FISP®

In-Situ Sampling Probes

User Guide

Preface

Revision History

Revision	Revision Date	Document Number
B	July 2016	FAI-001-007-B

If you have questions or comments regarding the contents of this guide, contact Flownamics technical support at 1-800-932-6989 or e-mail at techsupport@flownamics.com.

Flownamics Analytical Instruments, Inc.has made every effort to ensure the information in this guide is correct at the time of availability. Flownamics Analytical Instruments, Inc. reserves the right to make changes without notice as part of ongoing product development.

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Intended Use

The FISP® Sampling Probe is intended for life science applications only and is not intended to be used for medical or diagnostic procedures.

Use of this Manual

This manual should be read in its entirety before using the FISP In-Situ Sampling Probe. This manual is intended as instructions for its use. It provides information about the system's installation, operation, maintenance and troubleshooting.

Symbols Used in this Manual

The following symbols may be used in this manual:

Symbol	Description
	Note. Provides additional details.
	Stop. Understand and follow instructions so that system damage or data loss does not occur.
	Warning – Risk of Danger. Risk of personal injury to the operator or a safety hazard to the system or surrounding area.
	Biohazard. Take the necessary precautions when working with potentially infectious material.

Warnings and Precautions

Handling Requirements

It is essential that all users carefully read and observe the following safety information. Always follow basic safety precautions during use to reduce the risk of personal injury.

- Read, understand, and follow all information in this manual. Failure to do so could result in damage to the Seg-Flow Sampling System, injury to operating personnel, or poor system performance.
- Observe all  (Warning) and  (Stop) statements in this manual.
- Observe proper laboratory safety precautions, such as wearing personnel protective equipment and using approved laboratory safety procedures.

Ensure that this safety information is accessible for every person using FISP Sampling Probes. It is assumed that trained users are familiar with the necessary safety precautions for handling chemicals and biohazardous materials. Observe the following laws and guidelines:

- National industrial protection law
- Accident prevention regulations
- Material Safety data sheets (MSDS) of the reagent manufacturers

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1 Introduction

1.1 FISP® Sampling Probe Technology

1.1.1 FISP Sampling Probe Description

The FISP® sampling probe is an *in-situ* sampling device that permits the withdrawal of a sterile cell-free filtrate from any fermentation vessel or bioreactor equipped with an Ingold®, headplate or sanitary (tri-clamp) port. Additionally, the FISP sampling probe may be used for single-use bioreactors (SUB's) that are equipped with a standard pH or dissolved oxygen probe housing. The probe is designed to provide simple, efficient and consistent microfiltered sampling while maintaining a sterile interface between the vessel contents and the external environment.

The FISP sampling probe can be used in a wide range of processing industries including pharmaceutical, biotechnology, biofuels, chemical and brewing. In most applications the probe is installed prior to filling the vessel and is sterilized in-situ in the same manner as pH and dissolved oxygen sensors. Other applications may require the probe to be pre-sterilized prior to installation, in which the FISP sampling probe can be autoclaved by itself.

1.1.2 Ceramic Membrane Filtration

FISP ceramic membranes are designed for in-situ, microfiltered sampling of bioprocessing vessels and chemical reactors. The durable construction and inert nature of the membrane material allows the membrane to withstand a wide range of operating temperatures, pressures, viscosities, shear forces and chemicals. The hydrophilic nature of the membrane material makes it ideal for bacterial, fungal and cell culture applications. The ceramic membrane requires no pre-treatment and is compatible with steam-in-place (SIP) and clean-in-place (CIP) use.

1.1.3 Custom Applications

Standard FISP sampling probes are available in a variety of styles and sizes to fit almost any vessel size and port type. Custom sampling probe and membrane options are possible for allowing *in-situ* cell-free sampling for unique applications. Contact Flownamics for probe and membrane options and pricing. Contact information can be found on the back cover of this user guide.

2 FISP Configurations & Components

All sampling probes are pre-assembled at the factory and are shipped ready-to-use. The configurations and components for each type of sampling probe are described in the following sections.

2.1 D-Series FISP Sampling Probe

2.1.1 Standard Configuration

The D-Series FISP sampling probe is designed to fit into a 19 mm or 25 mm Ingold port of an SIP/CIP vessel. These probes can be fitted for either a standard or safety Ingold port. There are two basic configurations for the D-Series FISP sampling probe. The standard, or first, configuration is designed to connect to stainless steel (SS) HPLC tubing and has a SS reducer and fitting attached to the plug valve (Figure 2-1).

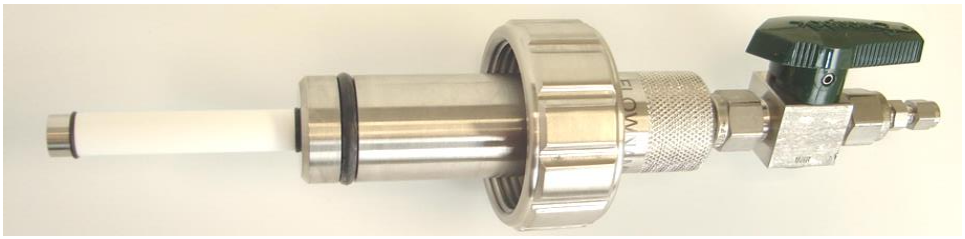


Figure 2-1

The components for this configuration are:

- Stainless steel (SS) probe body (19 or 25 mm)
- Ceramic membrane
- Main shaft EPDM o-rings (1 or 2)
- Membrane EPDM o-rings (2)
- SS end cap screw
- SS plug valve w/ SS reducer and fitting (standard)
- 1/8 inch hex wrench

2.1.2 Luer Configuration

The luer, or second, configuration uses a PEEK female luer connector in lieu of the SS reducer and SS fitting. This configuration is used for providing an on-line sample line connection for the Seg-Flow automated sampling system or other luer connector applications. With this option the SS ¼" Tubing Adapter and PEEK Female Luer to 10-32 Male Fitting are used to provide the luer connection. Figure 2-2 shows the D-Series FISP sampling probe with the PEEK luer connection configuration.

Figure 2-2

2.2 F-Series FISP Sampling Probe

The F-Series sampling probes are designed to fit both 12mm and 19 mm headplate ports and are available in a variety of immersion lengths. For 19 mm ports, a 12 to 19 mm Adapter/ PG 13.5 thread Adapter is required to install the sampling probe. Additionally, the F-Series FISP probes can be mounted into a 1.5 inch sanitary fitting using the 1.5 inch sanitary flange to 12 mm adapter.

2.2.1 Standard Configuration

The F-Series FISP sampling probes are available in two basic configurations. The standard, or first, configuration uses a PEEK female luer connector to provide a luer connection for the Seg-Flow automated on-line sampling system or other luer connector applications. Figure 2-3 shows the standard configuration for the F-Series FISP sampling probe.

Figure 2-3 (PG 13.5 nut not shown)

The following list details the components for the standard F-Series sampling probe configuration.

- SS probe body (12 mm)
- Ceramic membrane
- 12 mm PTFE beveled washer & EPDM o-ring (probe body)
- Membrane EPDM o-rings (2)
- SS end cap screw
- SS PG 13.5 retaining nut
- PEEK Female Luer to 10-32 Male Fitting
- 1/8 inch hex wrench

2.2.2 HPLC Tubing Configuration

The HPLC Tubing, or second, configuration uses a PEEK nut and ferrule for connecting PTFE, PEEK or other plastic HPLC tubing. In this case, the PEEK nut and ferrule are used in lieu of the PEEK female luer connector. Figure 2-4 shows the F-Series FISP sampling probe with the PEEK nut and ferrule connector.

Figure 2-4

2.3 Cell-containing Sampling Probe

In addition to the FISP sampling probes, Flownamics offers a stainless steel *in-situ* sampling probe for withdrawing cell-containing samples from a vessel. These probes may be used with autoclaveable glass vessels, SIP vessels and SUB's having a standard pH probe housing, i.e., Hyclone™ SUB. Like the F-series probes, the cell containing sampling probes (dip tubes) are designed to fit 12mm and 19 mm headplate ports. Like the F-Series sampling probes, these cell containing probes can be used with a 1.5 inch sanitary port using the 1.5 inch sanitary flange to 12 mm adapter. Figure 2-5 shows the cell-containing sampling probe. The cell containing sampling probe has a 6mm Outer Diameter, making it compatible with some compression style fittings employed in older bioreactor systems for the manufacturer's existing sample probe. The advantage of the Flownamics cell-containing sampling probe is the 1/32" inner diameter, mitigating hold up volume and purge requirements.

Figure 2-5

The components for the cell containing sampling probe are:

- Stainless steel probe body (6 mm)
- 6mm ID, 12mm OD compression fitting for PG 13.5 port
- PEEK Female Luer to 10-32 Male Fitting

3 Probe Assembly

The FISP sampling probe is shipped as a completed assembly. *Chapter 2, FISP Configurations & Components*, provides photos of the fully assembled D-Series FISP sampling probe, F-Series FISP sampling probe and the stainless steel cell-containing sampling probe. Refer to these figures as needed when inspecting the probe assembly.

Prior to using the FISP sampling probe, a careful inspection should be accomplished to ensure all components are in place and properly secured. In particular, check the mounted membrane to ensure that it is securely in place and that the end cap screw is tight. If the membrane is loose, carefully tighten the end cap screw using the membrane mounting procedure in *Section 7.1, Mounting the Membrane.*

4 Installation & Sterilization

4.1 Pre-installation Procedures

1. Inspect the membrane for any chips, cracks or flaking. If any of these conditions are present, replace the membrane using the membrane replacement procedure in *Section 7-1, Membrane Replacement*.
2. Inspect the probe and membrane shaft o-rings for chips, cracks or tears. If any of these are present, replace the damaged o-ring using the procedure in *Section 7-1, Membrane Replacement* and/or *Section 7-2, Probe Body O-ring and Washer Replacement*.
3. Ensure all o-rings are properly fitted and the end cap screw is securely tightened into the end of the membrane shaft. If not, carefully tighten the screw with the supplied hex wrench until it just stops.

DO NOT overtighten the end cap screw. Overtightening the end cap screw could result in damaging the membrane.

Although not required, Flownamics recommends performing a flux test of the membrane prior to installing the FISP sampling probe. This can be accomplished as follows:

1. Fill a luer lock tip syringe with deionized or purified water.
2. Connect the water-filled syringe to the FISP probe's luer connector.
3. Gently depress the syringe's plunger to push the water through the membrane. If liquid can pass through the membrane then the membrane flux test was successful.
4. If liquid cannot flow through the membrane, clean the membrane using the procedure in *Chapter 7, Probe Maintenance*.
5. If no flux can be achieved after cleaning the membrane, replace the membrane with a new one and repeat the flux test.

4.2 SIP & Glass Vessel Installation

4.2.1 Ingold® Port: D-Series FISP

1. Insert the D-Series FISP probe into the vessel's Ingold port until the retaining nut meets the port threads.
2. Screw the retaining nut onto the port threads until tightened.

MAKE SURE THE PROBE IS SECURELY TIGHTENED WITH THE RETAINER NUT PRIOR TO VESSEL PRESSURIZATION AND STERILIZATION.

3. Turn the plug valve to the off position to prevent the vessel's contents from leaking through the probe.

4. Once the plug valve is turned off, the probe is then ready for pressurization and sterilization.

4.2.2 Ingold® Port: F-Series FISP/Cell Containing Sample Probe

If a SegMod Tubing Assembly will be used with the Cell Containing probe or FISP sampling probe, connect the tubing assembly to the probe prior to inserting into the probe into the adapter. Use one of the following tubing connection procedures:

1. Non-sterile SegMod Tubing Assembly
    a. Connect an unsterile tubing assembly to the probe using the luer connector.
    b. Place the connected probe and tubing assembly into an approved autoclaveable container.
    c. Autoclave the probe and tubing assembly for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    d. Once the autoclave cycle is complete, remove the probe and tubing assembly from the autoclave and allow them to cool to room temperature

2. Sterile SegMod Tubing Assembly
    a. Place the probe into an approved autoclaveable container.
    b. Autoclave the probe for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    c. Once the autoclave cycle is complete, remove the probe from the autoclave and allow it to cool to room temperature
    d. Place the autoclaved probe in a Biosafety Cabinet (BSC)
    e. Remove the probe from the autoclave container and connect the sterile tubing assembly to the probe using the luer connector.

1. Insert the F-Series FISP probe or Cell Containing sampling probe into the 12 to 25 mm Ingold Port Adapter until the probe body o-ring completely seats into the adapter.
2. Screw the PG 13.5 nut into the adapter threads until tightened.
3. Insert the 12 to 25 mm Ingold Port Adapter into the vessel's Ingold port until the retaining nut meets the port threads.
4. Screw the retaining nut onto the port threads until tightened.

MAKE SURE THE PROBE IS SECURELY TIGHTENED WITH THE RETAINER NUT PRIOR TO VESSEL PRESSURIZATION AND STERILIZATION.

5. Turn the 316 SS shutoff valve to the off position to prevent the vessel's contents from leaking through the probe.
6. Once the plug valve is turned off, the probe is then ready for pressurization and sterilization.

4.2.3 Headplate Port

1. Insert the F-Series FISP probe or Cell Containing sampling probe into the vessel's headplate port until the probe body o-ring completely seats into the port.
2. For 12 mm ports, screw the PG 13.5 nut into the port threads until tightened. For 19 mm ports, use the 12 to 19 mm Adapter nut to secure the sampling probe into the headplate port.

MAKE SURE THE PROBE IS SECURELY TIGHTENED WITH THE RETAINER NUT PRIOR TO VESSEL PRESSURIZATION AND STERILIZATION.

3. Use the provided plug to seal the probe outlet during sterilization. Screw the threaded plug into the FISP outlet until tightened.
4. Once the plug is in place, the probe is then ready for pressurization and sterilization.

4.2.4 Sanitary Fitting

If a SegMod Tubing Assembly will be used with the Cell Containing probe or FISP sampling probe, connect the tubing assembly to the probe prior to inserting into the probe into the adapter. Use one of the following tubing connection procedures:

1. Non-sterile SegMod Tubing Assembly
    a. Connect an unsterile tubing assembly to the probe using the luer connector.
    b. Place the connected probe and tubing assembly into an approved autoclaveable container.
    c. Autoclave the probe and tubing assembly for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    d. Once the autoclave cycle is complete, remove the probe and tubing assembly from the autoclave and allow them to cool to room temperature

2. Sterile SegMod Tubing Assembly
    a. Place the probe into an approved autoclaveable container.
    b. Autoclave the probe for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    c. Once the autoclave cycle is complete, remove the probe from the autoclave and allow it to cool to room temperature
    d. Place the autoclaved probe in a Biosafety Cabinet (BSC)
    e. Remove the probe from the autoclave container and connect the sterile tubing assembly to the probe using the luer connector.

1. Insert the F-Series FISP probe or Cell Containing sampling probe into the 1.5 inch or 2.0 inch Sanitary Flange to 12mm adapter until the probe body o-ring completely seats into the adapter.
2. Screw the PG 13.5 nut into the adapter threads until tightened.
3. Insert the 1.5 inchor 2.0 inch Sanitary Flange to 12mm adapter into the vessel's 1.5 inch sanitary fitting. Ensure the adapter is seated completely into the fitting.
4. Secure the adapter to the vessel using the 1.5 inch sanitary fitting clamp. Ensure the clamp screw is completely tightened.

MAKE SURE THE 1.5 INCH SANITARY FLANGE TO 12 MM ADAPTER IS SECURELY TIGHTENED WITH THE SANITARY FITTING CLAMP PRIOR TO VESSEL PRESSURIZATION AND STERILIZATION.

5. If a 316 SS shut-off valve was supplied with the probe, ensure the valve luer connector is completely tightened on the FISP probe luer connector. Turn the valve to the off position to prevent the vessel's contents from leaking through the probe.
6. If a plug was provided with FISP probe, use it seal the probe outlet during sterilization. Screw the plug into the FISP outlet until completely tightened.
7. Once the valve is shut off or the plug is in place, the probe is then ready for pressurization and sterilization.

4.3 Single-Use Bioreactor Installation

The F-Series FISP sampling probe can be used with the ThermoFisher™ Hyclone™ SUB bioreactor or other single-use systems that allow standard pH probe connections. The sampling probe can be inserted into the autoclaveable insertion pouch in the same manner as a pH or dissolved oxygen probe. Figure 4-1 shows the sampling probe inserted into the Hyclone SUB's autoclaveable insertion pouch. Refer to the single-use bioreactor operator's manual for probe insertion and autoclaving instructions.

Figure 4-1

If a SegMod Tubing Assembly will be used with the Cell Containing probe or FISP sampling probe, connect the tubing assembly to the probe prior to inserting into the probe into the adapter. Use one of the following tubing connection procedures:

1. Non-sterile SegMod Tubing Assembly
    a. Connect an unsterile tubing assembly to the probe using the luer connector.
    b. Place the connected probe and tubing assembly into an approved autoclaveable container.
    c. Autoclave the probe and tubing assembly for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    d. Once the autoclave cycle is complete, remove the probe and tubing assembly from the autoclave and allow them to cool to room temperature

2. Sterile SegMod Tubing Assembly
    a. Place the probe into an approved autoclaveable container.
    b. Autoclave the probe for 30 minutes at 121° C. Do not autoclave the probe using a vacuum cycle.
    c. Once the autoclave cycle is complete, remove the probe from the autoclave and allow it to cool to room temperature
    d. Place the autoclaved probe in a Biosafety Cabinet (BSC)
    e. Remove the probe from the autoclave container and connect the sterile tubing assembly to the probe using the luer connector.

4.4 Autoclaving

If the FISP sampling probe or Cell Containing sampling probe requires sterilization prior to installation, the probe may be autoclaved. Also, the probe may be autoclaved *in-situ* with an autoclaveable vessel.

1. Place the probe in an approved autoclaveable container or install in an autoclaveable vessel.
2. Autoclave the probe for 30 minutes at 121° C.
3. Once the autoclave cycle is complete, remove the probe from the autoclave and allow it to cool.

To prevent a heat-related injury, allow the FISP or Cell Containing sampling probe to cool to ambient room temperature prior to installation or use.

5 Probe Operation

5.1 Using the FISP Sample Probe

Sampling can begin at anytime after establishing process conditions. A sample can be drawn by using the SegFlow® automated on-line sampling system, a peristaltic pump or the vessel's own pressure. For sample delivery, the FISP probe can be connected to most types of tubing, including C-Flex®, PharMed®, Teflon® or stainless steel using the nut, ferrule and/or adapter provided.

Samples may be drawn through the membrane at flow rates ranging between 0.25 to 5.0 ml/minute. For optimal performance the recommended range is 0.25 to 1.0 ml/minute; however, the end-user should define the appropriate sampling flow rate for their respective process.

Drawing samples at higher flow rates, i.e., 5.0 ml/minute could induce membrane fouling. For questions regarding membrane applications or sampling flow rates, see *Appendix C, Applications and Sampling Flow Rates*, or contact Flownamics Technical Support.

5.2 How the FISP Sampling Probe Works

Figure 5-1 illustrates how fluid delivery is conducted through the FISP sampling probe. The liquid sample is first drawn through the microporous membrane and into the probe's stainless steel groove network. The sample flows through the grooves and into central channel, which is then directed outside the vessel to an on-line analyzer, fraction collector or other collection device.

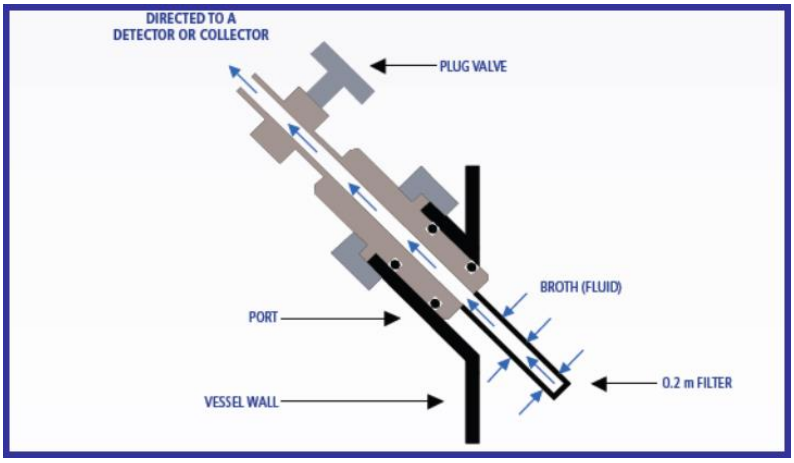


Figure 5-1

Sample flow rates are dependent on vessel fluid dynamics, i.e. viscosity, particulates. Additionally, membrane fouling is minimized by allowing the vessel's agitation to sweep across the membrane's surface, dislodging any particulates.

6 Probe Removal

Ensure the vessel has been cleaned, emptied and depressurized prior to removing the FISP sampling probe.

1. Remove the probe by unscrewing the probe's retainer nut and firmly pulling the probe from the port socket. Hold the knurled section when removing the probe from the vessel.

DO NOT PULL OR GRASP VESSEL FITTINGS OR VALVES when removing the probe from the vessel.

2. After removing the probe thoroughly rinse the probe under running water. Gently scrub the membrane's outer surface with a soft brush while continuing to rinse the probe. Rotate the probe while rinsing in order to clean the entire outer surface of the membrane.

    a. **For multiple-use applications**, the membrane should be maintained using the cleaning and maintenance procedures found in the *Chapter 7, Probe Maintenance*, of this user guide. This will ensure optimum probe and membrane performance.

    b. **For single-use applications (preferred)**, discard the membrane after each use per local laboratory guidelines. A new membrane should be installed onto the FISP sampling probe prior to each use. Use the membrane replacement procedure shown in *Chapter 7, Probe Maintenance*.

Used membranes and o-rings should be treated as biohazardous and/or chemical waste. Discard used materials in accordance with approved laboratory biohazard and/or chemical waste hazard protocols.

7 Probe Maintenance

For optimal performance, it is recommended to change the membrane O-rings after each use. In most cases, the membrane can be used up to 5 times if the cleaning procedure below is utilized after each run. However, the membrane life cycle is dependent on the vessel's internal environment and vessel operating conditions while the membrane is employed. Every process in which the FISP membrane is used is different and the FISP membrane performance will vary from one process to the next.

ALWAYS REPLACE THE MEMBRANE IF IT BECOMES CHIPPED, CRACKED OR THE CERAMIC MATERIAL STARTS TO FLAKE. These conditions will compromise the cell-free sampling and possibly the vessel's sterility. It is recommended for automated sampling using the Seg-Flow Automated Sampling system to replace the FISP membrane after every run.

7.1 Cleaning the Probe and Membrane

The ceramic membrane can be cleaned using either of the cleaning protocols provided below.

7.1.1 Cleaning the Membrane Mounted on the Probe

1. Remove the FISP sampling probe using the probe removal procedure shown in *Chapter 6, Probe Removal*.
2. Rinse the probe and membrane with running water while gently scrubbing the membrane with a soft brush.
3. After rinsing the probe and scrubbing the membrane, pump 0.5N NaOH through the probe and membrane at a rate of 0.5 – 1.0 ml/minute for 30 – 60 minutes. The NaOH solution should be pumped to waste and not recirculated.
4. Once the cleaning process is complete, pump deionized (DI) water or purified water (PW) through the probe and membrane at a rate of 0.5 – 1.0 ml/minute for at least 15 minutes to completely remove any residual NaOH. The DI water or PW should be pumped to waste and not be recirculated.
5. Place the supplied plug back onto the probe or turn the plug valve to the off position. Store the probe in a secure container or drawer, being careful that the surface of the membrane is protected from other objects coming in contact with it.

7.1.2 Cleaning the Membrane and Probe Separately

1. Remove the ceramic membrane using the membrane removal procedure in *Section 7.2, Membrane Removal*.
2. Rinse the membrane under running water while gently scrubbing the membrane with a soft brush.
3. Rinse the probe under running water.
4. After rinsing the probe and scrubbing the membrane, place the membrane in a recirculating bath with a mild non-alkaline detergent or 0.5N NaOH solution for 30 – 60 minutes. The membrane can

be immersed into the bath either alone or attached to the probe. The cleaning cycle may vary due to the membrane's condition.

5. An ultrasonic bath can be used for probe body cleaning. Cleaning the membrane in an ultrasonic bath can result in membrane damage if the membrane is allowed to rub against the surface of the bath. If an ultrasonic bath is used for membrane cleaning, please visually confirm the membrane surface integrity. If any cracking of flaking is seen on the membrane surface , replace the membrane.
6. After the cleaning cycle is complete, reinstall the membrane onto the probe, if removed. If the membrane needs to be reinstalled, use the membrane mounting procedure shown in *Section 7.3, Mounting the Membrane*.
7. With the membrane mounted, pump DI water or PW through the probe at a rate of 0.5 – 1.0 ml/minute for at least 15 minutes to completely remove the detergent or NaOH residue. Time may vary dependent on the condition of the membrane. The DI water or PW should be pumped to waste and not be recirculated.
8. Place the supplied plug back onto the probe or turn the plug valve to the off position. Store the probe in a secure container or drawer, being careful that the surface of the membrane is protected from other objects coming in contact with it.

7.1.3 Cleaning the Cell Containing Sampling Probe

1. Remove the sampling probe using the probe removal procedure shown in *Chapter 6, Probe Removal*.
2. Rinse the probe with running water while gently scrubbing the probe with a soft brush.
3. After rinsing the probe, pump 0.5N NaOH or mild non-alkaline detergent solution through the probe at a rate of 0.5 – 1.0 ml/minute for 15 – 60 minutes. The cleaning solution should be pumped to waste and not recirculated.
4. Once the cleaning process is complete, pump deionized (DI) water or purified water (PW) through the probe at a rate of 0.5 – 1.0 ml/minute for at least 15 minutes to completely remove any residual cleaning solution. The DI water or PW should be pumped to waste and not be recirculated.
5. Place the supplied plug back onto the probe. Store the probe in a secure container or drawer.

7.1.4 CIP Applications

The FISP sampling probe can be cleaned *in-situ* with a CIP process. The probe's wetted materials are compatible with most, if not all, CIP cleaning solutions and methods; however, some consideration is required for the ceramic membrane. Although the ceramic membrane provides good chemical resistance to alkalis and acids, the concentration and temperature of these cleaning reagents should not exceed 10% and 85°C, respectively. If the CIP process solution concentration and temperature will exceed these limitations, then the FISP sampling probe should be removed from the vessel and cleaned using either of the methods listed in *Sections 7.1.1* or *7.1.2*.

If the FISP sampling probe is cleaned using a CIP process, use the following rinsing procedure after the probe is removed from the vessel.

1. Rinse the probe with running water.
2. Pump deionized (DI) water or purified water (PW) through the probe at a rate of 0.5 – 1.0 ml/minute for at least 5 minutes to remove any residual cleaning solution. The DI water or PW should be pumped to waste and not be recirculated.

3. Store the probe in a secure container or drawer, being careful that the membrane surface of the probe is protected from other objects coming in contact with it.

The membrane can be used up to 10 times if the prescribed cleaning procedure is utilized after each use. However, the membrane lifecycle is dependent on the vessel's internal environment, vessel operating conditions and CIP process conditions while the membrane is employed. Ensure the membrane is inspected for cracks, chipping or flaking prior to each use.

7.2 Membrane Removal

Used membranes and o-rings should be treated as biohazardous and/or chemical waste. Discard used materials in accordance with approved laboratory biohazard and/or chemical waste hazard protocols.

Change the membrane o-rings each time the membrane is removed and reinstalled. This will ensure that (1) the membrane properly seals, (2) cell-free sampling integrity will not be compromised and (3) the sterile barrier between the vessel and the external environment will be maintained.

1. Remove the end cap screw using the supplied hex key wrench.
2. Remove the used membrane from the membrane shaft. Discard the used membrane.
3. Remove the membrane o-rings from the membrane shaft and end cap screw. Discard the used o-rings.

7.3 Mounting the Membrane

In the event that the ceramic membrane is loose or needs to be replaced, use the following procedure to mount the membrane. This procedure applies to both the D-Series and F-Series FISP sampling probes.

1. Place a membrane o-ring onto the membrane shaft. Slide the o-ring down the shaft until it seats against the main body of the probe (Figure 3-1). It is important to use a new set of membrane O-rings at each installation.

Figure 7-1

2. Place the ceramic membrane onto the membrane shaft, being careful not to chip the ends of the membrane. Slide the membrane until it seats against the mounted membrane o-ring (Figure 7-2

The membrane does not require any pre-treatment prior to use, however, dirt and oils from ungloved hands can potentially foul the membrane pores. Avoid handling the membrane with ungloved hands.

A chipped membrane will compromise the o-ring seal and possibly the vessel sterile barrier. If a membrane is chipped or cracked, it must be replaced with a new membrane.

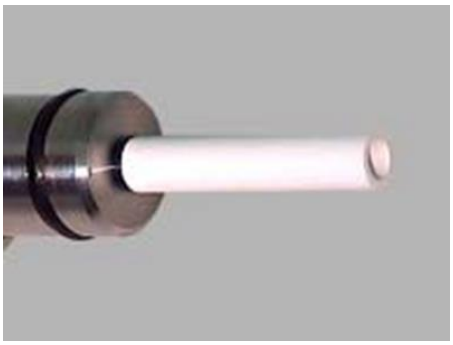


Figure 7-2

3. Place a second new membrane o-ring onto the threaded end of the end cap screw. Slide the o-ring until it seats against the shoulder of the screw (Figure 7-3).

Figure 7-3

4. Place the end cap screw into the end of the membrane shaft (Figure 7-4). Carefully tighten the screw with the supplied hex wrench until it just stops.

Figure 7-4

DO NOT overtighten the end cap screw. Overtightening the end cap screw could result in damaging the membrane.

Ensure all o-rings are properly fitted and the end cap screw is adequately tightened prior to mounting the FISP probe to the vessel.

7.4 Probe Body O-ring & Washer Replacement

The main shaft o-rings should be changed every 3-5 uses or each time the membrane O-rings are changed, depending on the user's application.

7.4.1 D-Series FISP Sampling Probe

The D-series FISP sampling probe will have either one or two probe body o-rings mounted, depending on the probe model used. Figure 7-5 shows a probe body with a single o-ring.

1. Remove the probe o-ring(s) from the o-ring groove. Slide the o-ring off the probe and discard.
2. Slide the new o-ring onto the probe shaft until the o-ring seats into the o-ring groove (Figure 7-5).

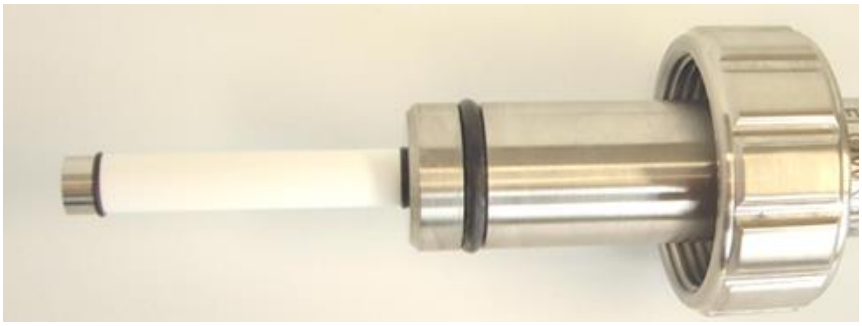


Figure 7-5

7.4.2 F-Series FISP & Cell Containing Sampling Probes

The F-series FISP sampling probe and the cell-containing sampling probe use a beveled washer and o-ring assembly to provide a seal with the vessel housing.

1. Remove the probe o-ring and beveled washer by sliding them off the probe body (Figure 7-6. Discard the used o-ring and beveled washer.

Figure 7-6

2. Slide the new beveled washer onto the probe body until it seat against the SS retaining ring (Figure 7-2)

Incorrect orientation of the beveled washer may compromise the vessel's sterile barrier. Ensure that the flat side of the beveled washer seats against the SS retaining ring and that the bevel faces towards the ceramic membrane (Figure 7-2). This will allow the o-ring to provide a sterile seal between the FISP sampling probe and vessel housing while in use.

3. Slide the new o-ring onto the probe body until the o-ring seats against the beveled washer (Figure 7-2).

8 Troubleshooting

8.1 Troubleshooting Basics

The troubleshooting guide below provides a means for determining the cause of a FISP sampling probe malfunction as well as the corrective measures that can be applied to remedy the problem. In order to more accurately diagnose the cause, gather as much information as possible prior to commencing the troubleshooting process.

If the malfunction cannot be resolved after using the troubleshooting guide, contact Flownamics Technical Support (contact information is in *Appendix F, Technical Assistance*). When corresponding please include the probe model and a detailed description of the problem.

8.2 Troubleshooting Guide

Ensure the vessel has been cleaned, emptied and depressurized prior to removing the FISP sampling probe.

Liquid is not able to flow through the FISP Sampling Probe

Cause: The D-Series FISP probe plug valve is shut off.

Solution: Check the plug valve and ensure it is in the open position.

Cause: Membrane is clogged.

Solution: If membrane clogging occurs during use, a backflush procedure can be used to unclog the membrane. Connect a transfer line or syringe to the FISP probe and introduce either a sterile liquid or sterile gas into the sampling probe. Use a flow rate that does not exceed 2 ml/min. Make sure that the liquid or gas used is compatible with your particular bioreactor process.

After the sample probe has been removed from the vessel, clean the membrane using the procedure in *Section 7.1, Cleaning the Probe and Membrane*. After the membrane has been cleaned perform a flux test on the membrane using the desired sampling rate. If the sampling rate can be achieved, then the membrane may be used. If the flow rate cannot be achieved, try using a new membrane. **NOTE:** The flow rate should not exceed the membrane flow rate specification.

Solution: The sampling flow rate may have been too high and induced membrane fouling. Try using a lower flow rate for your particular application. See *Appendix C, Applications & Sampling Flow Rates* for guidance.

Fluid exiting the FISP probe is not clear or looks like it may contain cells

Cause: The membrane is loose.

Solution: Retighten the end cap screw.

Cause: The membrane is cracked, chipped or flaking.

Solution: Replace the membrane.

Cause: A membrane o-ring is damaged.

Solution: Replace both membrane o-rings.

Liquid is able to flow through the FISP probe when the plug valve is shut off

Cause: The plug valve's internal components are worn or damaged.

Solution: Replace the plug valve. Contact Flownamics Technical Support for part availability and pricing.

9 Appendices

Appendix A FISP® Sampling Probe Specifications

D-Series FISP® Sampling Probe	
Probe Material	316 Stainless Steel (SS)
Port Type/Diameter	Ingold/19 mm & 25 mm Contact Flownamics for other port types.
Ingold Port Depth	40 mm: standard port - Probes P-D19-90-02 & P-D25-90-02 52 mm: safety port - Probe P-D25-115-02
Immersion Depth	90 mm: FISP Probes P-D19-90-02 & P-D25-90-02 f 110 mm: Probe P-D25-115-02
Probe Tubing Adapter	Standard: 316 SS reducer w/ 316 SS nut/ferrule When used with Seg-Flow® System: PEEK female luer to 10-32 male fitting
Dead Volume	0.5 ml (Dead volume includes both membrane and probe)
Plug Valve Materials	316 SS (main body), PTFE-coated SS (plug), PTFE-coated FKM (plug o-rings)
O-rings (all)	ethylene propylene diene monomer (EPDM)
Wetted Materials	316 SS, EPDM, Ceramic
F-Series FISP® Sampling Probe	
Probe Materials	316 SS (probe shaft) & PTFE (beveled washer)
Port Type/Diameter	Headplate/12 mm & 19mm (19 mm port requires 12 to 19 mm Adapter for PG 13.5 threads) 1.5 inch sanitary fitting: only when used with H-F-051, 1.5 inch Sanitary Flange to 12mm
Immersion Depth	120 mm, 200 mm, 310 mm & 410 mm
Probe Tubing Adapter	Standard: PEEK female luer to 10-32 male fitting Optional: 316 SS reducer w/ 316 SS nut/ferrule or PEEK 10-32 nut/ferrule
Dead Volume	0.3 – 1.0 ml (Dead volume includes both membrane and probe. Volume increases with probe length)
O-rings (all)	ethylene propylene diene monomer (EPDM)
Wetted Materials	316 SS, EPDM, Ceramic
Cell-containing Sampling Probe	
Probe material	316 SS (shaft), PTFE (compression fitting ferrule), PEEK (external reducer/connector)
Port Type/Diameter	Headplate/12 mm & 19mm (19 mm port requires 12 to 19 mm Adapter for PG 13.5 threads) 1.5 inch sanitary fitting: only when used with H-F-051, 1.5 inch Sanitary Flange to 12mm
Immersion Depth	200 mm , 350 mm and 500
Probe Tubing Adapter	Standard: PEEK female luer to 10-32 male fitting Optional: PEEK 10-32 nut/ferrule
Wetted Materials	316 SS
Probe Lifecycle	Reusable

FISP® Sampling Probe Specifications (continued)	
™ Ceramic Membrane	
Membrane Material	Ceramic
Length*	1.85 inches (47 mm)
Diameter*	0.375 inches (9.5 mm)
Pore size*	0.2 um (other options available)
Surface area	14.22 cm^2 (2.18 $in.^2$)
Operating Flow Rate**	0.2 – 2.0 ml/minute
Maximum Flow Rate**	3.0 ml/min
Maximum Operating Pressure	40 psi (2.7 bar)
Operating Temperature	0 – 121°C (32 – 250°F)
Sterilization Method	SIP or Autoclave
Sterilization Temperature	121 – 135°C (250 – 275°F)
Membrane Lifecycle***	5 cycles

*Values are for standard membrane specifications. Custom dimensions and pore sizes available. Contact Flownamics for availability and pricing.

**Flow rates are for typical bioprocessing applications. Flow rates are dependent on application and use.

***The membrane can be used up to 10 times if the prescribed cleaning procedure is utilized after each use. However, the membrane lifecycle is dependent on the vessel's internal environment and vessel operating conditions while the membrane is employed.

Appendix B FISP® Part Numbers

FISP Cell-Free and Cell-Containing Sampling Probe

Cell-Free Sampling: FISP® Sample Probes

In-situ sampling probe capable of withdrawing sterile, cell free samples from all types of bioreactors and fermenters. Allows direct on-line sample transfer from the vessel to the Seg-Flow System or other on-line analyzers and sample collectors.

Part Number	Description	
-	**D Series FISP® Sampling Probe** **Used for SIP/CIP vessels.** **For 1.5 inch Sanitary ports, use the 1.5 inch Sanitary Fitting adapter and F-series FISP probe (see accessories section below)** All D series Probes come fully assembled with ceramic membrane, o-rings, stainless steel ¼ inch tubing adapter and PEEK female luer to 10-32 male fitting. Allen wrench included.	
P-D25-90 -03	25 mm FISP Ceramic Sampling Probe, 90 mm Immersion (Includes needed fittings to connect to Seg-Flow) Fits 40 mm Ingold port depth.	
P-D19-90 -03	19 mm FISP Ceramic Sampling Probe, 90 mm Immersion (Includes needed fittings to connect to Seg-Flow) Fits 40 mm Ingold port depth.	Same configuration as P-D25-90-03, 25 mm FISP Sampling Probe
P-D25-115-03	25 mm FISP Ceramic Sampling Probe, 110 mm Immersion (Safety Port) (Includes needed fittings to connect to Seg-Flow) Fits 52 mm Ingold port depth. (Safety Port)	
-	**F Series FISP® Sampling Probe** Fits both 12 mm and 19 mm headplate ports. **For 19 mm ports, part A-F-07, 12 to 19 mm Adapter for PG 13.5 threads, is required.** All F series Probes come fully assembled with ceramic membrane, o-rings, PEEK female luer to 10-32 male fitting and PG13.5 nut. Black PEEK plug and Allen wrench included.	
P-F12-120-02	12 mm FISP Ceramic Sampling Probe, 120 mm Immersion Length	
P-F12-200-02	12 mm FISP Ceramic Sampling Probe, 200 mm Immersion Length	
P-F12-310-02	12 mm FISP Ceramic Sampling Probe, 310 mm Immersion Length	
P-F12-410-02	12 mm FISP Ceramic Sampling Probe, 410 mm Immersion Length	
Cell Containing Sample Probes		
-	Fits both 12 mm and 19 mm headplate ports. **For 19 mm ports, part A-F-07, 12 to 19 mm Adapter for PG 13.5 threads, is required. May be used up to 10 autoclave or SIP cycles.**	
P-DT12-200	12mm SS for Cell Containing Samples, 200mm	
P-DT12-350	12mm SS probe for Cell containing samples, 350mm Immersion Length	

P-DT12-500	12mm SS probe for Cell containing samples, 500mm Immersion Length	

FISP Sampling Probe Consumables

A-MR-01	0.2um 2" long Ceramic Membrane [2/Pkg] *Custom pore sizes available by request.*	
A-OR-01	Ceramic Membrane O-rings (D & F-series) [10/Pkg]	
A-OR-02	25 mm main shaft O-rings EPDM (D -series) [10/Pkg]	
A-OR-03	19 mm main shaft O-rings EPDM (D-series) [10/Pkg]	
A-OR-04	12 mm Teflon® washer with O-ring (F-series) [3/Pkg]	
A-F-03	10-32 PEEK plug (F-series) [5/Pkg]	
A-F-04	10-32 PEEK nut & Ferrule Combo (F-series) [5/Pkg]	
A-F-12	PEEK Female Luer to 10-32 Male Fitting [5/pkg]	
A-H-01	End Cap Screw [5/Pkg]	

FISP Sampling Probe Adapters

A-F-06	YSI Tubing Adapter [5/Pkg]	
A-F-07	12 to 19 mm Adapter for PG 13.5 threads	
A-F-11	Luer Adapter Fitting for D-series Probes **(Required if a 19 or 25 mm probe with part no. P-D19-90-02 or P-D25-90-02 or P-D25-115-02 is used with a Seg-Flow)**	
H-F-051	1.5” Sanitary Flange to PG 13.5 Adapter, 316 Stainless Steel **Must be used with F-series FISP probe.** **Includes 316SS shut-off valve for FISP probe outlet.**	
H-F-052	2.0” Sanitary Flange to PG 13.5 Adapter, 316 Stainless Steel **Must be used with F-series FISP probe.** **Includes 316SS shut-off valve for FISP probe outlet.**	

H-F-062	PG13.5 Male Thread to 12mm Compression Fitting	

FISP Sampling Probe Adapters (Continued)

H-F-063	12 to 25 mm Ingold Port Adapter For use with 200 mm F-series FISP sampling probe (P-F12-200-02) or 225 mm 316 SS sampling probe (P-DT12-225). **Must specify port depth: 40 mm or 52 mm**	
H-F-064	FISP F-Series Shut-Off Valve, 316 SS For use with any F-series FISP sampling probe or 316 SS sampling probe	

FISP Sampling Probe Replacement Parts

H-F-001	Retaining Nut, PG 13.5 316 Stainless Steel (F-series)	
H-F-002	Retaining Nut, 25mm (D-series)	
H-H-001	D-Series Plug Valve	
H-W-001	Hex Wrench	

FISP Sampling Probe Kits

K-D25-03	25 mm Probe Starter Kit Kit include (2) Ceramic Membranes, (10) 25mm Main Shaft O-rings, (10) Membrane Shaft O-rings, (5) 10-32 PEEK Nut and Ferrule Combo, & (5) End Cap Screws
K-D19-03	19 mm Probe Starter Kit Kit include (2) Ceramic Membranes, (10) 19mm Main Shaft O-rings, (10) Membrane Shaft O-rings, (5) 10-32 PEEK Nut and Ferrule Combo, & (5) End Cap Screws
K-F12-02	12 mm Probe Starter Kit Kit include (2) Ceramic Membranes, (3) 12mm O-rings and Teflon Washers, (10) Membrane Shaft O-rings, (5) 10-32 PEEK Plugs (5) 10-32 PEEK Nut and Ferrule Combo & (5) End Cap Screws

Appendix C Applications & Sampling Flow Rates

The FISP sampling probe technology has been used for various sampling and fluid management applications at lab, pilot and production scale. These applications include:

1. Vessel sampling for on-line process analytics (with and without the SegFlow system)
2. In-line microfiltration for off-line analytical sample collection
3. Small-scale perfusion systems
4. Reverse flow through ceramic membrane

Although typical sampling flow rates used with the RapidFlow™ ceramic membrane range between 0.5 – 2.0 ml/minute, it is the end-user's responsibility to determine the appropriate flow rate for their unique application and process condition.

The data shown in the following table has been generated by end users who have utilized the FISP technology for a minimum of one year. The majority of these results are from processes that have been in place for 2 or more years. Additionally, several of these FISP applications have been cGMP validated. The data provided should be used as a guide only.

FISP Sampling Flow Rates – Ceramic Membrane

Organism	Process Time (Hr)	Flow Rate (ml/min)
Bacterial Fermentation		
E. Coli K12	16	1.0
E. Coli K12	24 - 72	0.75
E. Coli K12	28	2.0
E. Coli K12	18	2.0
E. Coli recombinant.	40	1.5
Bacillus licheniformis	36	1.2
Bacillus subtilis	20	2.0
Mycobacter	45	1.0
Streptococcus pneumoniae	9	1.0
Fungal Fermentation		
Acremonium chrysogenum	160	1.0
Aspergillus awamori	180	1.0
Aspergillus niger	190	1.0
Cephalosporium acrimonium	200	1.0
Penicillium chrysogenum	200	0.7
Yeast Fermentation		
S. cerevisiae	48	1.0
S. cerevisiae	260	0.8
S. cerevisiae	24 - 150	1.0
Mammalian Cell Culture		
BHK 21, CHO	42 (Days)	0.5
Mouse hybridoma	41 (Days)	0.4
NSO	37 (Days)	0.75
Insect Cell Culture		
Spodoptera frugiperda	200	1.5
Spodoptera frugiperda	280	1.0
Spodoptera frugiperda	200	0.8

Appendix D Animal Derived Component Free Statement

FISP® Ceramic Membrane Animal Derived Component Free Certification

PRODUCT NAME: FISP® Ceramic Membrane

PRODUCT PART NUMBER: A-MR-01

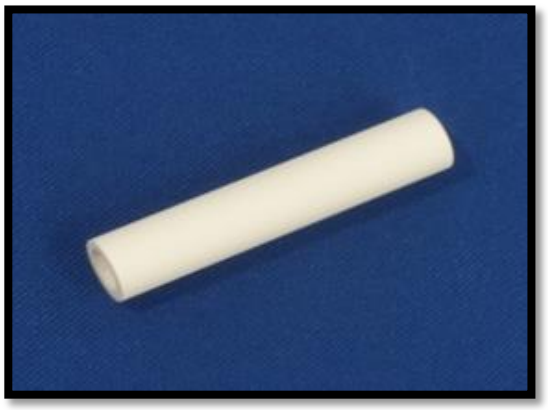
PRODUCT DESCRIPTION: Ceramic Membranes, 2/pkg

ANIMAL DERIVED COMPONENT FREE STATEMENT

No animal derived components or materials are used in the ceramic membrane manufacturing process. This certification applies to all FISP® ceramic membranes. The membranes are considered safe for use in pharmaceutical, biopharmaceutical, food and beverage applications.

Appendix E Membrane Certificate of Conformance

Every lot of ceramic membranes undergoes extensive physical and performance testing before release. A Certificate of Conformance is issued with each released batch to ensure adherence to specifications and for lot traceability. A Certificate of Conformance is provided upon request.



Appendix F Technical Assistance

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