Volume after dilution (mL)	Endotoxin concentration (EU/mL)
Reconstituted Stock Solution	5.0	5.0	1000	
Dilution 1	1.0 of 1000 EU/mL	1.0	2.0	500
Dilution 2	0.1 of 500 EU/mL	0.9	1.0	50
Dilution 3	0.1 of 50 EU/mL	0.9	1.0	5
Dilution 4	0.1 of 5.0 EU/mL	0.9	1.0	0.5
Dilution 5	0.1 of 0.5 EU/mL	0.9	1.0	0.05
Dilution 6	0.1 of 0.05 EU/mL	0.9	1.0	0.005

7.2.6 ThermoMax Setup

1. Turn on the instrument power switch. Click on SOFTMAX FOR WINDOWS, then the SOFTMAX icon.
2. Click on CONTROL function and drag to OPEN DRAWER.
3. Click on CONTROL function and drag to INCUBATOR, then activate.
4. Click on SET UP function and drag to INSTRUMENT and select the following:
   (i) Select KINETIC L1 under MODE
   (ii) Select wavelength 340 nm for TURBIDIMETRIC
   (iii) Select wavelength 405 nm for CHROMOGENIC
   (iv) Select Automix: ONCE
   (v) Set: 1-hour run time
   (vi) Set Read Interval: 00:14 sec
5. Click on SET UP function and drag to ANALYSIS
   (i) Select Onset Time (sec)
   (ii) Select Std Curve Fit: (log-log)

(iii) Select Spike Recovery Analysis

(iv) Select onset OD: 0.05

(v) Select onset limit: 0.2

6. Click on SET UP function and drag to TEMPLATE
7. Click and drag to select wells for:

(i) Blanks

(ii) Standard curve (0.05 to 5 EU/mL)

(iii) Unknown + dilution

(iv) Spike (0.5 EU/mL)

7.2.7 LAL Assay

Follow these steps to complete the LAL assay:

1. Place microtiter plate with lid on 37 °C heating block.
2. Remove lid and fill plate in duplicate with 100 µL of each:

(i) Blank (WFI)

(ii) 0.005 EU/mL standard

(iii) 0.05 EU/mL standard

(iv) 0.5 EU/mL standard

(v) 5.0 EU/mL standard

(vi) Unknown sample

(vii) PPC = Unknown sample + 0.5 EU/mL

(viii)(10 µL of 5.0 EU/mL of CSE standard)

(ix) Water flush (negative control)

3. Incubate plate with lid on for 10 minutes at 37 °C.
4. After incubation, place 100 µL of LAL reagent into each well within 2 minutes using a repeater pipette.
5. Click on CONTROL function and drag to READ PLATE.
6. Place plate in reader without the lid.

7.2.8 Interpreting Results

The LAL assay results are considered accurate if the determination meets the following criteria:

1. Linearity of the standard curve within the concentration range used must be verified. No less than 3 endotoxin standards, spanning the desired concentration range, should be assayed in duplicate. The coefficient of correlation shall be greater than or equal to the absolute value of 0.98 for the determination to be valid.
2. Each unknown sample must be accompanied by the corresponding spike or positive product control (PPC). The mean endotoxin concentration of the PPC must be within ±50% of the corresponding standard curve concentration.

7.2.9 Analyst Qualification

Run the appropriate standard curve in triplicate, performing regression analysis on each individual point ($r > 0.98$) by selecting standard values individually (i.e., Std 1, Std 2 and Std 3 = 0.05 EU/mL). The standard curve for KTA LAL is three point (2 log) including points: 0.05, 0.5 and 5.0 EU/mL and for Endochrome-K is four point (3 log) including points: 0.005, 0.05, 0.5, and 5.0 EU/mL.

The correlation coefficient must be greater than 0.98 to be acceptable for validation. Repeat until the validation is acceptable. (Must be done with both Chromogenic Kinetic and Turbidometric Kinetic Assay to be validated on both procedures.)

8. Biosafety Test

Results following are the report references for biological safety testing on components of T-Series cassettes with Omega membrane.

The following tests were performed on the components listed at the laboratories of:

Toxicon
225 Wildwood Ave
Woburn, MA 01801

Omega Membrane and Polyolefin Backing in Cassette Format

USP Biological Test for Plastics Class VI including:

- Systemic Injection Test
- Intracutaneous Reactivity Test
- Muscle Implantation Test
- Project No 88G 0156

Hemolysis Test — Direct Contact with Rabbit Blood

- Project No. 88G 0163

MEM Elution Cytotoxicity Test L929

- Project No. 88G 0164

Limulus Amebocyte Lysate and Total Oxidizable Substances Test

- Project No. 94G 2269

The following tests were performed on the components listed at the laboratories of:

STS, a division of Ethox International
7500 W. Henrietta Road
Rush, NY 14543
Tel: (585) 533-1672

Polypropylene Screen

Intracutaneous (Intradermal) Reactivity Test of Urethane — USP Method
STS Test No: T06-1391

STS Study No: GLP-2006-0179

Systemic Injection Test — USP Method
STS Test No: T06-1392

STS Study No: GLP-2006-0180

7 Day Muscle Implantation with Macroscopic Evaluation — USP Method
Systemic Injection Test — USP Method

STS Test No: T06-1393

STS Study No: GLP-2006-0181

USP Class Plastic Designation
Study Nos: GLP-2006-0179, GLP-2006-0180, GLP-2006-0181

Hemolysis Test — Saline Extract Method
STS Test No: T06-1394

STS Study No: GLP-2006-0182

MEM Elution Cytotoxicity Test — ISO Method
STS Test No: T06-1395

STS Study No: GLP-2006-0183

Polyurethane Encapsulant

Intracutaneous (Intradermal) Reactivity Test of Polyurethane — USP Method
STS Test No: T06-1443

STS Study No: GLP-2006-0186

Systemic Injection Test — USP Method
STS Test No: T06-1444

STS Study No: GLP-2006-0187

7 Day Muscle Implantation with Macroscopic Evaluation — USP Method Systemic Injection Test (USP Method)
STS Test No: T06-1445

STS Study No: GLP-2006-0188

USP Class Plastic Designation Study Nos: GLP-2006-0186, GLP-2006-0187, GLP-2006-0188 Hemolysis Test — Saline Extract Method
STS Test No: T06-1446

STS Study No: GLP-2006-0189

MEM Elution Cytotoxicity Test — ISO Method
STS Test No: T06-1447

STS Study No: GLP-2006-0190

Contact your local Pall representative for details on any of these test reports.

9. Glossary

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

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