DORSAN®
LIVING FILTRATION

Validation Guide

PTFE Hydrophilic Double Layer Filter Cartridges 0.2+0.2µm

Contents

1.Introduction

1.1 Product Overview

Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges are sterilize grade cartridge filters manufactured from hydrophobic PTFE membrane and polypropylene hardware providing high flow rates , low extractable, broad chemical compatibility. Double Layer Hydrophobic PTFE 0.2+0.2μm cartridge filters are 100% integrity tested during manufacturing t o assure sterilizing-grade performance.

The Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges are designed for removal of particles and the sterile filtration of gas/air/vent. The Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges are designed,developed and manufactured in accordance with an ISO 9001:2015 certified quality management system. These filters are manufactured in a controlled environment that meets the air quality standards of an ISO class 8 room with respect to viable and nonviable particulate and positive pressure.

1.2 Materials of Construction

1.2.1Component materials used in this product meets the FDA Indirect Food Additive requirements cited in 21 CFR 177-182.

Items	Material
Filter media	hydrophobic PTFE membrane
Support	polypropylene
Cage	polypropylene
Core	polypropylene
End caps	polypropylene with embedded stainless steel ring
O-ring	silicone

1.2.2Component materials used in this product meets the regulation(EC)No.1935/2004.

Items	Material
Filter media	hydrophobic PTFE membrane
Support	polypropylene
Cage	polypropylene
Core	polypropylene
End caps	polypropylene

1.3 Ordering Information

Application	Type	Material	Micron	Length	Adaptor	Sealing	R
D- Dorsan Biopharma Food&Beverage	PTFEDL Pleated Filter	DL DL-Double Layer PTFEDL	0.2+0.2µm	5″ 10″ 20″ 30″ 40″	E2-222/Flat with SS insert E3-222/Fin with SS insert E7-226/Fin with SS insert E6-226/Flat with SS insert	S S-Silicone E-EPDM N-NBR V-Viton F-PTFE F-Encapsuled Viton K-Encapsuled Silicone	

The Catalogue Number is shown as below:

Adaptor No.	Description	Top	Bottom
BLANK SPACE	DOE		
E7	226/Fin with SS insert		
E6	226/Flat with SS insert		
E2	222/Flat with SS insert		
E3	222/Fin with SS insert		

2. Validation Item

Retention	3.1 BCT (ASTM F838-20)	3.2 Integrity Test
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3. Test Methods and Results

3.1 Correlation of non-destructive integrity testing to liquid bacterial challenge with *Brevundimonas diminuta* (ATCC 19146) for sterilize grade filters

3.1.1 Introduction

The FDA guidelines on Sterile Products Produced by Aseptic Processing (2004) state, "A sterilizing filter is one which, when challenged with the micro-organism *Brevundimonas diminuta* (*B. diminuta*), at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent".

In order to meet the requirements of this guideline, liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146) were performed with Double Layer Hydrophobic PTFE 0.2+0.2µm filter cartridges using a minimum of $1x10^7$ colony forming units (CFU)/cm^2 of effective filtration area.

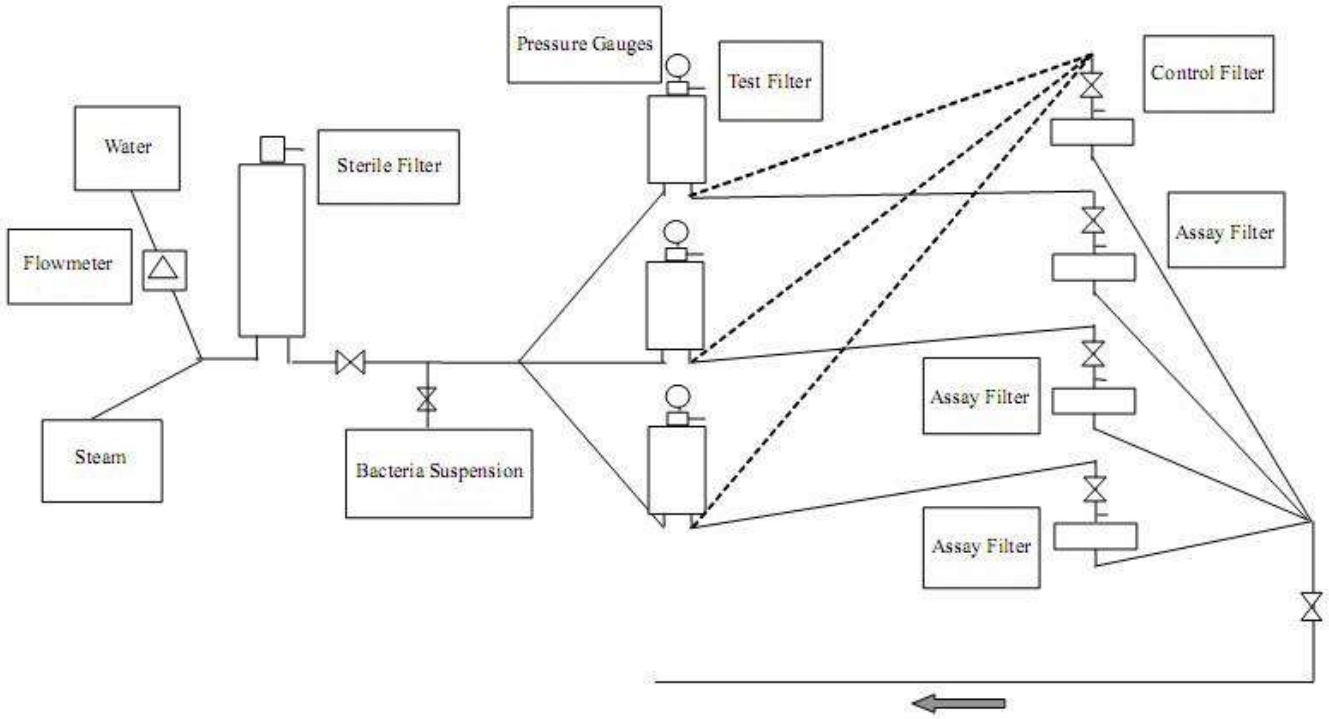
The correlation between microbial retention and a non-destructive integrity test is also an important aspect of the validation of sterilizing grade filters. The FDA guideline further states, "After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data". The integrity tests used during this validation study were the Water Intrusion and Bubble Point tests.

The Forward Flow Integrity Test
In the Forward Flow test, a filter is wetted with a suitable test liquid and a pre-determined gas pressure is applied to the upstream side of the filter assembly. After a stabilization period, the gas flow through the wetted membrane can be measured manually on the downstream side or on the upstream side, using sensitive flow measurement equipment such as the integrity test devices.

The Water Intrusion Integrity Test
The Water Intrusion test is performed on a dry filter. The upstream side of the filter assembly is filled with water and a pre-determined gas pressure is applied.The resulting water flow through the membrane can be measured directly on the upstream side using sensitive direct flow measurement equipment such as the integrity test devices.

The Bubble Point Integrity Test
In the Bubble Point test, a filter is wetted with a suitable test liquid. After a stabilization period, increasing the gas pressure to the upstream side of the filter assembly, using sensitive flow measurement equipment such as the integrity test devices to test the change point of the gas flow rate.

3.1.2 Summary of Methods



Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges with different batch lots were subjected to microbial challenge tests using an aqueous suspension of *Brevundimonas diminuta* (ATCC 19146).

The filter sample was installed in a housing and tested for integrity by the water intrusion or forward flow method.Prior to the challenge tests,the filters were wetted with 60:40(v/v)IPA/water,then installed in an appropriate housing, flushed with DI water, and then autoclaved at 121 °C for 30 minutes. The filter assembly was then aseptically connected to a pre-sterilized challenge apparatus.

An aqueous suspension of *Brevundimonas diminuta* was passed through the filter to achieve a challenge level of > 1 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area.

During the challenge test, the entire filter effluent was passed through a 0.2 μm rated recovery membrane on the downstream side of the test filter assembly. Following the challenge test,the recovery membrane was aseptically removed from the filter housing in a laminar flow cabinet and placed onto TSA plates.All agar plates were incubated at 30±2℃（86±3.6°F） for a minimum of two days.After incubation, the recovery membranes were examined for growth to determine whether bacteria had passed through the test filter during the challenge.

3.1.3Test Results

Table listed below indicates Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges that are water intrusion tested before and after bacterial challenge. The bacterial challenge was conducted using ASTM F838-20 to provide the necessary correlation between a bacterial challenge and a non-destructive integrity test.

Filter type: Double Layer Hydrophobic PTFE 0.2+0.2μm Challenge organism: *Brevundimonas Diminuta* (ATCC 19146)

Filter Serial Number	Water Intrusion mL/min	Challenging bacteria(CFU/10inch)	Sterile Effluent	LRV/ cm^2
312150611008	0.25	$1.26*10^{11}$	Yes	7.28
401080511021	0.27	$2.19*10^{11}$	Yes	7.52
401080511012	0.27	$1.26*10^{11}$	Yes	7.28
312150611005	0.28	$1.26*10^{11}$	Yes	7.28
312171511016	0.32	$3.05*10^{11}$	Yes	7.67
312150611004	0.34	$1.81*10^{11}$	Yes	7.44
401080511012	0.38	$2.19*10^{11}$	Yes	7.52
312171511005	0.41	$2.83*10^{11}$	Yes	7.63
312171511001	0.42	$3.05*10^{11}$	Yes	7.67
401080511008	0.45	$2.19*10^{11}$	Yes	7.52
401080511006	0.45	$1.81*10^{11}$	Yes	7.44
401080511017	0.46	$2.15*10^{11}$	Yes	7.51
312171511004	0.47	$1.68*10^{11}$	Yes	7.41
312171511006	0.48	$2.83*10^{11}$	Yes	7.63
312150611007	0.51	$2.15*10^{11}$	Yes	7.51
312171511009	0.52	$1.81*10^{11}$	Yes	7.44
312150611001	0.55	$1.68*10^{11}$	Yes	7.41
401080511009	0.60	$2.36*10^{11}$	Yes	7.55
312171511012	0.61	$2.83*10^{11}$	Yes	7.63
401080511001	0.66	$3.05*10^{11}$	Yes	7.67
312150611006	0.69	$2.36*10^{11}$	Yes	7.55
401080511010	0.72	$1.98*10^{11}$	Yes	7.48
401080511005	0.78	$1.68*10^{11}$	Yes	7.41
312171511008	0.82	$1.15*10^{11}$	Yes	7.24
401080511015	0.88	$1.98*10^{11}$	Yes	7.48
401080511013	0.90	$1.15*10^{11}$	Yes	7.24
312171511003	0.92	$2.36*10^{11}$	Yes	7.55
312150611009	1.05	$1.15*10^{11}$	Yes	7.24
312150611010	1.15	$2.15*10^{11}$	No	<7
312171511007	1.20	$1.98*10^{11}$	No	<7

3.1.4 Conclusions

A Typical Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges from production,

28pcs were found to pass the water intrusion integrity test. The table also indicates that these filter cartridges with Water Intrusion ≤1.05 mL/min/10"@ 2.6bar has sterile filtration efficiency when challenged with > 1 x 10^7 CFU per cm^2 of filtration area using *Brevundimonas diminuta*. Analysis in base of results of water intrusion, tested filter ≤1mL/min gave sterile effluent when challenged with LRV>7 of *B.diminuta*.

3.2Integrity Test Standard

In base of test results of validation test, water intrusion is approved as suitable test methods for Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges. We also test the bubble point before and after bacterial challenge. Bubble point ≥1.2bar at 20℃ has sterile filtration efficiency.

The Integrity test value standard is as below:

Test liquid	DI water
Temperature	20±5℃(68±9°F)
Test pressure	2.6bar
Water intrusion	≤1mL/min/10"

Test Liquid	60:40(v/v)IPA/Water
Temperature	20±5℃
Test gas	Air
Bubble Point	≥1.2bar

3.3 Flow Characteristic

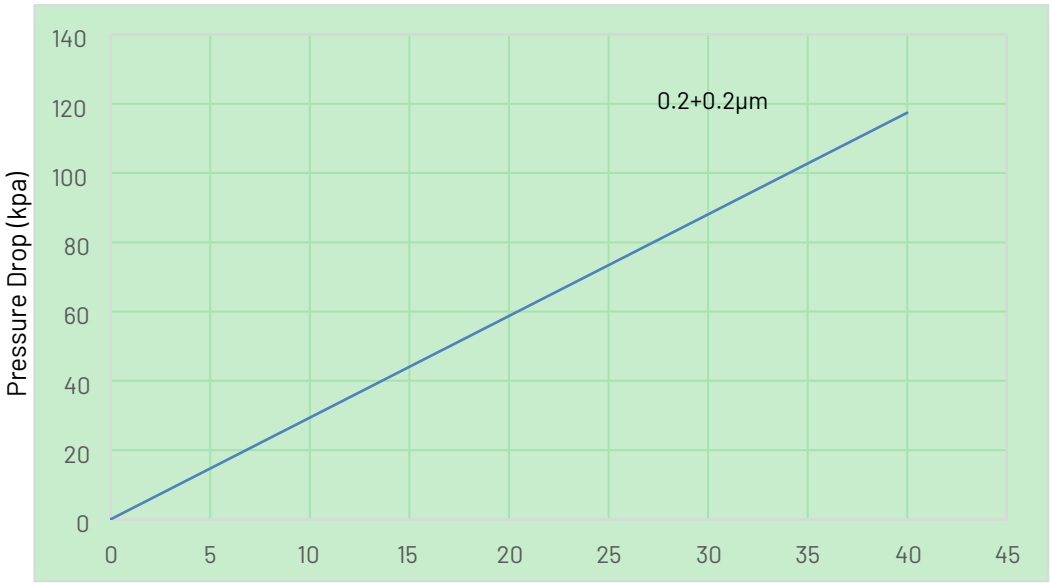
3.3.1 Summary of Methods

Typical Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges from production were used for the tests. The filters were tested water intrusion@2.6bar. After the integrity test, the filter cartridge wetted by 60:40(v/v)IPA/water, then flushed with DI water.

Standard production Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges (10", EFA 0.7m^2) were installed with flow rate test system device, which adjust the flow rate and pressure drop by auto-valve. Then take the continuous records with flow rate, upstream & downstream pressure value, temperature etc.

3.3.2 Test Results

Here is the flow chart of filter cartridge.



3.3.3 Conclusions

These data can be used to assist users in sizing filter systems.

3.4 Maximum Operating Conditions

3.4.1Summary of Methods

Typical Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges from production were used for the tests. The filters were tested water intrusion@2.6bar. After the integrity test, the filter cartridge wetted by 60:40(v/v)IPA/water, then flushed with DI water.

Standard production Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges(10", EFA 0.7m^2) were installed with pressure drop test system device, which adjust the upstream and downstream pressure by auto-valve.

Use Standard test dust to increase the pressure drop till 5.0bar,temperature 25℃ and keep the pressure for 30mins, total 4 cycles. Then test the integrity.

Use Standard test dust to increase the pressure drop till 2.0bar,temperature 80℃ and keep the pressure for 30mins, total 3 cycles. Then test the integrity.

3.4.2 Test Results

Filter Serial Number	Integrity Test（Before the pressure test）		Integrity Test（After the pressure test）	
	Water Intrusion(mL/min@2.6bar)	Bubble Point(bar)	Water Intrusion(mL/min@2.6bar)	Bubble Point(bar)
401080511026	0.32	1.658	0.28	1.721
312171511017	0.41	1.732	0.39	1.758
312171511019	0.35	1.621	0.33	1.651
312150611020	0.42	1.708	0.41	1.712
312150611022	0.38	1.687	0.36	1.705

The Integrity test value standard is water intrusion ≤1mL/min@2.6bar,bubble point ≥1.2bar,test temperature 20±5℃.

3.4.3 Conclusions

Here is the Max. pressure drop and temperature of filter cartridge.

Maximum Differential Pressure (Forward)	Maximum Operating Temperature
5.0bar @ 25 ℃ 2.0bar @ 80 ℃	80°C

3.5 Steam Sterilization(Thermal Stability)

3.5.1Summary of Methods

The purpose of these tests was to determine the effects of repeated exposure to in-line steam or autoclave cycles on filter integrity using standard Double Layer Hydrophobic PTFE 0.2+0.2μm filters from production.The filters were tested water intrusion@2.6bar.

During this autoclave study, filters were steamed using high initial differential pressures (1.0bar (14.5 psi)) at 125°C (257°F). The tests were performed in 30 minutes cycles in the forward (out to in) direction.

During the on-line steam sterilization,These filter cartridges were installed in stainless steel housing and steamed in place in the forward(out to in) direction using saturated steam at constant pressure and flow while ensuring effective condensate drainage.After each steam-in-place cycle the filters were cooled by passing dry compressed air through them.

Integrity tests maintained integrity after Sterilized in place and Autoclave to determine the ability of the filter to provide a sterile filtration.

3.5.2 Test Results

Water Intrusion (mL/min) after the following number of 30 minutes autoclave cycles

Serial No.	0Cycles	10cycles	30cycles	50cyecles	100cycles	150cycles
401080511002	0.38	0.41	0.39	0.42	0.40	0.39
401080511004	0.65	0.68	0.66	0.69	0.67	0.68
401080511016	0.48	0.45	0.49	0.47	0.50	0.49
401080511019	0.62	0.61	0.63	0.62	0.64	0.65
312171511002	0.70	0.71	0.69	0.70	0.68	0.69
312171511010	0.71	0.69	0.70	0.72	0.70	0.68
312171511011	0.35	0.34	0.36	0.32	0.35	0.37
312150611013	0.45	0.47	0.46	0.45	0.46	0.50
312150611015	0.68	0.66	0.66	0.68	0.67	0.66
312150611018	0.54	0.55	0.56	0.54	0.55	0.58

The Integrity test value standard is water intrusion ≤1mL/min@2.6bar,test temperature 20±5℃.

Water Intrusion (mL/min) after the following number of 30 minutes on-line steam cycles

Serial No.	0Cycles	10cycles	20cycles	30cyecles	40cycles	50cycles
401080511028	0.46	0.45	0.48	0.47	0.45	0.44
401080511030	0.38	0.36	0.35	0.37	0.36	0.39
312171511019	0.42	0.43	0.45	0.43	0.41	0.43
312171511020	0.39	0.38	0.42	0.41	0.40	0.38
312150611020	0.52	0.54	0.53	0.53	0.52	0.51

The Integrity test value standard is water intrusion ≤1mL/min@2.6bar,test temperature 20±5℃.

3.5.3 Conclusions

Double Layer Hydrophobic PTFE 0.2+0.2μm filters have been demonstrated to be capable of withstanding multiple in-line steam/autoclave sterilization cycles.

The data presented in this section support the following product claims for in-line steaming/autoclaving these filters.

cartridges:

Sterilized in Place				Autoclave		
Temp.	Time	ΔP	Cycles	Temp.	Time	Cycles
135℃	30min	30kPa	50	125℃	30min	150

3.6 Cleanness

3.6.1Gravimetric Extractable

Typical Double Layer Hydrophobic PTFE 0.2+0.2μm filter Cartridge from production were used for the tests.

3.6.1.1Summary of Methods

Preparation of Filter Samples

Extractables tests were performed on typical production filter cartridges (10inch, 0.7m^2), which had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminium foil and autoclaved for half hour at 121°C, using a slow exhaust cycle. Visible droplets of water remaining on the filter elements were allowed to evaporate at room temperature before the extraction was performed.

Extraction Procedure

Dynamic extraction tests were performed. The test filters were immersed in 1800 ml of extraction fluid in a clean measuring cylinder for 24 hours. For four hours the filter was gently moved up and down. This movement created flow through the filter membrane as a result of the pressure head that was created each time the element was partially lifted out of the liquid.

Analysis of Material Extracted

After the extraction, 1500mL of the extraction liquid was evaporated to dryness and the non-volatile extractable were determined gravimetrically.

3.6.1.2 Test Results

Extraction Fluid	Filter serial number	Gravimetric Extractable (milligrams per Filter)	Average(milligrams)
50:50（v/v） Ethyl alcohol/Water	401080511020	12.9	14.0
	312171511014	15.4	
	312171511015	13.8	

3.6.1.3 Conclusions

The extractable determined of Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridge were depended by different solvent. The extractable levels under different solvents, different solubility, different temperature and different contact time are not consistent, so it is recommended to test under actual process conditions.

3.6.2 Fiber Releasing

Typical Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridge from production were used for the tests.

3.6.2.1 Summary of Methods

Filters were autoclaved for one cycle of 30 minutes, 121℃,and wetted with 60%IPA,then flushed with a total of 10 liters of 0.1μm filtered water at a flow rate of 1 L per minute. The filtrate was passed through a 0.65μm black gridded disc filter to collect any fibers removed from the filter. Filters were then integrity tested to verify that only integral filters were used in the test.

3.6.2.2 Test Results

Double Layer Hydrophobic PTFE 0.2+0.2μm Filter – Fiber Shedding Results		
Filter serial number	**Number of Fibers in filtrate**	**Water Intrusion(mL/min/10"@ 2.6bar,20℃)**
312150611016	0	0.52
401080511025	0	0.43
312171511018	0	0.39

The Integrity test value standard is water intrusion ≤1mL/min@2.6bar,test temperature 20±5℃.

3.6.2.3 Conclusions

The Double Layer Hydrophobic PTFE 0.2+0.2μm from production don't have Fiber releasing and meet the request of FDA 21 CFR 210.3(b)(6).

3.7 Biological Safety

3.7.1 Biological Tests

3.7.1.1 Summary of Methods

These filters are non-toxic per USP Class VI Biological Tests forPlastics.
Systemic Injection Test, Intracutaneous Test as well as Implantation Test were performed to determine the toxicity of this filters. This testing was performed by an independent laboratory.

3.7.1.2 Conclusions

The materials used in Double Layer Hydrophobic PTFE 0.2+0.2μm
filter cartridges from production met the specifications for Biological Reactivity Tests, in vivo, listed in the current revision of the United States Pharmacopeia (USP) for Class VI –121 °C Plastics.

3.7.2Bacterial Endotoxin: LAL Test

3.7.2.1Summary of Methods

The test filters were soaked with endotoxin-free water. The aqueous extract was tested with an LAL reagent and all tubes were incubated at 37±1℃ for 60±2min.

3.7.2.2 Test Results

Extracts from filters contain <0.25EU/mL endotoxin units per milliliter per the USP Bacterial Endotoxins Test. The results are shown in the following table.

PTFE 0.2+0.2μm filter--Bacterial Endotoxin: LAL Test per USP(+Clotted;-Not Clotted)					
Filter serial number	**Positive Control**	**Negative Control**	**Positive control of test solution**	**Test solution**	**Test results （EU/mL）**
401080511023	+ +	- -	+ +	- -	<0.25
401080511024			+ +	- -	<0.25
312171511021			+ +	- -	<0.25
312171511022			+ +	- -	<0.25
312150611019			+ +	- -	<0.25
312150611017			+ +	- -	<0.25

3.7.2.3 Conclusions

The Double Layer Hydrophobic PTFE 0.2+0.2μm filter cartridges from production met the specifications for USP Bacterial Endotoxins Test.

3.8 Chemical Compatibility

The chemical compatibility of Double Layer Hydrophobic PTFE 0.2+0.2μm filters is shown in the chart below. Recommendations are based upon static soak for 72 hours at 25℃ and 1.0 atmosphere (14.5 psi,1.01 bar absolute) pressure. Dynamic

(operating) conditions at moderate temperatures(±10% fluctuation) will not change the recommendations, but high liquid temperature may do so insome cases.

NOTE: This data is intended to provide expected results when filtration device are exposed to chemicals under static conditions for 48hours at 25℃, unless otherwise noted, membrane integrity was tested by bubble point.

This chart is intended only as a guide. User should verify chemical compatibility with a specific filter under actual use condition, such as various temperatures, pressure, and concentration.

R = Resistant.
L = Limited resistance
N = Not resistant
- = No data

Chemicals	PTFE Membrane filter	PP Plastic Parts	Silicone O-ring	EPDM O-ring	Viton O-ring
Acetic Acid, glacial	R	R	L	L	N
Acetic Acid, 25%	R	R	R	L	L
Acetic Acid, 10%	R	R	L	L	L
Hydrochloric acid, conc. 35%	R	R	N	N	R
Hydrochloric acid, 20%	R	R	N	N	R
Hydrochloric acid, 3.3%	R	R	-	N	R
Nitric Acid， conc .67%	R	R	N	-	R
Nitric Acid， 25%	R	R	L	L	R
Sulfuric Acid， conc. 96%	R	N	N	-	R
Sulfuric Acid， 16%	R	R	N	-	R
Ammonium Hydroxide 3N,5.7%	R	R	R	-	R
Ammonium Hydroxide 6N,11.4%	R	R	R	-	R
Potassium Hydroxide， 15%	R	R	N	R	R
Sodium Hydroxide3N， 11%	R	R	R	R	R
Sodium Hydroxide， 22%	R	R	R	R	R
Amyl Alcohol	R	R	N	R	R
Benzyl Alcohol	R	R	L	-	R
Butanol	R	R	L	-	R
Isopropanol	R	R	R	-	R
Methanol	R	R	R	R	N
Ethylene glycol	R	R	R	-	R

Glycerol	R	R	R	R	R
Propylene glycol	R	R	R	-	R
Ethyl ether	R	R	N	N	N
Tetrahydrofuran	R	R	N	N	N
Tetrahydrofuran，50% v-v	R	R	-	N	N
Acetone	R	R	R	R	N
Cyclohexanone	R	R	L	L	N
Methyl Ethyl Ketone (MEK)	R	R	N	R	N
Methyl Isobutyl Ketone (MIBK)	R	R	N	R	N
Amyl acetate	R	R	N	R	N
Butyl Acetate	R	R	R	-	N
Cellusolve Acetate	R	R	R	-	N
Ethyl Acetate	R	R	L	N	N
Isopropyl acetate	R	R	L	R	N
Methyl acetate	R	R	N	R	N
Carbon Tetrachloride	R	R	N	N	R
Chloroform	R	R	N	N	R
Ethylene dichloride	R	R	N	-	R
Methylene Chloride	R	R	N	N	L
Tetrachloroethylene	R	R	N	-	R
Trichloroethane	R	R	N	N	-
Benzene	R	N	N	N	R
Toluene	R	N	N	N	R
Xylene	R	N	N	N	R
Cottonseed	R	R	R	-	R
Peanut	R	R	R	-	R
Formaldehyde 37%	R	R	L	R	R
Formaldehyde 4%	R	R	R	R	R
Hexane	R	R	N	-	R
Acetonitrile	R	R	N	R	R
Dimethyl Formamide(DMF)	R	R	R	N	L
Dimethylsulfoxide(DMSO)	R	R	N	N	N
Kerosene	R	-	N	N	R
Pyridine	R	L	N	N	N
Petroleum spirits	R	R	N	N	-
Hydrogen Peroxide	R	R	R	R	-
Ozone	R	R	N	L	-
Phenol	R	R	-	-	-

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