Membrane Solutions

VALIDATION GUIDE

BY MS VALIDATION SERVICE CENTER

NuggetMem™ TFF CASSETTE



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1 Overview

This document provides validation support information for the NuggetMem™ series of tangential flow filtration (TFF) cassettes, including data from tests such as biosafety, extractables, chemical compatibility, physical properties, and performance, as well as information on use conditions (e.g., temperature limits, chemical limits, cleaning, flushing, integrity testing, and operating procedures).

The methods and information contained in this guide are designed to provide users with relevant guidance for validating NuggetMem™ series TFF cassettes under actual use conditions.

1.1 Filtration Process Validation

Ultrafiltration cassettes are a typical TFF membrane separation technology solution widely used in pharmaceutical processes, especially in downstream purification of biopharmaceuticals, including solution concentration, diafiltration, desalting, and separation/purification of components based on molecular size. Process validation using TFF cassettes is an indispensable key step to ensure product safety and efficacy, and is an important component of cGMP regulations.

Since TFF ultrafiltration cassettes need to be integrated into complex systems, the three-stage system validation should be executed in the following order: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ).

1.2 Installation Qualification (IQ)

Prior to IQ, it is necessary to confirm that all required documents for the cassette have been received (including operating instructions and Certificate of Analysis/Data). During IQ execution, the cassette model must first be confirmed and installation must be performed according to the instructions; detailed installation steps such as torque settings should be included. Additionally, this step requires confirming that all required cassette documents (operating instructions and qualification certificates) have been received.

1.3 Operational Qualification (OQ)

After installing the cassette, validation personnel test and document the cassette's operating range, including limit conditions. OQ does not necessarily have to be performed within the customer's production area. Validation personnel conduct relevant studies and challenges using water or other simulated fluids. As part of OQ, operating procedures also need to be validated and documented.

1.4 Performance Qualification (PQ)

During PQ, the filtration process parameters for producing the final product using the cassette are tested under actual operating conditions, including installation, sanitization, concentration, diafiltration, product recovery, and cleaning. Elements involved in the PQ stage include chemical compatibility and retention characteristics. Since the purpose of validating a process is to ensure the process achieves its intended objectives,

PQ provides the most in-depth process validation data. This data comes from the process itself under intended operating conditions. PQ does not necessarily need to provide data for system operational design limits (alarm conditions), as the process conditions may never reach these limits.

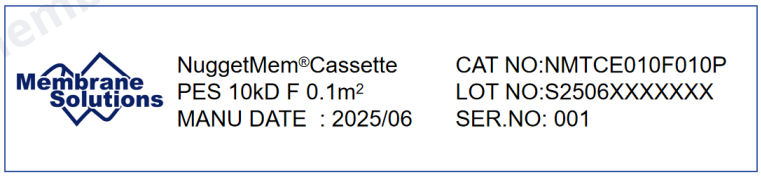
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2 Product Specifications

2.1 Packaging Information

Cassettes are individually packaged in aluminum foil. The side of the cassette is printed with the following information:

- Company Name
- Cassette Product Model
- Molecular Weight Cut-Off (MWCO)
- Effective Membrane Area
- Cassette Lot Number
- Cassette Serial Number
- Manufacturing Date



Delivered cassettes also include 2 silicone gaskets and a Quality Assurance Certificate. User Manual and MSDS documents are available upon request.

2.2 Product Model

The product model provides specific information about the cassette. Refer to Table 1.

Table 1: Product Model Cross-Reference

NuggetMem® TFF UF Cassette Ordering Information					
Series Name	Membrane Material	MWCO	Flow Channel Type	Effective Membrane Area	Application Grade
NMTC	E	100	F	010	P
NMTC = NuggetMem® TFF Cassette	E = PES	001 = 1kDa 002 = 2kDa 003 = 3kDa 005 = 5kDa 008 = 8kDa 010 = 10kDa 020 = 20kDa 030 = 30kDa 050 = 50kDa 100 =100kDa 300 =300kDa	F = Fine C = Coarse V = Suspended	001 = 0.01m² 002 = 0.02m² 010 = 0.10m² 050 = 0.50m² 250 = 2.50m²	P = BioPharm Process F = Food and Beverage W = Water

NuggetMem® TFF MF Cassette Ordering Information					
Series Name	Membrane Material	Removal Rating	Flow Channel Type	Effective Membrane Area	Application Grade
NMTC	E	011	V	010	P
NMTC = NuggetMem® TFF Cassette	E = PES V = PVDF	011 = 0.1μm 022 = 0.22μm 045 = 0.45μm	V = Suspended	002 = 0.02m² 010 = 0.10m² 050 = 0.50m² 250 = 2.50m²	P = BioPharm Process F = Food and Beverage W = Water

2.3 Lot Number - Serial Number

The following information for each cassette can be traced through its unique lot and serial number:

- Manufacturing Date
- Production Material Information
- Diffusion Flow / Integrity Test Data
- Pressure Drop
- Water Flux Rate

2.4 Materials of Construction

Refer to Table 2 below.

Table 2: Cassette Material Composition

Material Composition	
Filter Membrane	PES,PVDF
Sieve Mesh	PP
Encapsulating Material	Silicone
Gasket	Silicone

2.5 Dimensions and Applications

According to size, Membrane Solutions offers 5 size specifications of ultrafiltration cassettes. For the same flow channel type, different sizes share the same channel length and height, facilitating process linear scale-up, suitable for laboratory, pilot, and production stages. Refer to Table 3 for specific dimensions.

Table 3: Cassette Dimensions and Applications

Membrane Area & Dimensions (m^2)	Length*Width* Height (mm)	Application
0.01	210*55.5*1.7	R&D, Laboratory
0.02	210*55.5*3.4	R&D, Laboratory
0.1	210*55.5*15	R&D, Laboratory, Pilot Scale
0.5	213*178*16	Pilot Scale, Production Scale
2.5	213*178*75	Pilot Scale, Production Scale

2.6 Operating Pressure and Temperature

Cassette operation has limit requirements for pH, pressure, and temperature. Refer to Table 4 for specific requirements.

Table 4: Cassette Operating pH, Pressure, and Temperature Ranges

pH Range	Feed Pressure	Operating Temperature
1.0~14.0	⩽5.5bar	4.0°C~50.0°C

3 Validation Procedures

3.1 Low, Ambient, and High-Temperature High-Pressure Operation Validation.

Ultrafiltration cassettes are operated in water under high pressure (feed side) for 5-hour cycles at low, ambient, and high temperatures. After each cycle, diffusion flow/integrity, pressure drop, and normalized water flux rate are tested. This cycle is repeated 4 times for a cumulative total of 20 hours.

3.1.1 High Pressure, Low Temperature Validation

3.1.1.1 Validation Conditions: Feed pressure at 5.5 bar, water temperature 2-8°C, test after 5 hours of operation, repeat cycle 4 times

3.1.1.2 Procedure

(1) Install the cassette in the holder, test and record the initial diffusion flow/integrity, pressure drop, and normalized water flux.

(2) Adjust pump speed, retentate valve, and permeate valve to achieve a feed pressure of 5.5 bar and a retentate flow rate of 5-10 L/min/m^2.

(3) Use a chiller to lower the temperature to between 2-8°C. Start the timer when the water temperature reaches this range.

(4) After 5 hours of recirculation, drain the system and remove the cassette from the holder.

(5) Test and record the cassette's diffusion flow/integrity, pressure drop, and normalized water flux at ambient temperature.

(6) Repeat the above steps 4 times.

3.1.1.3 Validation results are shown in Table 5

Table 5: Test results of the cassette under high pressure and low temperature conditions

Cassette Product Model	Lot Number	Cycle (Number)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m^2/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m^2
NMTCE010F050P	240408001	0	20	123	0.7
		4	23	120	0.7
NMTCE020F050P	240426002	0	10	120	0.8
		4	12	119	0.8

Conclusion: Under low-temperature, high-pressure conditions, after 4 cycles totaling 20 hours, the cassette's diffusion flow/integrity, pressure drop, and normalized water flux are all within the normal range with no significant changes.

3.1.2 High Pressure, Ambient Temperature Validation

3.1.2.1 Validation Conditions: Feed pressure at 5.5 bar, ambient water temperature 20-25°C, test after 5 hours of operation, repeat cycle 4 times

3.1.2.2 Procedure

(1) Install the cassette in the holder, test and record the initial diffusion flow/integrity, pressure drop, and normalized water flux.

(2) Adjust pump speed, retentate valve, and permeate valve to achieve a feed pressure of 5.5 bar and a retentate flow rate of 5-10 L/min/m².

(3) Control the water temperature within 20-25°C and start the timer.

(4) After 5 hours of recirculation, drain the system and remove the cassette from the holder.

(5) Test and record the cassette's diffusion flow/integrity, pressure drop, and normalized water flux at ambient temperature.

(6) Repeat the above steps 4 times.

3.1.2.3 Validation results are shown in Table 6

Table 6: Test results of the cassette under high pressure and ambient temperature conditions

Cassette Product Model	Lot Number	Cycle (Number)	IDiffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240408001	0	20	123	0.7
		4	21	124	0.7
NMTCE020F050P	240426002	0	10	120	0.8
		4	10	113	0.8

Conclusion: Under ambient temperature, high-pressure conditions, after 4 cycles totaling 20 hours, the cassette's diffusion flow/integrity, pressure drop, and normalized water flux are all within the normal range with no significant changes.

3.1.3 High Pressure, High Temperature Validation

3.1.3.1 Validation Conditions: Feed pressure at 4 bar, water temperature 50-55°C, test after 5 hours of operation, repeat cycle 4 times

3.1.3.2 Procedure

(1) Install the cassette in the holder, test and record the initial diffusion flow/integrity, pressure drop, and normalized water flux.

(2) Adjust pump speed, retentate valve, and permeate valve to achieve a feed pressure of 4 bar and a retentate flow rate of 5-10 L/min/m².

(3) Use a thermostatic bath to raise the temperature to between 50-55°C. Start the timer when the water temperature reaches this range.

(4) After 5 hours of recirculation, drain the system and remove the cassette from the holder.

(5) Test and record the cassette's diffusion flow/integrity, pressure drop, and normalized water flux at ambient temperature.

(6) Repeat the above steps 4 times.

3.1.3.3 Validation results are shown in Table 7

Table 7: Test results of the cassette under high pressure and high temperature conditions

Cassette Product Model	Lot Number	Cycle (Number)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240408001	0	20	123	0.7
		4	24	118	0.7
NMTCE020F050P	240426002	0	10	120	0.8
		4	10	114	0.8

3.2 Chemical Compatibility

Cassettes may come into contact with many chemicals during sanitization and cleaning. To assess the impact of common chemicals on cassette performance, changes in performance are tested after the cassette is exposed to chemical substances, simulating typical worst-case scenarios.

3.2.1 Validation Protocol

Cassettes are soaked under the following 4 conditions, then flushed, and their diffusion flow/integrity, pressure drop, and normalized water flux are tested.

(1) 0.5M Sodium Hydroxide solution, soaked at 45°C for 28 days.

(2) 0.5M Sodium Hydroxide + 400ppm Sodium Hypochlorite solution, soaked at 45°C for 7 days.

(3) 0.1M Nitric Acid solution, soaked at 45°C for 28 days.

(4) 40% Ethanol solution, soaked at ambient temperature for 28 days.

3.2.2 Procedure

(1) Install the cassette in the holder, circulate the test solution within the cassette for 15 minutes.

(2) Then remove the cassette from the holder and soak it in a package containing the test solution. Seal the package and store it under the corresponding temperature conditions.

(3) After the specified duration, remove the cassette from the package, install it in the holder, and flush the cassette with purified water for 30 minutes.

(4) Test diffusion flow/integrity, pressure drop, and normalized water flux.

3.2.3 Validation Results & Conclusion

3.2.3.1 Compatibility study test results for 0.5 M Sodium Hydroxide solution at 45°C are shown in Table 8

Table 8: Test results of the cassette after immersion in 0.5 M Sodium Hydroxide solution

Cassette Product Model	Lot Number	Test Time (day)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240513001	0	14	103	0.6
NMTCE010F050P	240513001	7	13	115	0.8
NMTCE010F050P	240513001	14	18	101	0.8
NMTCE010F050P	240513001	21	20	105	0.8
NMTCE010F050P	240513001	28	19	110	0.8

Conclusion: After soaking in 0.5 M Sodium Hydroxide solution at 45°C for 28 days, the water flux, diffusion flow, and pressure drop values for all cassettes are within the normal range.

3.2.3.2 Compatibility study test results for 0.5 M Sodium Hydroxide + 400 ppm Sodium Hypochlorite solution at 45°C are shown in Table 9

Table 9: Test results of the cassette after immersion in 0.5 M Sodium Hydroxide + 400 ppm Sodium Hypochlorite solution

Cassette Product Model	Lot Number	Test Time (day)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240513002	0	15	108	0.7
NMTCE010F050P	240513002	7	31	112	0.7

Conclusion: After soaking in0.5M Sodium Hydroxide + 400ppm Sodium Hypochlorite solution at 45°C for 7 days, the water flux, diffusion flow, and pressure drop values for all cassettes are within the normal range.

3.2.3.3 Compatibility study test results for 0.1 M Nitric Acid solution at 45°C are shown in Table 10

Table 10: Test results of the cassette after immersion in 0.1 M Nitric Acid solution

Cassette Product Model	Lot Number	Test Time (day)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240516002	0	16	112	0.7
NMTCE010F050P	240516002	7	14	120	0.7
NMTCE010F050P	240516002	14	18	110	0.7
NMTCE010F050P	240516002	21	20	115	0.8
NMTCE010F050P	240516002	28	20	110	0.8

Conclusion: After soaking in 0.1M Nitric Acid solution at 45°C for 28 days, the water flux, diffusion flow, and pressure drop values for all cassettes are within the normal range.

3.2.3.4 Compatibility study test results for 40% Ethanol solution at 25°C are shown in Table 11

Table 11: Test results of the cassette after immersion in 40% Ethanol solution

Cassette Product Model	Lot Number	Test Time (day)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m²/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m²
NMTCE010F050P	240509003	0	20	108	0.8
NMTCE010F050P	240509003	7	12	112	1
NMTCE010F050P	240509003	14	20	117	1
NMTCE010F050P	240509003	21	20	121	0.9
NMTCE010F050P	240509003	28	19	114	0.8

Conclusion: After soaking in 40% Ethanol solution at 25°C for 28 days, the water flux, diffusion flow, and pressure drop values for all cassettes are within the normal range.

3.2.4 Chemical Compatibility

The chemical compatibility table for the cassette is shown in Table 12

Table 12: Chemical compatibility table for the cassette

Chemical Reagent	Compatible
0.5M SODIUM HYDROXIDE	PASS
0.5M SODIUM HYDROXIDE+400PPM SODIUM HYPOCHLORITE	PASS
40% ETHANOL	PASS
0.1M NITRIC ACID	PASS
ACETIC ACID (5%)	PASS
CITRIC ACID (1%)	PASS
FORMALDEHYDE (1%)	PASS
GLYCERIN (50%)	PASS
GUANIDINE (0.1M)	PASS
HYDROGEN PEROXIDE (1%)	PASS
PHOSPHORIC ACID (0.1M)	PASS
SODIUM DODECYL SULFATE (0.01M)	PASS
UREA (25%)	PASS
ACETONITRILE (50%)	PASS

3.3 Cleaner Compatibility Study

During cleaning, cassettes are often exposed to extreme conditions such as extreme pH (2 to 14), high temperature, and high pressure to remove residual product and foulants. This experiment is designed to verify the impact of these extreme conditions on ultrafiltration cassettes.

3.3.1 Validation Protocol

Selected cassettes are validated using 2 solutions and conditions. The cassettes are tested for diffusion flow, pressure drop, and normalized water flux in water.

(1) 0.1M Phosphoric Acid solution, recirculated for 18 hours at 45°C and a feed pressure of 4 bar.

(2) 0.5M Sodium Hydroxide solution, recirculated for 18 hours at 45°C and a feed pressure of 4 bar.

3.3.2 0.1M Phosphoric Acid Solution Protocol

3.3.2.1 0.1M Phosphoric Acid Procedure

(1) Determine the initial diffusion flow, pressure drop, and normalized water flux of the cassette.

(2) Prepare 4L of 0.1M Phosphoric Acid solution and heat it to 45±5°C.

(3) Open the feed and retentate outlet valves.

(4) Adjust pump speed and retentate valve to achieve a feed pressure of 4 bar and a retentate flow rate of 5-10 L/min/m^2.

(5) Use a thermostatic bath to raise the temperature to 45±5°C. Start the timer when the water temperature stabilizes at 45°C.

(6) After 3 hours of recirculation, drain the system and test the cassette's diffusion flow, pressure drop, and normalized water flux at ambient temperature.

(7) Repeat the above steps 6 times, for a total cumulative recirculation time of 18 hours.

3.3.2.2 Phosphoric acid test results & conclusions are shown in Table 13

Table 13: Phosphoric acid test results for the cassette

Cassette Product Model	Lot Number	Recirculation Time (H)	Diffusion Flow / Integrity (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m^2/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m^2
NMTCE010F050P	240408001	0	23	107	0.7
		18	29	108	0.6
NMTCE020F050P	240426002	0	13	109	1
		18	8	105	1

Conclusion: After a cumulative 18 hours of recirculation in 0.1M Phosphoric Acid solution at 45°C and a feed pressure of 4 bar, the cassette's diffusion flow, pressure drop, and normalized water flux are all within the normal range.

3.3.3 0.5M Sodium Hydroxide Solution Protocol

3.3.3.1 0.5M Sodium Hydroxide Procedure

(1) Determine the initial diffusion flow, pressure drop, and normalized water flux of the cassette.

(2) Prepare 4L of 0.5M Sodium Hydroxide solution and heat it to 45±5°C.

(3) Open the feed and retentate outlet valves.

(4) Adjust pump speed and retentate valve to achieve a feed pressure of 4 bar and a retentate flow rate of 5-10 L/min/m^2.

(5) Use a thermostatic bath to raise the temperature to 45±5°C. Start the timer when the water temperature stabilizes at 45°C.

(6) After 3 hours of recirculation, drain the system and test the cassette's diffusion flow, pressure drop, and normalized water flux at ambient temperature.

(7) Repeat the above steps 6 times, for a total cumulative recirculation time of 18 hours.

3.3.3.2 0.5 M Sodium Hydroxide test results & conclusions are shown in Table 14

Table 14: 0.5 M Sodium Hydroxide test results for the cassette

Cassette Product Model	Lot Number	Recirculation Time (H)	Diffusion Flow (ml/min) @1 bar	Normalized Water Permeability NWP (L/h/m^2/bar)	Pressure Drop (bar) @ Tangential Flow Rate 6 L/min/m^2
NMTCE010F050P	240408003	0	25	116	0.8
		18	23	115	0.8
NMTCE020F050P	240426002	0	13	109	1
		18	10	115	1

Conclusion: After a cumulative 18 hours of recirculation in 0.1M Phosphoric Acid solution at 45°C and a feed pressure of 4 bar, the cassette's diffusion flow, pressure drop, and normalized water flux are all within the normal range.

3.4 Hold-Up Volume / Dead Volume

3.4.1 Procedure

(1) Internal volume is determined by measuring the weight change before and after filling with water, assuming 1 gram of water equals 1 mL.

(2) Five 0.5 m^2 cassettes, one 2.5 m^2 cassette, and four 0.1 m^2 cassettes. Record the dry weight of the cassettes.

(3) The 0.5 and 2.5 m^2 cassettes are tested individually, while the 0.1 m^2 cassettes are tested as a set of 4 cassettes clamped in the holder. The volume of the respective holder is determined (subtracted to determine the cassette-only volume).

(4) Assemble the cassette(s) in the holder and measure the total weight.

(5) Apply 4-5 PSI air to the permeate side to minimize water flow through the membrane. Then fill the feed side of the device with water and weigh again. The difference between the initial and final weights approximates the weight of water in the feed channels of the cassette(s).

(6) Close the feed valve, fill the permeate channel with water, and re-weigh. Similarly, the weight difference approximates the weight of water in the permeate channel(s).

(7) The 0.5 and 2.5 m^2 cassettes are tested individually, once each. The 0.1 m^2 cassettes are tested as a set of 4, repeated 3 times.temperature, and high pressure to remove residual product and foulants. This experiment is designed to verify the impact of these extreme conditions on ultrafiltration cassettes.

3.4.2 Test Results

3.4.2.1 Cassette hold-up volume/dead volume test results are shown in Table 15

Table 15: Cassette hold-up volume/dead volume test results

Cassette Product Model	Lot Number	Flow Channel	Cassette Area (m^2)	Cassette Feed Channel Volume (ml)	Cassette Permeate Channel Volume (ml)	Cassette Total Volume (ml)
NMCTE010F001P	240408003	F	0.01	2.1	1.3	3.4
NMCTE010F002P	240426002	F	0.02	4.0	3.2	7.2
NMCTE010F010P	S25010200001	F	0.1	16	15	31
NMCTE010F050P	S25012000001	F	0.5	40	58	98
NMCTE010F250P	S25011000003	F	2.5	143	233	376

3.4.2.2 Cassette holder dead volume is shown in Table 16

Table 16: Cassette holder dead volume

Holder Manufacturer	Holder Area (m^2)	Holder Feed Channel Volume (ml)	Holder Permeate Channel Volume (ml)	Holder Total Volume (ml)
NMCTE010F001P	F	0.01	2.1	1.3
NMCTE010F002P	F	0.02	4.0	3.2
NMCTE010F010P	F	0.1	16	15

3.5 Cassette Flushing Procedure Validation

To validate the effectiveness of the flushing steps for removing preservatives from new cassettes, the TOC, pH, and conductivity of the flush water after cleaning are tested to ensure they are reduced to acceptable standards.

3.5.1 Procedure

(1) Perform pre-flushing of the new cassette according to the Cassette User Manual.

(2) Prepare 4L of 0.5M Sodium Hydroxide solution and heat it to 40-45°C.

(3) Open the feed and retentate outlet valves.

(4) Adjust pump speed and retentate valve to achieve a inlet liquid pressure of 1 bar and a retentate flow rate of 5-10 L/min/m^2. Sanitize for 45 minutes.

(5) Flush the system with purified water and drain the flushed sodium hydroxide.

(6) Flush with purified water for 60 minutes.

(7) Collect flush water from the retentate and permeate outlets at 10, 20, 30, 40, 50, and 60 minutes.

(8) Test TOC, pH, and conductivity at the different time points.

3.5.2 Test Results

3.5.2.1 Validation of cassette flushing procedure, flush water TOC test results are shown in Figure 1

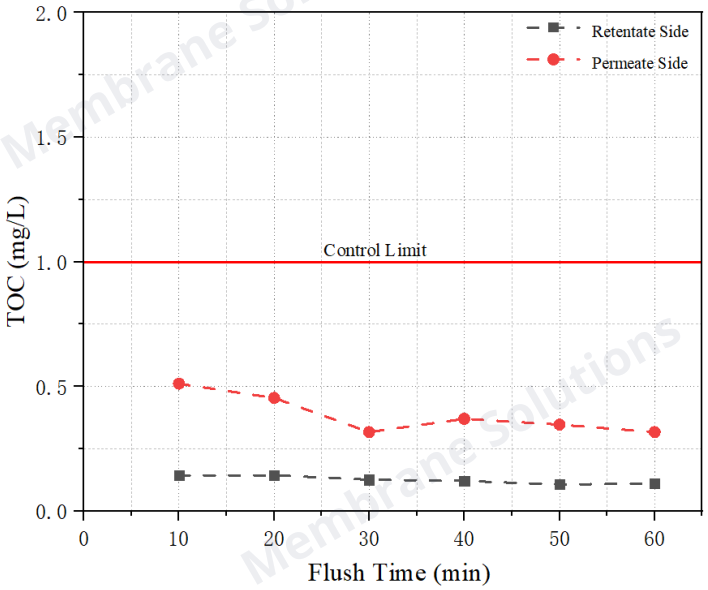


Figure 1: Cassette Flush Test – TOC

Conclusion: After 10 minutes of purified water flushing, the TOC content of the flush water from both the retentate and permeate ends is below 1 ppm, meeting quality requirements. After 30 minutes, the TOC content of the flush water from both ends approaches stability.

3.5.2.2 Validation of cassette flushing procedure, conductivity results are shown in Figure 2

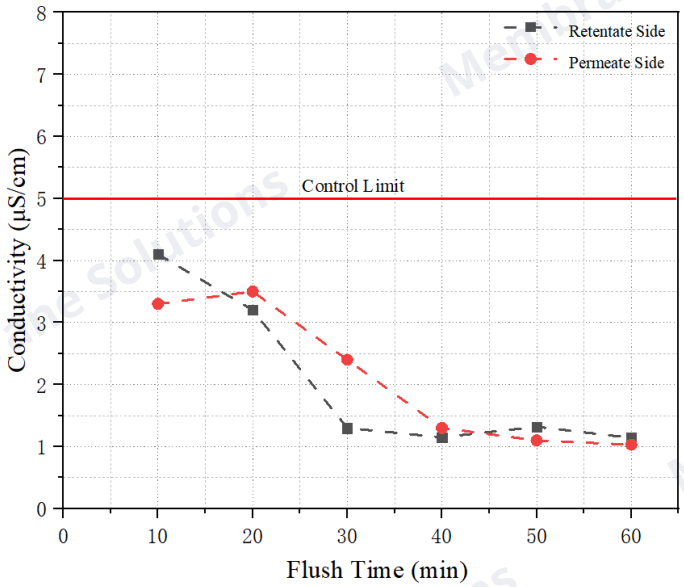


Figure 2: Cassette Flush Test – Conductivity

Conclusion: After 10 minutes of purified water flushing, the TOC content of the flush water from both the retentate and permeate ends is below 1 ppm, meeting quality requirements. After 30 minutes, the TOC content of the flush water from both ends approaches stability.

3.5.2.3. Validation of cassette flushing procedure, pH results are shown in Figure 3

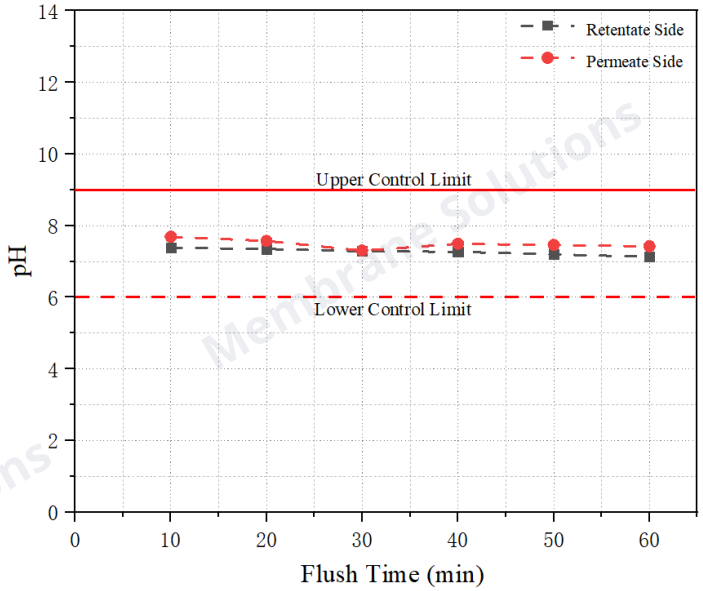


Figure 3: Cassette Flush Test – pH

Conclusion: After performing the cleaning procedure, the final pH of the flush water from both the retentate and permeate ends is within the range of 6-9, meeting specifications.

3.6 Bacterial Endotoxins Validation

Endotoxins are components of the cell wall of Gram-negative bacteria called lipopolysaccharides. Lipopolysaccharides are toxic to the host. The bacterial endotoxin test includes two methods: the gel-clot method and the photometric method, using Limulus Amebocyte Lysate (LAL) reagent to qualitatively or quantitatively detect endotoxins. This validation is to assess the initial endotoxin content and the effectiveness of the recommended flushing steps.

3.6.1 Procedure

(1) Pre-flush the new cassette according to the Cassette User Manual to ensure the system is endotoxin-free.
(2) Prepare 4L of 0.5M Sodium Hydroxide solution and heat it to 40-45°C.
(3) Open the feed and retentate outlet valves.
(4) Adjust pump speed and retentate valve to achieve a TMP of 1 bar and a retentate flow rate of 5-10 L/min/m^2. Sanitize for 45 minutes.
(5) Flush the system with endotoxin-free water and drain the flushed sodium hydroxide.
(6) Clean with endotoxin-free water for 30 minutes, then drain the system.
(7) Collect flush water from the retentate and permeate ends and send for endotoxin testing.

3.6.2 Test Results

Bacterial endotoxin validation test results are shown in Table 17.

Table 17: Bacterial endotoxin validation test results for the cassette

Cassette Product Model	Lot Number	Endotoxin Result (EU/ml)	
		Retentate	Permeate
NMCTE010F050P	S25030700001/001	<0.25	<0.25
NMCTE010F050P	S25030700001/006	<0.25	<0.25
NMCTE010F050P	S25030700001/007	<0.25	<0.25

Conclusion: After flushing and sanitization, the bacterial endotoxin content is <0.25 EU/mL. The bacterial endotoxin level complies with the water for injection (WFI) quality standard specified in the *Chinese Pharmacopoeia.*

3.7 Bacterial Endotoxins Challenge Validation

The inherent endotoxin content of new cassettes is limited. The purpose of this validation is to verify the effectiveness of the post-use cassette flushing steps through an endotoxin challenge.

3.7.1 Procedure

(1) Recirculate a solution containing 500-1000 EU/mL endotoxins for 1 hour at a feed flow rate of 5 L/min/m^2 and a TMP of 1 bar.

(2) Flush with purified water for 30 minutes and completely drain the system.

(3) Prepare 4L of 0.5M Sodium Hydroxide solution and heat it to 40-45°C.

(4) Open the feed and retentate outlet valves.

(5) Adjust pump speed and retentate valve to achieve a TMP of 1 bar and a retentate flow rate of 5-10 L/min/m^2. Sanitize for 45 minutes.

(6) Flush the system with endotoxin-free water and drain the flushed sodium hydroxide.

(7) Clean with endotoxin-free water for 30 minutes, then drain the system.

(8) Collect flush water from the retentate and permeate ends and send for endotoxin testing.

3.7.2 Test Results

Endotoxin challenge test results are shown in Table 18.

Table 18: Bacterial endotoxin validation test results for the cassette

Cassette Product Model	Lot Number	Endotoxin Result (EU/ml)	
		Retentate	Permeate
NMCTE010F050P	S25030700001/001	<0.25	<0.25
NMCTE010F050P	S25030700001/006	<0.25	<0.25
NMCTE010F050P	S25030700001/007	<0.25	<0.25

Conclusion: After flushing and sanitization, the bacterial endotoxin content is <0.25 EU/mL. The bacterial endotoxin level complies with the water for injection (WFI) quality standard specified in the *Chinese Pharmacopoeia.*

3.8 Cassette Particulate Shedding Validation

The cassette itself does not shed any fibers. The purpose of this validation is to test the flush water after cleaning cassettes for particulate shedding, ensuring no particles or fibers are detected.

3.8.1 Procedure

(1) Pre-flush the new cassette according to the Cassette User Manual.

(2) After pre-flushing, drain the system.

(3) Open the feed and retentate outlet valves.

(4) Adjust pump speed and retentate valve to achieve a TMP of 1 bar and a retentate flow rate of 5-10 L/min/m^2. Flush with 20L of purified water.

(5) Collect the final flush water from the retentate and permeate ends every 10 minutes, for a total of 6 collections, and send for particle counting.

3.8.2 Test Results

Results are shown in Figure 4 and Figure 5

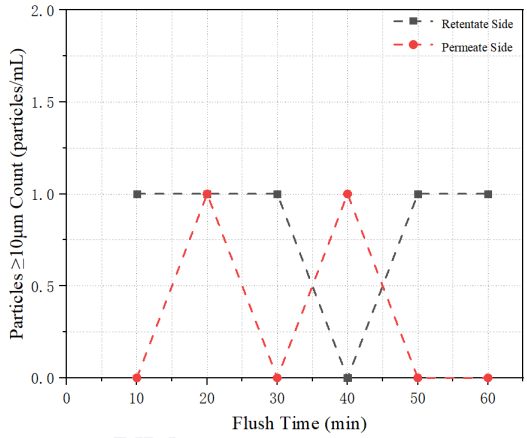


Figure 4: Cassette Flush Test – Particles ⩾10 μm Count

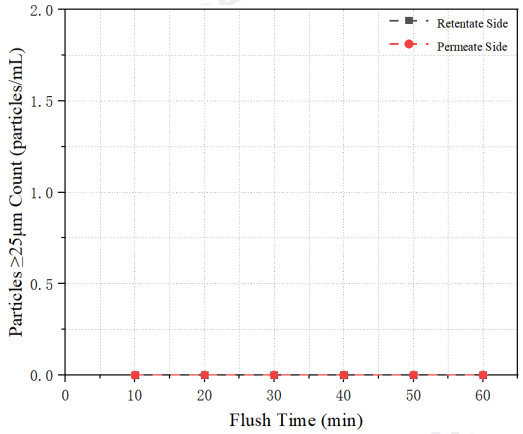


Figure 5: Cassette Flush Test – Particles ⩾25 μm Count

Conclusion: After cassette flushing, starting from the 10-minute mark, the flush water particles are ⩽3 particles/mL (⩾25μm) and ⩽25 particles/mL (⩾10μm), complying with the Chinese Pharmacopoeia 2020 Edition, Part IV, General Chapter 0903 "Insoluble Particulate Matter Test" (equivalent to USP <788>).

3.9 Cassette Linear Scale-up Validation

Cassette linear scale-up testing is a validation method used to assess the performance consistency of filtration cassettes at different operational scales, mainly used in process scale-up in fields such as biopharmaceuticals and water treatment.

3.9.1 Procedure

(1) Prepare five ultrafiltration cassettes with molecular weight cut-off (MWCO) of 10 kDa and areas of 0.01 m^2, 0.02 m^2, 0.1 m^2, 0.5 m^2, and 2.5 m^2, respectively.

(2) Install and flush the cassettes according to the Cassette User Manual.

(3) Open the feed and retentate outlet valves.

(4) Adjust pump speed and retentate valve to achieve a TMP of 1 bar and a retentate flow rate of 5-10 L/min/m^2.

3.9.2 Test Results

Test results are shown in Table 19

Table 19: Test results for the cassette

Membrane Area	Cassette Product Model	Tangential Flow Rate @1 bar (L/min/m^2)	Normalized Water Permeability (NWP) @1 bar (L/m^2/h)
0.01 m^2	NMCTE010F001P	5.1	120
0.02 m^2	NMCTE010F002P	5.5	114
0.1 m^2	NMCTE010F010P	5.5	100
0.5 m^2	NMCTE010F050P	5.0	104
2.5 m^2	NMCTE010F250P	4.8	119

3.10 Cassette Extractables Validation

Cassette extractables validation is a critical study to assess chemical substances that may be released under process conditions, ensuring the safety of pharmaceuticals or biological products.

3.10.1 Procedure

(1) Place a clean evaporating dish in an oven at 105°C and dry for 0.5h. Transfer it to a desiccator and cool for 0.5h, then weigh. Repeat the drying, cooling, and weighing steps until the difference between two consecutive weighings does not exceed 0.3mg. Record the last weighing result as the mass of the empty evaporating dish.
(2) Disassemble the sample into the support layer and the membrane sheet. Flush each separately with purified water for 1h, then air dry.
(3) Cut the membrane sheet and support layer into suitable sizes. Place them separately into stoppered erlenmeyer flasks. Add extraction solution at a ratio of 6dm^2 : 1L. Soak at 22°C for 24h.
(4) Transfer 60mL of the extraction solution into the constant weight empty evaporating dish. Evaporate to near dryness on a water bath at 100°C. Place the dish in an oven at 105°C for 0.5h, then remove and place in a desiccator to cool for 0.5h, and weigh. Repeat the drying, cooling, and weighing steps until constant weight is achieved (the mass difference between two weighings does not exceed 0.3mg).
(5) Perform a blank test simultaneously.

3.10.2 Calculation of Results

Calculate the non-volatile residue using the following formula:

$$X = \frac{\left[\left(m_1 - m_0\right)_{Sample} - \left(m_1 - m_0\right)_{Blank}\right]}{V_0} \times \frac{100}{6}$$

Where —

x: Non-volatile residue content in the sample, in milligrams per square meter (mg/m^2).

m_1: Mass of the evaporating dish with solvent residue, in milligrams (mg).

m_0: Mass of the empty evaporating dish without solvent residue, in milligrams (mg).

V_0: Volume of extraction solution used for the test, in liters (L).

100/6: Coefficient, in liters per square meter (L/m^2).

3.10.3 Test Results

Results and conclusions are shown in Table 20

Table 20: Non-volatile residue data for the cassette

Sample ID	Extraction Solution	Extraction Conditions	Sample Material	Unit	Result
64861-01	Purified Water	22°C, Static, 24h	Membrane Sheet	mg/m^2	<LOD (300)
			Support Layer	mg/m^2	<LOD (300)
	50% Ethanol	22°C, Static, 24h	Membrane Sheet	mg/m^2	333.3
			Support Layer	mg/m^2	833.3

Note: LOD = Limit of Detection

3.11 USP<87> In Vitro Biosafety

This test is designed to determine the biological reactivity of mammalian cell cultures following contact with elastomeric, plastic, and other polymeric materials that have direct or indirect patient contact, or with specific extracts prepared from the test material.

3.11.1 Procedure

(1) The membrane sheet of the sample was flushed for 1h, then sterilized at 121°C for 15 minutes; the support layer was flushed for 1h, then sterilized at 121°C for 15 minutes. The negative and positive controls were sterilized at 121°C for 15 minutes.

(2) Test article extraction conditions are shown in Table 21.

Table 21: Test article extraction conditions

Test Group	Sample-to-Extractant Ratio	Centrifugation/ Filtration	Extractant	Extraction Conditions
Negative Control	$3cm^2/1mL$	No	MEM with 10% FBS	37°C, 120 rpm oscillation, 24h
Positive Control	$3cm^2/1mL$	No		
64861-01 (Membrane)	$6cm^2/1mL$	No		
64861 (Support Layer)	0.1g/1mL	No		

(3) Testing for USP <87> In Vitro Biological Reactivity.

L-929 cells were cultured in advance using MEM medium containing 10% fetal bovine serum and antibiotics (Penicillin 100 IU/mL, Streptomycin 100 g/mL) in an incubator at 37°C with 5% CO_2.

The prepared L-929 cells were digested with 0.25% trypsin, and the resulting cell suspension was centrifuged at 1000 rpm for 5 minutes. The supernatant was removed, fresh medium was added to resuspend the cells, and then a cell count was performed. The cell density was adjusted to 1×10^5 cells/mL. The cell suspension was seeded into a 6-well plate at 2 mL per well and incubated for 24h at 37°C in a 5% CO_2 incubator.
After a monolayer formed, the plates were removed, the supernatant was aspirated, and 2mL of test sample extract (100%), blank control (MEM with 10% FBS), positive control (100%), and negative control (100%) were added to the respective wells. Each group had 3 replicates. The plates were returned to the 37°C, 5% CO_2 incubator and cultured for an additional 48h. After 48h, cytomorphological observation was performed under an inverted microscope.
(4)Acceptance criteria are shown in Table 22

Table 22: Acceptance criteria

Grade	Toxicity Level	Morphological Description
0	Non-Toxic	Discrete granules in the cytoplasm; no cell lysis.
1	Slight	⩽20% of cells are rounded, loosely adherent, and show no intracytoplasmic granules; occasional cell lysis.
2	Mild	>20% to ⩽50% of cells are rounded and show no intracytoplasmic granules; no extensive cell lysis or clear areas between cells.
3	Moderate	>50% to <70% of the cell layer consists of rounded or lysed cells.
4	Severe	The cell layer is nearly or completely destroyed.

3.11.2. Results and Conclusion

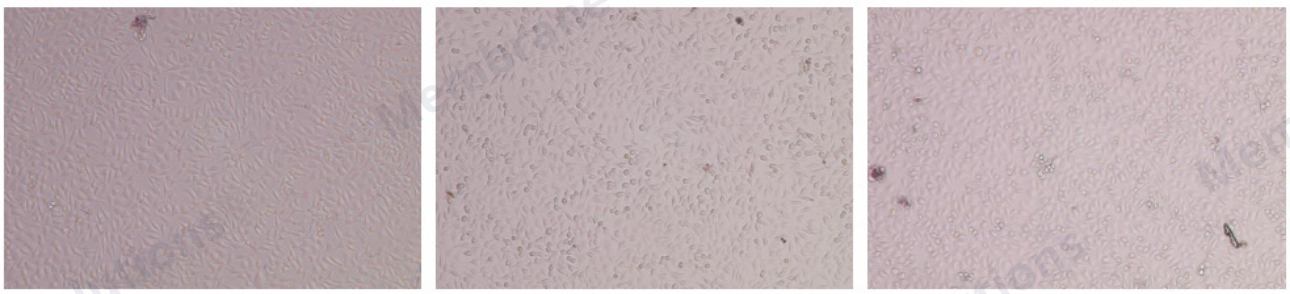
Cell morphology is shown in Table 23.

Table 23: Cell morphology

Sample Name/Model	Sample Lot No.	Sample ID	Cytomorphological Observation				Toxicity Level	Individual Conclusion
			Replicate 1	Replicate 2	Replicate 3	Reactivity Grade		
Cassette (Membrane Sheet)	NA	64861-01	1	1	1	1	Slight	Pass
Cassette (Support Layer)	NA	64861-01	1	1	1	1	Slight	Pass
0.1% ZDEC Polyurethane Film	A-243K	ositive Control	4	4	4	4	Severe	NA
High Density Polyethylene Film	C-241	egative Control	0	0	0	0	Non-Toxic	NA
MEM with10% FBS	20251223001	Blank Control	0	0	0	0	Non-Toxic	NA

Test results are shown in Figure 6

64861-01(membrane)



64861-01(support layer)

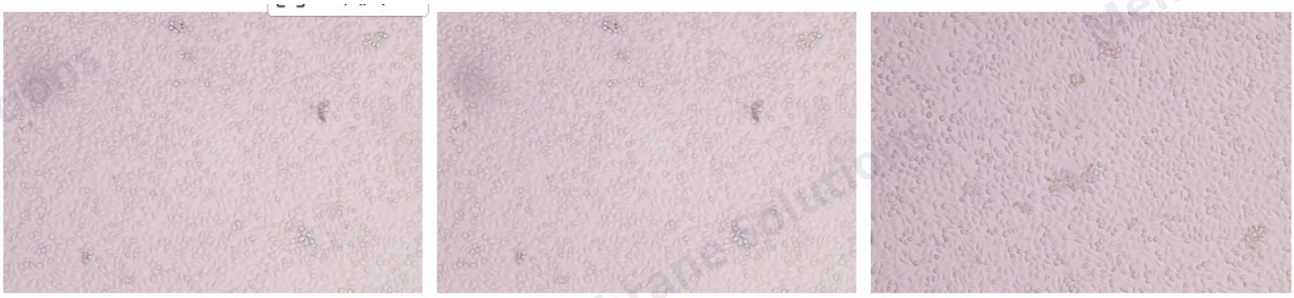


Figure 6: Sample test results

Conclusion: Under the conditions of this test, the sample extract did not show potential cytotoxicity to L-929 cells.

3.12 Shelf Life of New and Old Cassettes

For unopened new cassettes, the recommended shelf life is at least 2 years from the date of manufacture. To ensure performance, it is recommended to store cassettes sealed in their unopened original packaging at 4°C to 25°C, avoiding direct sunlight.

Under appropriate conditions and with proper use, cleaning, storage, and maintenance, the service life of cassettes typically exceeds 1 year. However, the shelf life or service life of cassettes that have been used or removed from their original packaging cannot be determined. The actual service life of a cassette will depend on numerous factors, such as the quality and complexity of the feed solution contacted, the composition of process fluids, process temperature, operating pressure, and cleaning protocols. Therefore, customers should validate the reuse life of cassettes in their own process. Membrane Solutions makes no warranty or guarantee regarding the performance of reused cassettes.

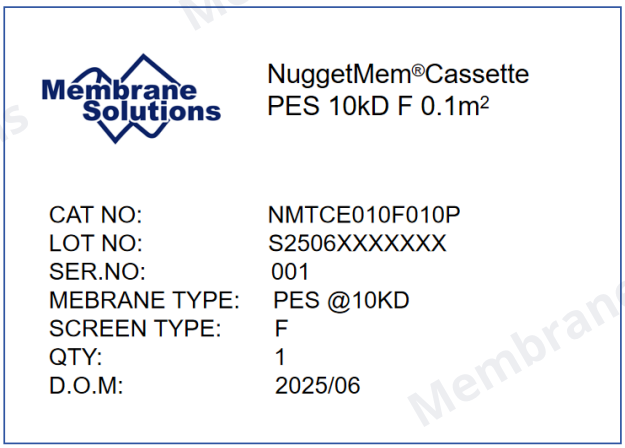
Please refer to the recommended storage conditions in the User Manual.

4 Quality Assurance

Membrane Solutions establishes a quality management system for product R&D, manufacturing, and sales in accordance with the ISO 9001:2015 standard. The quality policy, objectives, and requirements are determined. A process approach is adopted, and through monitoring and measurement of the system, its effective operation and continuous improvement are ensured to meet customer requirements and applicable legal and regulatory requirements.

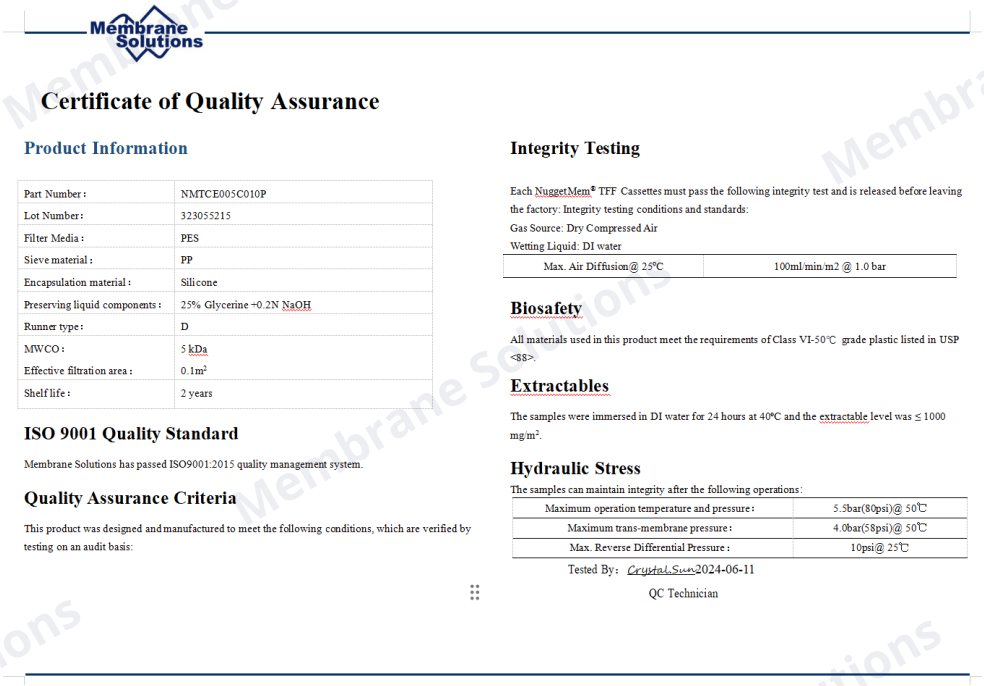
Each cassette undergoes 100% diffusion flow testing, visual inspection, and is stamped with a cassette serial number during production. It is then filled with preservation solution, sealed, and packaged in an aluminum foil bag.

Each cassette's packaging bag and carton have labels. The label content includes: model, serial number, and lot number.



Each cassette has a unique serial number and is accompanied by an individual Quality Assurance Certificate.

Example of Quality Assurance Certificate



Membrane Solutions

Certificate of Quality Assurance

Product Information

Part Number :	NMTCE005C010P
Lot Number :	323055215
Filter Media :	PES
Sieve material :	PP
Encapsulation material :	Silicone
Preserving liquid components :	25% Glycerine +0.2N NaOH
Runner type :	D
MWCO :	5 kDa
Effective filtration area :	0.1m²
Shelf life :	2 years

ISO 9001 Quality Standard

Membrane Solutions has passed ISO9001:2015 quality management system.

Quality Assurance Criteria

This product was designed and manufactured to meet the following conditions, which are verified by testing on an audit basis:

Integrity Testing

Each NuggetMem® TFF Cassettes must pass the following integrity test and is released before leaving the factory: Integrity testing conditions and standards:
Gas Source: Dry Compressed Air
Wetting Liquid: DI water

Max. Air Diffusion@ 25℃	100ml/min/m2 @ 1.0 bar

Biosafety

All materials used in this product meet the requirements of Class VI-50℃ grade plastic listed in USP <88>.

Extractables

The samples were immersed in DI water for 24 hours at 40℃ and the extractable level was ≤ 1000 mg/m².

Hydraulic Stress

The samples can maintain integrity after the following operations:

Maximum operation temperature and pressure :	5.5bar(80psi)@ 50℃
Maximum trans-membrane pressure :	4.0bar(58psi)@ 50℃
Max. Reverse Differential Pressure :	10psi@ 25℃

Tested By: Crystal.Sun 2024-06-11

QC Technician

Membrane Solutions

MS-TM-E-0035-EN A/1

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