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1.0 Purpose

This SOP describes the proper care and use of the PLANOVA® ultrafiltration device.

2.0 Scope

This SOP applies to Biopharmaceutical Development Program (BDP) personnel using the PLANOVA® ultrafiltration device in CGMP processes.

3.0 Authority and Responsibility

3.1 The Manager, Large Scale Purification, BDP has the authority to define this procedure

3.2 BDP personnel are responsible for the implementation of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Preface

4.1 This SOP describes the general procedure to filter a protein solution using a PLANOVA® filter, manufactured by Asahi Chemical Industry Co., Ltd. When preparing to carry out a protein solution filtration test or virus removal test using the PLANOVA® device, or when purifying protein solutions such as biological products, read and follow this procedure carefully.

4.2 PLANOVA® 15N, PLANOVA® 20N, and PLANOVA® 35N are process filters used for reducing the viral burden of biological products and other protein solutions. PLANOVA® 75N is a process filter used for removing particles from biological products and other protein solutions.

4.3 PLANOVA® consists of BMM (Bemberg Microporous Membrane) hollow fibers, a plastic housing, caps, and a sealant. The device is filled with purified water and pre-sterilized by the manufacturer. A wide range of surface areas (0.001 m^2 to 1.0 m^2) is available. PLANOVA® filters are designed by the manufacturer to be single-use devices.

4.4 Further information can be obtained by referencing the “Instruction for Use,” the “Filtration Procedure,” and the “Standard Operation Procedure of Integrity Test” manuals which are available from Asahi.

5.0 General Precautions

5.1 Visually inspect the device prior to use (see Diagram 1). Do not use if any of the following are observed.

5.1.1 Sterilization bag is damaged.

5.1.2 A nozzle cap is removed or broken.

5.1.3 Filling water is below ¾.

5.1.4 Filling water leaked into the sterilization bag.

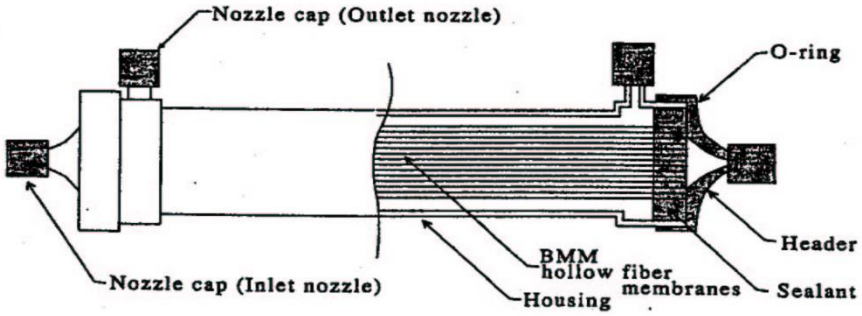
5.1.5 Filling water is frozen.

5.1.6 Other problems exist.

Diagram 1

Diagram of PLANOVA

PLANOVA is a hollow fiber membrane filter as shown below :



5.2 Check devices with surface areas of 1.0 m2, 0.3 m2, and 0.12 m2 for looseness of the filter header. If loose, retighten fully by hand from outside of the sterilization bag.

5.3 When handling, transporting, and storing the device, pay attention to the following to maintain the integrity of the filter.

5.3.1 Avoid strong shocks to the device.

5.3.2 Do not allow the filling water to freeze.

5.3.3 Do not allow the hollow fibers to dry out.

5.3.4 Store the device at 4°C - 30ºC.

5.3.5 Do not use beyond the sterilization expiration date.

5.4 Perform Leakage Test on the PLANOVA® filter as per section 8.2.

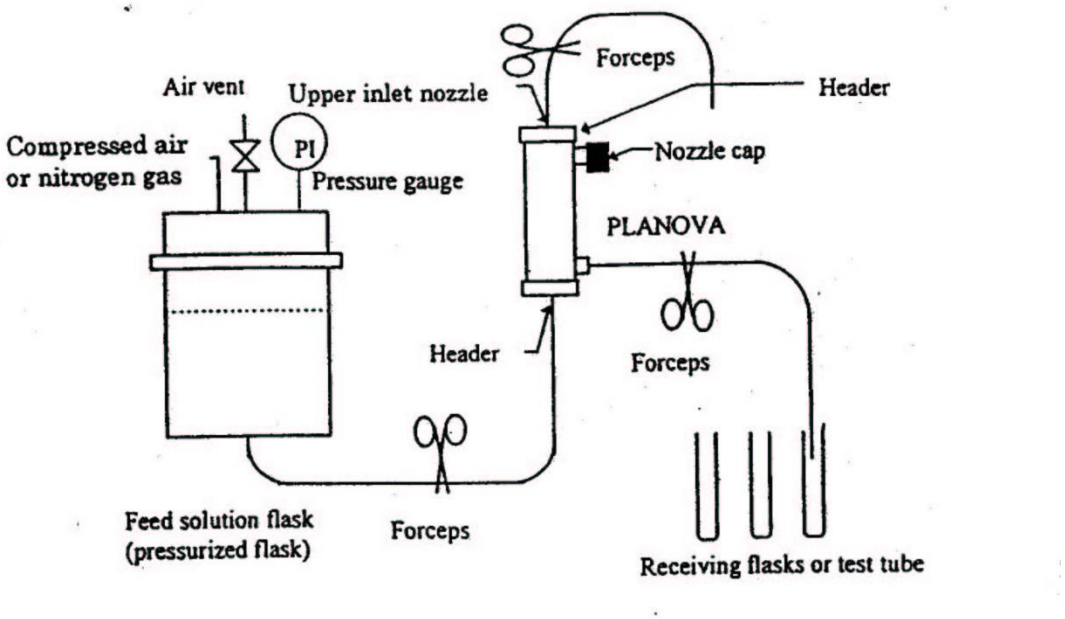
6.0 Filtration Procedure (Dead-end and Constant Pressure Filtration w/Pressure Tank)

6.1 Assembly

6.1.1 Assemble the filtration system according to Diagram 2.

NOTE: Prepare tubing capable of withstanding pressure exceeding 1.0 bar (14.2psi). Secure all tubing connections with cable ties. Before disconnecting any tubing, release the internal pressure on the system and confirm that it is zero.

Diagram 2



6.1.2 Drain the water from outside of the hollow fibers in the filter.

NOTE: It is advisable to connect the feed solution flask and filter after completely filling the tubing with buffer in order to remove the air from inside of the hollow fibers more easily.

6.2 Pre-Washing/Conditioning

NOTE: The objective of the pre-washing/conditioning step is to remove substances leached out from the hollow fibers at the time of autoclaving and to saturate (condition) the filter with the appropriate buffer.

6.2.1 Add the required volume of buffer to the feed solution flask, taking capacity of the tubing into account. The amount of buffer needed will be a minimum of 2X the value shown in Table 1.

6.2.2 Close the feed solution flask outlet line.

6.2.3 Set the pressure below 1.0 bar, preferably in the range of 0.2 - 0.8 bar (3.0 - 12.0 psi). Do not exceed 1.0 bar of filtration pressure.

6.2.4 Open the feed solution flask outlet line, discharge the buffer from the retentate of the filter and remove the air from inside of the hollow fibers. The volume of solution to be discharged from the retentate should be at least the value listed in Table 1.

Table 1

Unit	Surface Area (m^2)	Pre-washing/Conditioning Volume (mL)
PLANOVA® 15N	0.001	10
	0.01	30
	0.12	360
	0.3	900
	1.0	3,000
PLANOVA® 20N	0.001	10
	0.01	30
	0.12	360
	0.3	900
	1.0	3,000
PLANOVA® 35N	0.001	10
	0.01	30
	0.12	360
	0.3	900
	1.0	3,000
PLANOVA® 75N	0.001	10
	0.01	30
	0.3	900
	1.0	3,000

6.2.5 Open the bottom permeate then clamp the tubing on retentate with forceps. The volume of the filtrate collected should be at least the value reported in Table I.

6.2.6 After completion of the pre-washing/conditioning process, close the feed solution flask outlet line. Be careful not to allow air to enter the tubing or filter, since the filtration of protein solution is started only by replacing the buffer solution.

6.3 Filtration

6.3.1 Release the pressure of the feed solution flask and replace the buffer with protein solution.

6.3.2 Adjust to a **constant** pressure between 0.2 and 0.8 bar. **Do not** exceed 1.0 bar of filtration pressure.

6.3.3 Open the feed solution flask outlet line before starting the filtration.

6.3.4 Record data from the filtration process at periodic intervals as required, either in the BPR or Form 15102-01, (Attachment 1).

NOTE: The effective life of the filter is exhausted when the flow rate decreases to 1/10 of its starting value.

6.3.5 Immediately before the original protein solution to be filtered is exhausted, close the feed solution flask outlet line.

NOTE: Make sure that no air enters the tubing or filter during this process, since the post-washing follows immediately.

6.4 Post-washing

NOTE: The object of post-washing is to improve the recovery rate by pushing out the protein remaining in the filtration system (tubing and filter) by washing with buffer.

6.4.1 Release the pressure on the feed solution flask and add the desired quantity of buffer.

6.4.2 Pressurize the flask and open the outlet line to start the post-washing process.

6.4.3 It is recommended to take aliquots of the filtrate during the post-washing process and monitor by absorbance @ 280 nm if applicable.

6.4.4 Stop the post-washing process when the absorbance @ 280 nm is ≤ 0.05 OD.

7.0 Filtration Procedure (Dead-end and Constant Flow Filtration w/Peristaltic Pump)

7.1 Calibration of Pump Speed

7.1.1 Determine the relationship between the pump speed and the flow rate beforehand. See Table 1A for recommended initial filtration flow rates for each type of filter. Pre-washing flow rates can be 5X greater than the initial filtration flow rate. **Do not** exceed 1.0 bar of filtration pressure.

NOTE: If the pump speed is too low, maintenance of constant flow is difficult. It is recommended to select pump tubing having an inside diameter which ensures a pump speed of 10 rpm or higher.

Table 1A Selection of Pump Flow rate (mL/min)

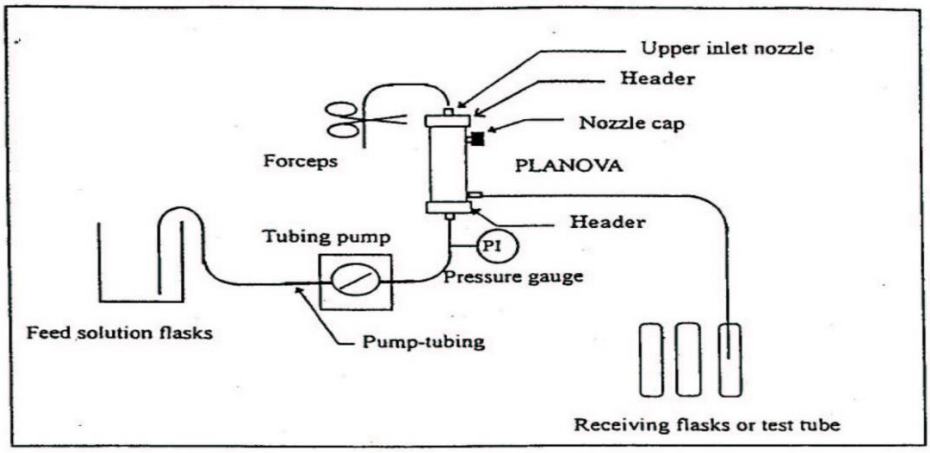
Surface Area (m^2)	PLANOVA® 15N	PLANOVA® 20N	PLANOVA® 35N	PLANOVA® 75N
0.001	0.1-5.0	0.2-10	2.5-25	10-150
0.01	1.5-35	3-100	25-200	100-1500
0.12	25-350	50-1200	300-2500	N/A
0.3	50-1000	100-3000	450-7500	3500-55,000
1.0	200-3,000	400-10,000	2,500-20,000	10,000-150,000

7.2 Assembly

7.2.1 Assemble the filtration system according to Diagram 3.

NOTE: Prepare tubing capable of withstanding pressure exceeding 1.0 bar (14.2 psi). Secure all tubing with cable ties. Before disconnecting any tubing, release the internal pressure on the system and confirm that it is zero.

Diagram 3



7.2.2 Drain the water from outside of the hollow fibers in the filter.

NOTE: It is advisable to connect the feed solution flask and filter after completely filling the tubing with buffer in order to remove the air from inside of the hollow fibers more easily.

7.3 Pump Flow Rate Adjustment (setting of filtration flow rate)

7.3.1 Put the required volume of buffer in the feed solution flask and operate the pump.

7.3.2 Discharge the buffer from the retentate and remove the air from the inside of the hollow fibers. The volume of buffer to be discharged from the upper inlet nozzle should be at least the value listed in Table 1.

7.3.3 Close the retentate with forceps and adjust the pump speed so that the pressure gauge on the filter reads below 1.0 bar, preferably in the range of 0.2 - 0.8 bar (3.0 - 12.0 psi). Do not exceed 1.0 bar of filtration pressure. See section 5.1 for recommended flow rates. Measure the buffer volume coming out of the bottom permeate of the filter with a graduated cylinder and stopwatch.

7.4 Pre-Washing/Conditioning

NOTE: The objective of the pre-washing/conditioning step is to remove substances leached out from the hollow fibers at the time of autoclaving and to saturate (condition) the filter with the appropriate buffer.

7.4.1 Add the required amount of buffer (at least 2X the value shown in Table I) into the feed solution flask and operate the pump. Remember to consider the capacity of the tubing.

7.4.2 Discharge the buffer from the retentate and remove the air from inside of the hollow fibers. The volume of solution to be discharged from the upper inlet nozzle should be at least the value reported in Table 1.

7.4.3 Close the tubing on the retentate with forceps or similar item and filter the buffer. The volume of filtrate collected should be at least the value reported in Table 1.

7.4.4 After completion of the pre-washing/conditioning process stop the pump. Be careful not to allow air to enter the tubing or filter, since the filtration of protein solution is started only by replacing the buffer solution.

7.5 Filtration

7.5.1 Replace the buffer in the feed solution flask with protein solution.

7.5.2 **Do not** exceed 1 bar of pressure on the filtration system.

7.5.3 Turn the pump on and begin filtration of the protein solution.

7.5.4 Record data from the filtration process at periodic intervals as required, either in the BPR or use Form 15102-01 (Attachment 1).

NOTE: **Do not** exceed 1.0 bar of filtration pressure on the system. If it is anticipated that the pressure may exceed 1.0 bar, decrease the flow rate or discontinue the process.

7.5.5 Immediately before the original protein solution to be filtered is exhausted, turn off the pump.

NOTE: Make sure that no air enters the tubing or filter during this process, since the post-washing follows immediately.

7.6 Post-washing

NOTE: The object of post-washing is to improve the recovery rate by pushing out the protein remaining in the filtration system (tubing and filter) by washing with buffer.

7.6.1 Add the required quantity of buffer to the feed solution flask.

7.6.2 Operate the pump to start the post-washing process.

7.6.3 It is recommended to take aliquots of the filtrate during the post-washing process and monitor by absorbance @ 280 nm.

7.6.4 Stop the post-washing process when the absorbance @ 280 nm is ≤ 0.05 OD.

8.0 Integrity Testing for PLANOVA®

8.1 General Description

8.1.1 Each individual PLANOVA® must be submitted for testing as indicated in Table 2.

Table 2

PLANOVA® type	Pre-use	Post-use
15N	Leakage Test	Leakage Test Gold Particle Test (GPT)
20N	Leakage Test	Leakage Test Liquid Forward Flow (LFR) Test or Gold Particle Test (GPT)
35N	Leakage Test	Leakage Test Gold Particle Test (GPT)
75N	Leakage Test	Leakage Test

8.1.2 The recommended order for these procedures is generally as follows:

- Pre-use Leakage Test.
- Post-use Leakage Test.
- Post-use Washing.
- Post-use Gold Particle Test (GPT) or Liquid Forward Flow Test (LFR).

NOTE: The Post-Use Gold Particle Test or the Liquid Forward Flow Test is not performed for the PLANOVA® 75N.

8.2 Leakage Test

8.2.1 The Leakage Test (pre- and post-use) is performed to confirm that the PLANOVA® filter is free from pinholes and other membrane damage, by observing change concerning the occurrence of continuous air bubble release through the filter membrane.

8.2.2 Assemble the PLANOVA® as shown in Diagram 4.

8.2.3 With both the Feed/Retentate lines open, slowly increase the pressure to 98 ± 4.9 kPA, 1.0 ± 0.05 bar, 14.2 ± 0.7 psi.

8.2.4 After achieving this pressurization, allow the buffer to discharge out of the top Permeate.

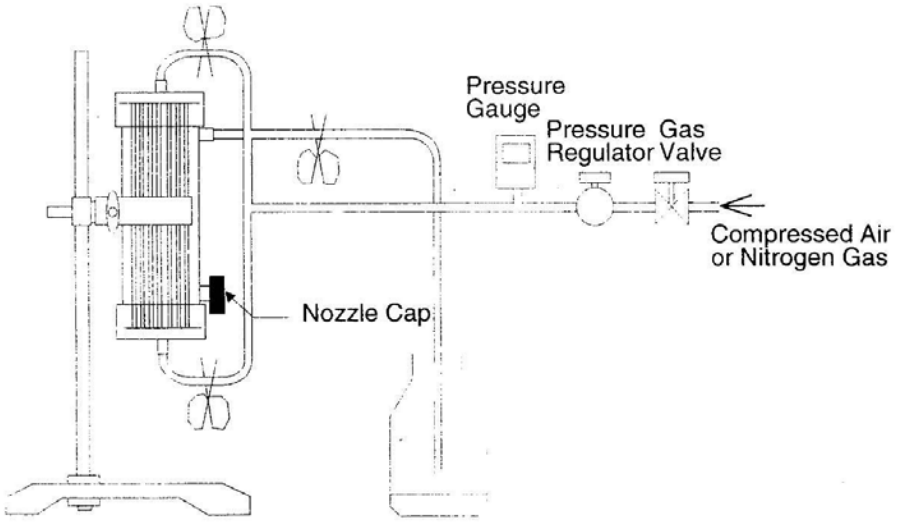
8.2.5 After the buffer has been allowed to discharge, turn the filter horizontally with both permeate ports facing upward.

8.2.6 Observe the filter for continuous bubbling, which would indicate a leak in the filter membrane. Brief bubbling may be observed, which is just the release of residual air in the membrane, but no continuous bubbling should be observed.

8.2.7 The PLANOVA® is assessed to have passed the Leakage Test if no bubbles are observed for a period of 20 seconds at 1.0 ± 0.05 bar (14.0 - 15.0 psi).

Diagram 4

Leakage Test System Configuration



8.3 Post-use Washing of PLANOVA®

8.3.1 The PLANOVA 15N, PLANOVA® 20N, and the PLANOVA® 35N must be washed before it is submitted to the Gold Particle Test (GPT) or the Liquid Forward Flow Test (LFR). The washing procedure removes residual proteins from the hollow fibers.

NOTE: Washing of the PLANOVA® 75N is generally unnecessary.

8.3.2 The Washing procedure consists of the following steps:

- Washing with NaOH-Triton mixed solution OR SDS solution
- Washing with WFI to remove the Washing Solution.

8.3.3 Prepare the 0.25M NaOH + 0.5% Triton X-100 Solution in the following manner.

NOTE: Preparation of Solution is based on a 1.0 m^2 Filter. Proportionally adjust amounts as required for individual filters (See Table 3).

8.3.3.1 0.25M NaOH + 0.5% Triton X-100 Solution

8.3.3.1.1 Measure and add 1.5 L of WFI to a container able to hold at least 2 L with a stir bar.

8.3.3.1.2 Add 20 g of NaOH to the water. Stir solution until NaOH is completely dissolved.

8.3.3.1.3 Add 10 g of Triton X-100. Stir solution until the Triton is completely dissolved.

8.3.3.1.4 Bring total volume up to 2 L with WFI.

8.3.3.1.5 Store at room temperature.

Table 3

Filter Surface Area (m^2)	Minimum Vol. of WFI Required	Minimum Vol. NaOH/Triton X-100 Solution OR SDS Solution Required
0.001	10 mL	6 mL
0.01	35 mL	15 mL
0.12	550 mL	300 mL
0.3	1150 mL	450 mL
1.0	3500 mL	1500 mL

8.3.4 Prepare the 0.25M NaOH + 1.0 wt% SDS Solution

8.3.4.1 Measure and add 1.5L of WFI to a container able to hold at least 2L with a stir bar.

8.3.4.2 Add 20g of NaOH to the water. Stir solution until NaOH is completely dissolved.

8.3.4.3 Add 20 g of SDS. Stir solution until SDS is completely dissolved.

8.3.4.4 Bring total volume up to 2L with WFI.

8.3.4.5 Store at RT.

8.4 Planova Assembly

8.4.1 Assemble the PLANOVA® as shown in Diagram 2 or 3 depending on whether a peristaltic pump or pressure tank is to be used

8.4.2 Flush the filter with ≥ Volume V3 (Table 4) of 0.25M NaOH + 0.5% Triton X-100 OR SDS Solution through the retentate, making sure to remove all residual air.

8.4.3 Close the retentate with forceps, etc. Flush filter with ≥ Volume V4 (Table 4) of 0.25M NaOH + 0.5% Triton X-100 OR SDS Solution through the permeate.

8.4.4 Flush the filter ≥ Volume V1 (Table 4) of WFI through the retentate, making sure to remove all residual air.

8.4.5 Close the retentate with forceps, etc. Flush filter with ≥ Volume V2 (Table 4) of WFI through the permeate

Table 4

Filter Surface Area (m^2)	WFI Retentate (V1)	WFI Permeate (V2)	Washing Solution Retentate (V3)	Washing Solution Permeate (V4)
0.001	5 mL	5 mL	5 mL	1 mL
0.01	5 mL	30 mL	5 mL	10 mL
0.12	150 mL	400 mL	150 mL	150 mL
0.3	150 mL	1000 mL	150 mL	300 mL
1.0	500 mL	3000 mL	500 mL	1000 mL

8.5 Gold Particle Test for PLANOVA® 15N, 20N and 35N Filters

8.5.1 The Gold Particle Test (GPT) is performed to determine the integrity of the PLANOVA® Filter and is recommended by Asahi as (together with the Post-use Leakage Test) a component procedure of the Post-use Integrity Test for each individual PLANOVA® Filter (except for the 75N) following use in virus filtration.

8.5.2 The Gold Particle Test is a destructive test.

8.5.3 The PLANOVA® Filter must first be washed as described in Section 8.3 of this SOP, before it is subjected to the GPT.

8.5.4 Inspection and Dilution of the Asahi Gold Particle (AGP) Solution (AGP-HA15, AGP-HA35, AGP-HA20.

NOTE: Always vigorously mix the Gold Particle Solution before any use.

8.5.4.1 Dilute the Gold Particle Solution 1:10 in WFI (1 part GPS into 9 parts WFI).

8.5.4.2 Measure the absorbance of the Diluted Gold Particle Solution as described in Table 5.

8.5.4.3 Compare the results to the Standard Values shown in Table 5 both values for the corresponding Diluted Gold Particle Solution must be in range to be deemed acceptable.

8.5.4.4 If the Gold Particle Solution is acceptable, continue to section 8.4.5, if not notify the Supervisor. Contact Asahi if needed.

Table 5

Size Filter	Wavelength (nm)	Standard Values
15N	A530 (Amax)	1.100 ± 0.150 (0.950~1.250)
15N	A530/A520 (Ratio)	1.042 ± 0.020 (1.022~1.062)
20N	A526 (Amax)	1.050 ± 0.150 (0.900~1.200)
20N	A530/A520 (Ratio)	1.005 ± 0.020 (0.985~1.025)
35N	A535 (Amax)	1.050 ± 0.150 (0.900~1.200)
35N	A550/A530 (Ratio)	0.964 ± 0.030 (0.934~0.994)

8.5.5 Preparation, Dilution and Inspection of Gold Particle Solution for Integrity Testing

NOTE: Preparation of Solution is based on a 1.0 m^2 Filter. Proportionally adjust amounts as required for individual filters (see Table 6 and Attachment 4).

8.5.5.1 Preparation of Diluting Solution (0.27% SDS).

8.5.5.1.1 Measure 2.7 g of SDS and place it in a clean flask.

8.5.5.1.2 Measure and add 997 mL of WFI.

8.5.5.1.3 Mix until in solution.

8.5.5.2 Dilution of Gold Particle Solution.

8.5.5.2.1 Vigorously Mix the Gold Particle Solution.

8.5.5.2.2 Add 110 mL of Gold Particle Solution to a clean flask.

8.5.5.2.3 Add 990 mL of the Diluting Solution (0.27% Sodium Lauryl Sulfate (SDS)) and mix well.

8.5.5.3 Inspection/Acceptance of Diluted Gold Particle Solution.

8.5.5.3.1 Inspect solution as in section 8.4.4.1 to 8.4.4.4.

8.5.5.3.2 Only Diluted Gold Particle Solution that is deemed acceptable can be used for the Integrity test. Performance of the Gold Particle Test

8.5.5.4 Place the Feed line into the Diluted Gold Particle Solution, ensuring that no air enters the tubing.

8.5.5.5 With the permeate line closed and the retentate line open, turn on the pump and allow a small volume of solution to pass through the retentate line (5-10 mL). If any air was allowed to enter the tubing in section 8.4.5.4, this is an opportunity to remove it.

8.5.5.6 Stop the pump. Close the retentate line and open the permeate line.

8.5.5.7 Turn on the pump and adjust the flow to achieve a pressure of 0.25-0.30 Bar and collect the volume V1 from Table 6 and discard.

8.5.5.8 With the pump continuing to run at the same flow rate, collect volume V2 from Table 6, in a clean new container.

8.5.5.9 Stop the pump and close all the lines.

Table 6

Filter Surface Area (m^2)	V1 (mL)	V2 (mL)
0.001	0.5	0.5
0.01	5	5
0.12	60	60
0.3	150	150
1.0	500	500

8.5.6 Measurement of the optical density of the Gold Particle Filtrate (V2).

NOTE: The measurements should be read using the same cell rinsing with WFI between measurements.

8.5.6.1 Using WFI, blank the spectrophotometer at the wavelength specified in Table 7.

8.5.6.2 Measure the absorbance of the WFI (Aw1).

8.5.6.3 Measure the absorbance of the Gold Particle Filtrate from section 6.4.6.5.

8.5.6.4 Measure the absorbance of WFI again (Aw2) and calculate the mean value of Aw1 and Aw2 as Awm.

8.5.6.5 Measure the absorbance of the Diluted Gold Particle Solution (Amax).

Table 7

Size Filter	Wavelength (nm)
15N	A530
20N	A526
35N	A535

8.5.7 Calculate the gold particle removal rate for the filtrate sample using the following equation.

$$N = \text{Log}_{10} (Amax \div (A - Apvp - Awm))$$

Where:

Amax = absorbance of Diluted GPS at wavelength in Table 7

A= absorbance of Filtrate Sample at wavelength in Table 7

Awm = mean absorbance of WFI at wavelength in Table 7

Apvp = absorbance of the 0.25% PVP Solution described in the COA of the AGP-HA at wavelength in Table 7

NOTE: If A – Apvp – Awm is < 0.001, then give A – Apvp – Awm a value = 0.001.

See Table 8 for the minimum acceptable value of N.

Table 8

Filter	Minimum Acceptable Value for N
15N	2.03
20N	1.40
35N	1.84

8.6 Liquid Forward-flow Test for PLANOVA® 20N

NOTE: The Liquid Forward Flow Test is not required if the Gold Particle Test has already been performed.

8.6.1 The Liquid Forward-flow Test (LFR) is performed to determine the integrity of the PLANOVA® 20N, and is recommended by Asahi as (together with the Post-use Leakage Test) a component procedure of the Post-use Integrity Test for each individual PLANOVA® 20N following use in virus filtration.

8.6.2 The Liquid Forward-flow Test is a destructive test.

8.6.3 The PLANOVA® 20N must first be washed as described in Section 8.3 of this SOP, before it is subjected to the LFR.

8.6.4 Preparation of LFR test solutions

NOTE: It is very important that the materials and solutions be stored at the LFR Test Temperature within ± 1°C. Any variation in temperature will affect the test results.

8.6.4.1 Place the volume of Ammonium Sulfate from Table 9 in a clean flask.

8.6.4.2 Add the appropriate volume of WFI from Table 9.

8.6.4.3 Allow the solution to stir until the Ammonium Sulfate is completely in solution.

8.6.4.4 Add the appropriate volume of Isopropyl Alcohol into the flask and allow it to stir vigorously for ≥ 30 minutes.

8.6.4.5 Allow the solution to sit undisturbed for ≥ 30 minutes.

8.6.4.6 Verify that the solution has two clearly-separated layers. The upper layer is referred to as the Low Specific Gravity solution or LSG, and the bottom layer is referred to as the High Specific Gravity Solution or HSG.

8.6.4.7 Remove the LSG and HSG solutions separately into two different containers. This can be performed using a peristaltic pump and tubing. Begin by removing the HSG solution (bottom layer). Carefully insert the tubing into the lower phase of the solution and remove all but a small portion of the HSG. Next, using fresh tubing, remove the LSG solution (upper layer) being sure not to remove any of the HSG solution with the LSG solution.

Table 9

Filter Surface Area (m^2)	Solution Required (g)	Ammonium Sulfate (g)	WFI (g)	Isopropyl Alcohol (g)
0.001	1000	190	630	180
0.01	1000	190	630	180
0.12	1000	190	630	180
0.3	1400	266	882	252
1.0	4200	798	2646	756

8.6.5 Inspection of the Test Solutions

8.6.5.1 The LSG and HSG solutions are inspected by measurement of their specific gravity. This can be performed using a float hydrometer or other device capable of measuring down to 0.001 or less.

8.6.5.2 Pour the solution into a graduated cylinder and measure its temperature and specific gravity.

8.6.5.3 Confirm that the test solutions are within the prescribe ranges in Table 10.

Table 10

Solution Temperature (°C)	Specific Gravity of LSG Solution	Specific Gravity of HSG Solution
26	0.8795-0.8855	1.1195-1.1220
25	0.8815-0.8875	1.1195-1.1225
24	0.8835-0.8890	1.1200-1.1225
23	0.8850-0.8910	1.1200-1.1230
22	0.8870-0.8925	1.1200-1.1230
21	0.8885-0.8945	1.1205-1.1230
20	0.8905-0.8965	1.1205-1.1235
19	0.8925-0.8980	1.1210-1.1235
18	0.8940-0.9000	1.1210-1.1240
17	0.8960-0.9015	1.1210-1.1240
16	0.8975-0.9035	1.1215-1.1240
15	0.8995-0.9055	1.1215-1.1245
14	0.9015-0.9070	1.1220-1.1245

NOTE: Round the temperature to the nearest whole number.

8.6.6 LFR Measurement

8.6.6.1 Set up the filter apparatus as shown in Diagram 3.

8.6.6.2 Drain all of the solution out of the filter from both the feed inlet and the two permeates.

8.6.6.3 Close both permeates when finished and fill the hollow fibers with HSG solution being sure to remove as much air as possible. Allow the HSG solution to flow through the retentate until the required volume, Va (Table 11) is achieved.

8.6.6.4 Close the retentate, and open both permeate lines. Tilt the filter to about 30° from vertical with the permeate lines facing downward.

8.6.6.5 Allow the HSG solution to flow through the permeate lines until the required volume, Vb (Table 11) is achieved.

8.6.6.6 Tilt the filter to about 30° from vertical with the permeate lines facing upward.

8.6.6.7 Close the bottom permeate line and continue the filtration of the HSG solution until it reaches the upper permeate line, thus filling the filter housing with HSG solution.

8.6.6.8 Immediately, stop filtration and close the top permeate line.

8.6.6.9 Clear the feed line completely of the HSG solution.

8.6.6.10 Place the filter in a vertical position, remove the feed line (if not already removed), and open the retentate line. This should completely empty the hollow fibers of any HSG solution.

8.6.6.11 Using the LSG solution, completely fill the feed line with solution and reconnect the feed line to the filter.

8.6.6.12 Open the retentate line and allow the LSG solution to fill the hollow fibers taking care to remove any excess air. Allow this to continue until the required volume, Vc is achieved.

8.6.6.13 Tilt the filter to about 30° from vertical with the permeate lines facing downward.

8.6.6.14 Open the top permeate line and adjust the flow to achieve the correct LFR Test Pressure (Table 12).

8.6.6.15 Adjust the height of the feed solution to within 3 cm of the top permeate line and allow the system to stand for ≥ 10 minutes.

8.6.6.16 Readjust the height of the feed solution to within 3 cm of the top permeate line and allow the system to stand for ≥ 5 additional minutes.

8.6.6.17 Begin measurement of the leakage rate by collecting the solution exiting the top permeate line into a graduated container.

8.6.6.18 Stop the collection of fluid from the top permeate line when the volume corresponds to 30 – 50 mL/m2 (ex. For a 0.3 m2 filter, the collection range would be 10 – 17 mL) and record the time of collection in minutes.

8.6.7 Calculate the LFR for the filter by the equation below.

LFR (mL/min/m^2) = leakage volume (ml)/leakage period (min)/surface area (m^2)

8.6.8 The LFR values which meets this LFR standard in Table XII is deemed acceptable.

Table 11

Filter Surface Area (m^2)	HSG Solution Required (Va)	HSG Solution Required (Vb)	LSG Solution Required (Vc)
0.001	5 –10 mL	1 mL	50 mL ~
0.01	5 –10 mL	10 mL	50 mL ~
0.12	70 –90 mL	30 mL	70 – 90 mL
0.3	70 –90 mL	300 mL	70 – 90 mL
1.0	200 –250 mL	1000 mL	200 – 250 mL

Table 12

LFR Test Pressure (Bar)	LFR Test temperature	LFR Standard ($mL/min/m^2$)
1.00	26	32 or less
	25	38 or less
	24	44 or less
	23	51 or less
0.92	24	16 or less
	23	23 or less
	22	30 or less
	21	37 or less
	20	44 or less
0.85	21	22 or less
	20	27 or less
	19	32 or less
	18	37 or less
0.77	18	21 or less
	17	26 or less
	16	30 or less
	15	35 or less

9.0 References and Related Documents

PLANOVA® SOP Integrity Test Manual, Version 1.2, 7/1/04.

LANOVA® SOP Integrity Test Manual, LFR Edition, Version 1.1, 12-01-01.

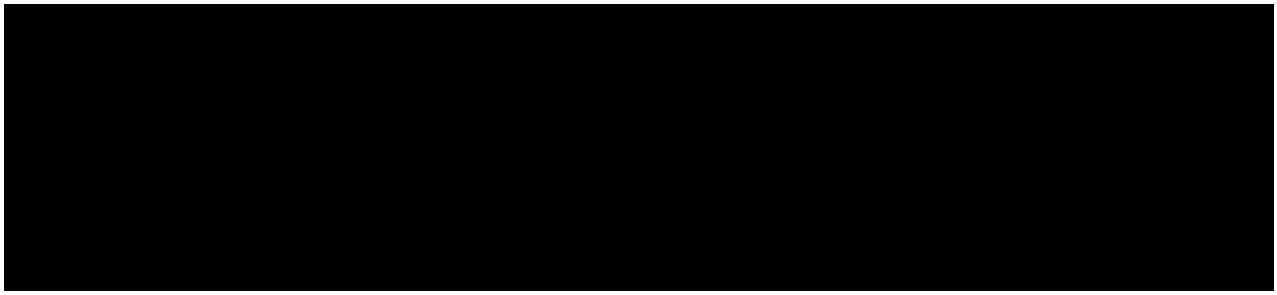
Form 15102-01 *PLANOVA® Filtration Data.*

Form 15102-02 *Integrity Test of Planova® Filter Using the Gold Particle Test.*

Form 15102-03 Integrity Test of Planova® Filter Using the Liquid Forward Flow (LFR) Test,

Attachment 1 PLANOVA Gold Particle Integrity Test: Modified Quantities for 500mL Solutions

10.0 Change Summary



Attachment 1

PLANOVA Gold Particle Integrity Test: Modified Quantities for 500mL Solutions

0.27% SDS Solution:

Since - 0.0027 x 997mL WFI = 2.7 g SDS;

Then - 0.0027 x 500mL WFI = 1.35 g SDS

Therefore: 1.35g SDS + 500mL WFI = 0.27% SDS Solution

Post Wash Solution (0.25M NaOH + 0.5% Triton X-100):

C= n/V C = concentration of solution (in moles/Liter)

n = moles of solute

V = Volume of solvent (always in Liters)

0.25M NaOH = n / (0.5L WFI)

n = (0.25M NaOH)(0.5L WFI) → (0.25 m/L)(0.5 L) = 0.125 moles of NaOH

Molecular weight of NaOH: 40 g

0.125 moles of NaOH x 40 g NaOH per mole = 5 g NaOH needed for solution

0.5% Triton X-100 = 0.005 x 500mL WFI = 2.5 g Triton X-100 needed for solution

Post Wash Solution (0.25M NaOH + 1.0% SDS):

C= n/V C = concentration of solution (in moles/Liter)

n = moles of solute

V = Volume of solvent (always in Liters)

0.25M NaOH = n / (0.5L WFI)

n = (0.25M NaOH)(0.5L WFI) → (0.25 m/L)(0.5 L) = 0.125 moles of NaOH

Molecular weight of NaOH: 40 g

0.125 moles of NaOH x 40 g NaOH per mole = 5 g NaOH needed for solution

1.0% SDS = 0.01 x 500mL WFI = 5g SDS needed for solution

Therefore: 5 g NaOH + 2.5 g Triton X-100 + 500mL WFI = *0.25M NaOH + 0.5% Triton X-100*

Therefore: 5 g NaOH + 5g SDS + 500mL WFI = *0.25M NaOH + 1.0% SDS*