

# BIOPROFILE® FLEX2

## ESM Instructions for Use Manual



*nova*  
biomedical®



## Preface

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### BioProfile® FLEX2 ESM Instructions for Use Manual

#### Ordering Information

The *BioProfile® FLEX2 ESM Instructions for Use Manual* can be ordered from Nova Biomedical Order Services. Write or call:

Nova Biomedical  
200 Prospect Street  
Waltham, MA 02454-9141 U.S.A.

Telephone: 1-800-822-0911  
FAX: 1-800-316-1178 (in the U.S.A.) or  
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## 1 Introduction

This manual provides all necessary instructions for the routine operation and maintenance of the BioProfile FLEX2 External Sampling Module (ESM). Please read this manual carefully. It has been prepared to help you achieve optimal performance from your External Sampling Module. If the ESM is used in a manner not specified by Nova Biomedical, the safety and/or use of the equipment may be impaired. When used properly, the ESM is designed to maintain the sterility of the cell culture vessel(s) to which it is interfaced.

The BioProfile FLEX2 ESM is designed to be used as an accessory sample handling device for the BioProfile FLEX2 Analyzer. The ESM serves to integrate the BioProfile FLEX2 Analyzer with other commercially available cell culture sample handling systems not manufactured by Nova Biomedical. This manual details the integration of the FLEX2 with the ambr® 15 Cell Culture or ambr® 250 High-Throughput microbioreactor systems from Sartorius Stedim Biotech. This section introduces the ESM and covers the intended use and operational overview, including system requirements for both the FLEX2 and ESM.

**NOTE:** *Nova Biomedical is only responsible for the service and support of those devices exclusively manufactured by Nova Biomedical. All service and support of any external device or system connected to or integrated with the BioProfile FLEX2 ESM is the responsibility of the End User and/or the external system manufacturer.*

### 1.1 About This Manual

This manual is for the Nova Biomedical BioProfile FLEX2 ESM. Throughout this manual:

**NOTE** indicates especially important information.

**CAUTION** indicates information that is critical to avoid system damage or incorrect sample handling.

**WARNING** indicates possible hazard to the operator.

### 1.2 Safety

Personnel operating the BioProfile FLEX2 + ESM must be proficient in the operation and maintenance procedure for the device. The following safety procedures must be followed:

#### General Safety

1. Read the safety and operating instructions before operating the system.
2. Retain the safety and operating instructions for future reference.
3. Observe all warnings on the ESM and in the operating instructions.
4. Follow all operating and use instructions.
5. Do not install the system within 5 feet of a water source.
6. Use only on a bench or a stand that is recommended by the manufacturer.
7. Place the system so that its location or position does not interfere with its proper ventilation.

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8. Place the system away from heat sources.
9. Connect the system to a power supply only of the type described in the operating instructions or marked on the device.
10. Do not defeat the safety purpose of the polarized or grounding type plug.
11. Route power and communication cords so that they are not likely to be walked on or pinched by items placed upon or against them, paying particular attention to cords at plugs, power sockets, and at the point where they exit from the system.
12. The system should be cleaned only as recommended by the manufacturer.
13. Take care not to let objects or liquids fall into the system.
14. The ESM should be serviced only by qualified service personnel.
15. Do not attempt to service the system beyond that described in the operating instructions. All other servicing should be referred to qualified service personnel.
16. Do not attempt to remove the system cover or the syringe pump safety guard.

### Electrical Safety

1. To reduce the risk of electric shock, do not remove the system cover.
2. There are NO operator serviceable parts inside the ESM.
3. Servicing must be performed only by qualified service personnel.
4. Before changing the fuses, unplug the power cord.
5. Replace the fuses with only the same type and rating.
6. To reduce the risk of fire or electric shock, do not expose the system to water.
7. The ESM is supplied with a main, non-rewireable plug for the intended country.
8. Ensure that the wall outlet receptacle is properly wired and earth grounded.
9. DO NOT use a 3-to-2 wire plug adapter.
10. DO NOT use a 2-wire extension cord or a 2-wire multiple-outlet power strip.

### Chemical and Biological Safety

1. Observe all precautionary information printed on the original reagent container.
2. Operate the system in the appropriate environment.
3. Take all necessary precautions when using toxic materials to prevent the generation of aerosols.
4. Wear appropriate laboratory attire, e.g., safety glasses, gloves, lab coat, and breathing apparatus when working with hazardous materials.
5. Dispose of all waste solutions according to company standard operating procedures.
6. The ESM Reagent Cartridge contains a waste pouch where biological material will be collected. This waste pouch is considered a biohazard and should be disposed of according to company procedures.

**WARNING:** *Cell culture samples are potential sources of infectious agents. Handle all sample and flow path components (Sample Cup, Sample Tubing, Reagent Cartridge, etc.) with care. Gloves and personal protective equipment are recommended.*



## 1.3 System Requirements

### Electrical Requirements

- Two grounded, 3-wire receptacles within 5 feet of the system are required for operation.
- It is recommended that the BioProfile FLEX2 Analyzer and the ESM are connected to a 1100 – 1200 VA Universal Power Supply (UPS) to maintain the power load in the event of a power outage.

### FLEX2

- *Operating Voltage:*
- *Operating Frequency:*
- *Nominal Power Consumption (without Osmo):*
- *Nominal Power Consumption (with Osmo):*
- *Fuses (2):*

### Fuse Requirements

90 - 264 VAC  
50 - 60 Hz  
480 Watts  
590 Watts  
Slo-Blo (SB) 8A or T8A/250V

### ESM

- Operating Voltage:
- Operating Frequency:
- Fuse Rating:

100 to 120, 220 to 240 VAC  
50 - 60 Hz  
Slo-Blo (SB) 2A or T8A/250V

### Environmental Specifications

- The BioProfile FLEX2 ESM should be operated indoors. Keep the working area around the system free of dirt & debris, corrosive fumes, and excessive temperature changes.
- Ambient operating temperature between 15°C to 30°C (59°F to 86°F)
- Altitude up to 2500 meters (8202 Feet)
- Relative Humidity of 20 – 85% (without condensation)
- Installation Category (II)
- Pollution Degree (2)

### Dimensions (ESM unit only)

- Height: 6.9 in. (17.53 cm)
- Width: 9.7 in. (24.64 cm)
- Depth: 18.2 in. (46.23 cm)

### Weight:

- 8.6 lbs. (3.9 kg) Without Reagent Cartridge
- 17.8 lbs. (8.1 kg) With Reagent Cartridge

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## 1.4 Intended Use and Tests Performed

The BioProfile FLEX2 External Sampling Module is intended for automatic delivery of cell culture or media samples to the BioProfile FLEX2 Analyzer from a fluid handling device that is capable of both delivering samples to the ESM Sample Cup and communicating with the FLEX2 Host application software, such as the ambr® 15 or ambr® 250 automated bioreactor systems from Sartorius Stedim Biotech. Integration of the BioProfile FLEX2 analyzer and any applicable external fluid handling device via the ESM enables an automated “online” analysis for quantitative determination of the 16 key cell culture parameters offered by the BioProfile FLEX2 system (depending on module configuration), including: pH, pO<sub>2</sub>, pCO<sub>2</sub>, glutamine (Gln), glutamate (Glu), glucose (Gluc), lactate (Lac), ammonium (NH<sub>4</sub><sup>+</sup>), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), Total Cell Density (TCD), Viable Cell Density (VCD), Viability (%), Live Cell Diameter, and Osmolality (Osm). Using the directly measured results and the vessel temperature entered by the operator, the BioProfile FLEX2 + ESM offers the following calculated parameters:

- Temperature Corrected pH (for entered values other than 37°C)
- Temperature Corrected pO<sub>2</sub> (for entered values other than 37°C)
- Temperature Corrected pCO<sub>2</sub> (for entered values other than 37°C)
- Air Saturation
- CO<sub>2</sub> Saturation
- HCO<sub>3</sub><sup>-</sup> (Bicarbonate) Concentration

**NOTE:** *The BioProfile FLEX2 ESM Module is an accessory sample delivery system for the BioProfile FLEX2 Analyzer, not an analytical device.*

## 1.5 Operation Overview

Sampling is performed by the ambr® liquid handler robot arm, which draws a sample from a selected ambr® vessel and deposits it into the ESM sample cup. The ESM automatically transfers the sample from the cup to the BioProfile FLEX2 Analyzer for analysis. Sample assay panels are configured on the FLEX2 and made available for selection when scheduling analyses from the ambr® user interface. The FLEX2 communicates with ambr® over TCP/IP to allow for sample scheduling, data transfer, and feedback control. The initialization and maintenance of the ESM is predominantly facilitated through the BioProfile FLEX2 user interface, but some functions may also be performed through the ambr® user interface. All control schemes based on FLEX2 results such as feedback control are managed in the ambr® software.

**NOTE:** *Integration of the FLEX2 with ESM requires an ESM top plate in place of the 96-well plate sampling deck. The ESM top plate houses the ESM sample port, pinch valve, and air detector. Once an ESM is installed, the FLEX2 will no longer offer 96-well plate sampling, but Manual and Load-and-Go Carousel sampling will still be available.*



Figure 1.1. FLEX2 + ESM integrated with ambr® 15 system

## 2 System Details

### 2.1 ESM15 vs. ESM250

The ESM15 serves to integrate the FLEX2 with the ambr® 15, and the ESM250 serves to integrate the FLEX2 with the ambr® 250 automated bioreactor system. In either configuration, the components, set up, operation, and maintenance required for the External Sampling Module are largely the same, with a few key differences. When pertinent, sections in this manual will use "ESM15" or "ESM250" notation to distinguish between different sets of guidelines. In sections where the External Sampling Module is referred to generally as "ESM," one can assume the guidelines are shared between both the ESM15 and ESM250 configurations.

### 2.2 ESM System Details

All flow components and consumables critical to ESM operation are shared between the ESM15 and ESM250. The key difference lies in the ESM software, which is customized for the integrated fluid handling system (i.e. ambr® 15 or ambr® 250). The External Sampling Module consists of the components listed below and pictured in Figures 2.1 and 2.2.

- A multi-port syringe pump that draws the sample from the ESM sample cup to the sample port inside the BioProfile FLEX2 Analytical Unit (AU) and controls the flow of ESM reagents from the ESM Reagent Cartridge.
- An External Tubing Set (replaceable by the End User) for delivery of sample material from the ESM sample cup to the FLEX2, supply of System Fluid solution and Depro solution from the reagent cartridge to the ESM sample cup for flow path cleaning, and evacuation of residual sample waste.
- A Reagent Cartridge Bay with a needle shroud manifold and internal tubing for supply of reagents from the ESM Reagent Cartridge used for flow path priming and cleaning, and for evacuation of waste.
- A COM port for communication between the ESM and FLEX2 and control of hardware components.
- An AD/Valve port for control of ESM flow components.
- A power entry module for independent power supply to the ESM.



Figure 2.1 ESM Front View



Figure 2.2 ESM Rear View

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The ESM is installed using an accessory pack which is configured for either ambr® 15 or ambr® 250 integration. In either case, the accessory pack contains the following components necessary for FLEX2 + ambr® integration:

- An ESM Reagent Cartridge that contains:
  - System Fluid Solution
  - Internal Waste Collection Pouch
  - Deproteinizing Solution
  - Performance Check Solution
- A power cable for separate power supply to the ESM
- A serial cable for communication between the ESM and FLEX2
- An AD/VALVE cable for control of flow components
- A Connectivity Kit for communication between the Bridge computer and ambr® computer (See Section 3.3 for more information)
- A sample cup for sample delivery from ambr®

### 2.3 ESM Sample Cup

While the reagent cartridge and required communication and power cables are shared between the ESM15 and ESM250, the key difference between the two accessory packs is the ESM sample cup. The acrylic sample cup is provided by Nova and mounted by Sartorius on the ambr® deck. It serves as the transfer point where the ambr® robot arm deposits a cell culture sample for delivery to the FLEX2. There are two different sample cups—one that is suitable for both ambr® 15 and ambr® 250 integration (PN 59975, Fig. 2.3A), and one that is designed for the ambr® 15 + Analysis Module (PN 60094, Fig. 2.3B). The ESM15 accessory pack includes both cups to accommodate either configuration (i.e. ambr® 15 with or without Analysis Module); whereas the ESM250 accessory pack includes only PN 59975, which is suitable for integration with the ambr® 250 with or without an Analysis Module.

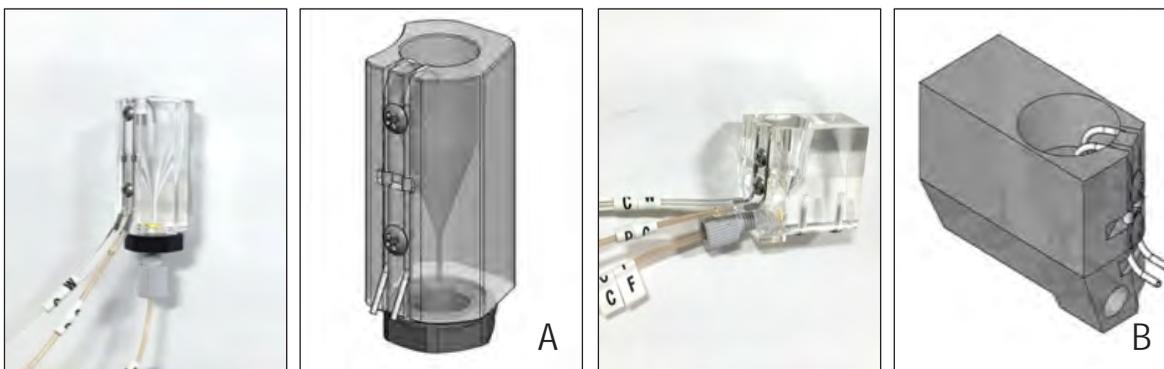


Figure 2.3 ESM Sample Cup for A) ambr® 15/250, PN 59975 B) ambr® 15 + Analysis Module, PN 60094

### 2.4 FLEX2 + ESM/ambri® Integration Overview

The BioProfile FLEX2 ESM is part of an integrated system as shown in Figure 2.4. An online analysis is scheduled from the ambr® computer, and the ambr® robot arm draws a sample from the selected microculture vessel, positions itself above the ESM sample cup and deposits the sample. The ESM syringe pump automatically drains the sample from the cup, drawing it to the ESM air detector inside the FLEX2 analytical unit to ensure there is sufficient sample volume. Then, the ESM pinch valve closes, and the FLEX2 sample probe aspirates the sample from the ESM sample port. Sample analysis by the FLEX2 proceeds as usual, depending on the modules configured for the selected FLEX2 Sample Type. Residual sample is deposited into the ESM reagent cartridge waste pouch by the ESM syringe pump. The ESM then performs a routine flowpath cleaning using reagents from the ESM cartridge. Measured and calculated sample results are stored in the FLEX2 Historical Results and transmitted to the ambr® computer.



Figure 2.4 System Integration Overview

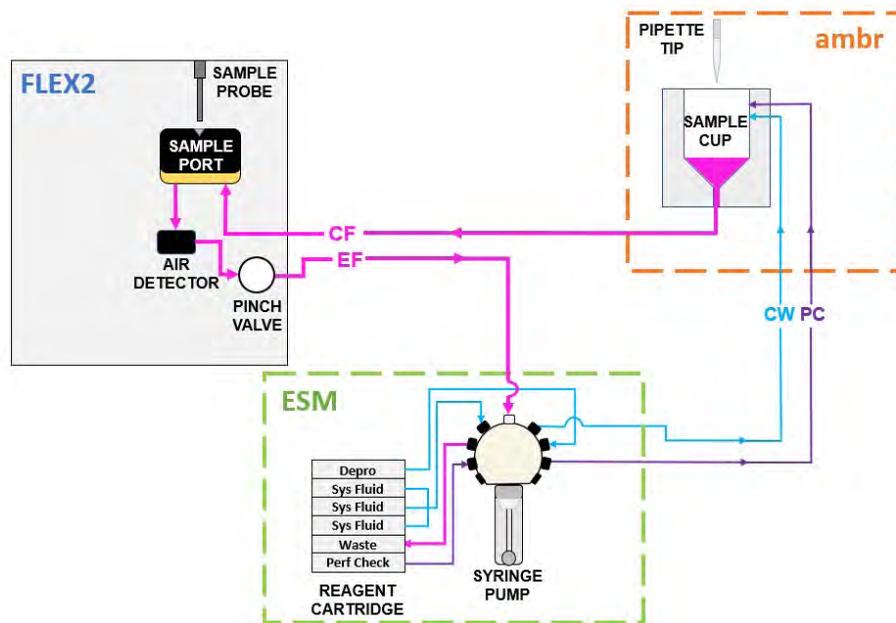


Figure 2.5 FLEX2 + ESM/ambri® Integration Diagram

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### 3 Implementation

This section serves as a site preparation and implementation guide for the End User when purchasing a BioProfile FLEX2 + ESM system for integration with an ambr® 15 or ambr® 250 microbioreactor system. It provides details concerning spatial requirements and networking considerations. The End User is responsible for site preparation leading up to the installation, which may involve: 1) Procuring a sterile hood or biosafety cabinet (BSC) and preparing access holes on the left side of the hood (ambr® 15 only), and/or 2) Verifying there is adequate bench space for the FLEX2 + ESM to the left of the ambr®. It is the End User's responsibility to prepare this space by obtaining a bench or table to hold the FLEX2 + ESM and ensuring accessibility of a power supply and corporate/laboratory network port (if so desired). Note, electrical and environmental specifications for both pieces of Nova Biomedical equipment are outlined in Section 1.3. For specific information about the ambr® 15 or ambr® 250 system, please contact Sartorius.

**CAUTION:** *The FLEX2 + ESM should be installed on a stable, vibration-free surface. The system should not be installed on the same bench top or within proximity to any high-speed centrifuge system. Since these systems often create a significant amount of vibration, placement of the BioProfile FLEX2 and/or BioProfile ESM near or on the same bench top may impact sample results. Ensure the surface holding the FLEX2 + ESM does not contact the chiller or ambr® system.*

#### 3.1 ESM Installation

The scheduling and implementation of events during ESM installation requires a coordinated effort and will be dependent upon what equipment is available at a given time. Ideally, the FLEX2, ESM, and ambr® would all be present, and both a Nova and Sartorius representative would be on site together for a coordinated installation of all equipment. When this is not the case, all parties will carry out their respective responsibilities. The BioProfile FLEX2 analyzer can be installed with the ESM Module included, or it can be upgraded to ESM-Ready configuration later. The BioProfile FLEX2 ESM should only be installed by a trained and qualified Nova Field Support Specialist (FSS). During installation, the FSS will unpack the ESM and place it in position, upgrade the FLEX2 hardware to ESM-Ready configuration (if needed), ensure the FLEX2 software is V3.2 or newer, install the required ESM consumables, and verify FLEX2 + ESM flow.

The ESM sample cup is included in the accessory pack provided by Nova Biomedical at the time of installation. Although the cup is supplied by Nova, Sartorius is responsible for sample cup installation as they must teach the cup's location to the ambr®'s liquid handler arm and plumb fluid lines from the cup to the FLEX2 + ESM once it is situated on the ambr® fluid deck. Lastly, Sartorius and Nova personnel will work together to verify communication between the FLEX2 Bridge computer and ambr® control computer.

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## 3.2 Spatial Considerations

This section serves to inform the End User of the FLEX2 and ESM component dimensions and tubing specifications so that the required space next to the ambr® system can be prepared prior to install.

### 3.2.1 FLEX2 + ESM Component Dimensions

Measurement	FLEX2 Without Osmometer	FLEX2 With Osmometer	ESM
Height	23.5 in. (59.69 cm)	23.5 in. (59.69 cm)	6.9 in. (17.53 cm)
Width	16.75 in. (42.55 cm)	24.75 in. (62.86 cm)	9.7 in. (24.64 cm)
Depth	25.0 in. (63.50 cm)	25.0 in. (63.50 cm)	18.2 in. (46.23 cm)
Weight*	less than 125 lbs. (56.7 kg)	less than 145 lbs. (65.8 kg)	17.8 lbs. (8.1 kg)

\*With applicable reagent cartridge(s) installed

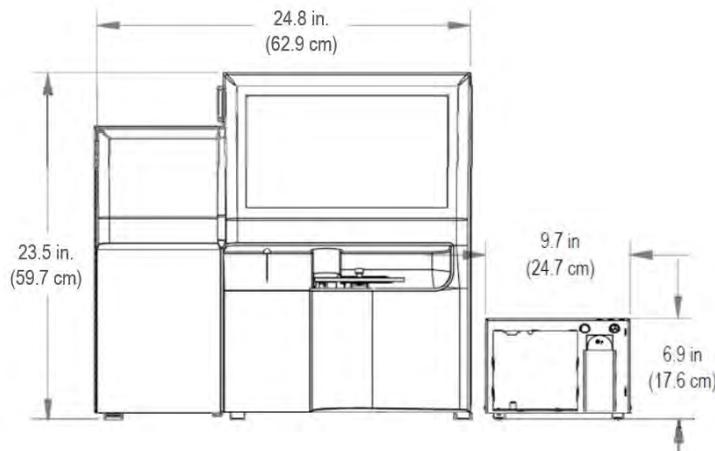


Figure 3.1 Front View- FLEX2 + Osm + ESM

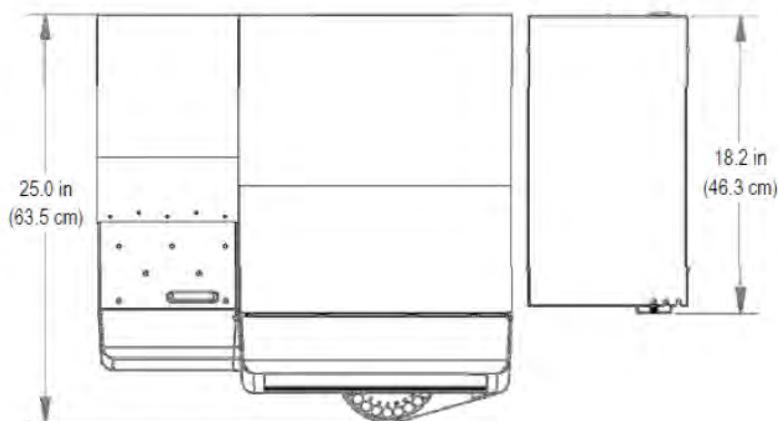


Figure 3.2 Top View- FLEX2 + Osm + ESM

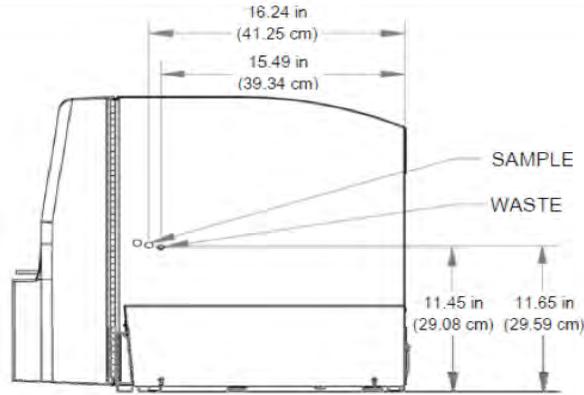


Figure 3.3 Right Side View- FLEX2 + Osm + ESM

## 3.2.2 ESM Fluid Line Details

The table below details the different lines of tubing that comprise the ESM External Tubing Set (PN 59209). It should be noted that there is a single part number for the ESM Tubing Set, whether it is to be installed on an ESM15 or ESM250. The FLEX2 + ESM must sit directly to the left of the ambr®; and their positioning in relation to the ambr® is restricted by the lines in red. The FLEX2 and ESM must be close enough together such that the Waste (EF) line is not under strain, and the FLEX2 must be close enough to the ambr® such that the Sample (CF) line is not under strain.

Table 3.2 ESM Fluid Line Details			
Tubing Line	Length	Connection	Function
Sample (CF)	36 in. (91.4 cm)	Right side of FLEX2 cover to ESM Sample Cup (mounted on ambr® deck)	Drains contents of Sample Cup and delivers it to FLEX2 for analysis
Waste (EF)	22 in. (55.9 cm)	Right side of FLEX2 Cover to ESM Syringe Pump	Evacuates sample waste from FLEX2 + ESM flowpath
Cup Wash (CW)	48 in. (121.9 cm)	ESM Syringe Pump to spout on ESM Sample Cup	Delivers fluid from ESM Reagent Cartridge to Sample Cup for priming and cleaning
Performance Check (PC)	48 in. (121.9 cm)	ESM Syringe Pump to spout on ESM Sample Cup	Delivers PC solution from ESM Reagent Cartridge to Sample cup for analysis by the FLEX2

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## 3.2.3 ESM15/ambr® 15 Spatial Requirements

The FLEX2 and ESM15 must be placed on a surface to the left of the ambr® 15 system. The maximum distance the FLEX2 can reside from the ambr® is constrained by the sample (CF) tubing line which runs from the base of the sample cup to the right-side cover of the FLEX2. The FLEX2 + ESM must be close enough together such that the waste (EF) tubing line, which runs from the FLEX2 right side cover to the ESM syringe pump, is not stretched or crimped. If the ambr® resides inside a laminar flow hood or biosafety cabinet (BSC), the ambr® should reside as close to the left side wall as space will allow. Additionally, it is recommended that any valves or other protruding structures on the left side BSC wall be removed or relocated, as they may interfere with placement of the ambr® 15 and proper routing of the ESM tubing lines. The estimated bench space needed to the left of the ambr® 15 for the FLEX2 + ESM15 is provided in Tables 3.3 and 3.4, and accounts for ESM positioning, FLEX2 ventilation, and FLEX2 front panel door and Osmo door clearance (when applicable).

The FLEX2 + ESM15 components should be arranged on the bench top to the left of the ambr® according to one of the following options:

- **Option 1** – ESM placed between FLEX2 and ambr®.
  - Ensure the right side of the FLEX2 resides no further than 12.0 in (30.4 cm) from the left outer wall of the BSC.

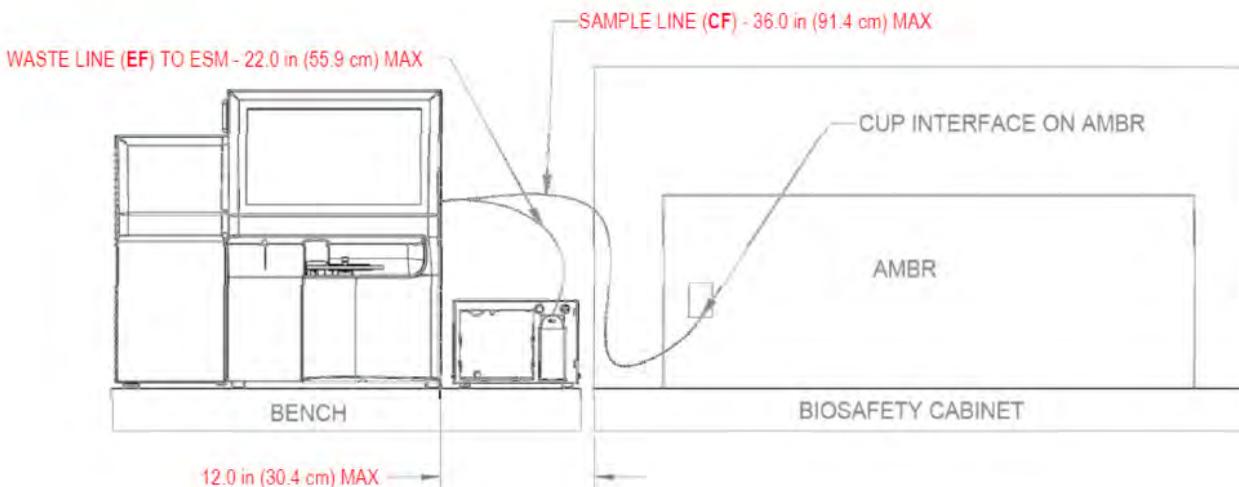


Figure 3.4 Option 1: ESM Between FLEX2 & ambr®

Table 3.3 Estimated Bench Space Required (Left of ambr® 15)- Option 1

Configuration	FLEX2 w/o Osmo	FLEX2 + Osmo
ESM next to FLEX2	34.8W x 31.0D x 29.4H	42.6W x 31.0D x 29.4H
Inches (centimeters)	(88.4W x 78.8D x 74.7H)	(117.4W x 78.8D x 74.7H)

- **Option 2** – ESM placed below FLEX2. This arrangement is required for ambr®15 with Analysis Module.\*
  - When obtaining a shelf or cart to support the ESM, ensure that the top of the ESM resides no further than 4.0 in (10.1 cm) from the base of the FLEX2.
  - Ensure that the right side of the FLEX2 resides no further than 5.0 in. (12.7cm) from the left outer wall of the BSC.
  - The right side of the FLEX2 and the right side of the ESM should be approximately in-line vertically.
  - The FLEX2 should be positioned forward (proud) enough so that the main door is not impeded when opening or closing.

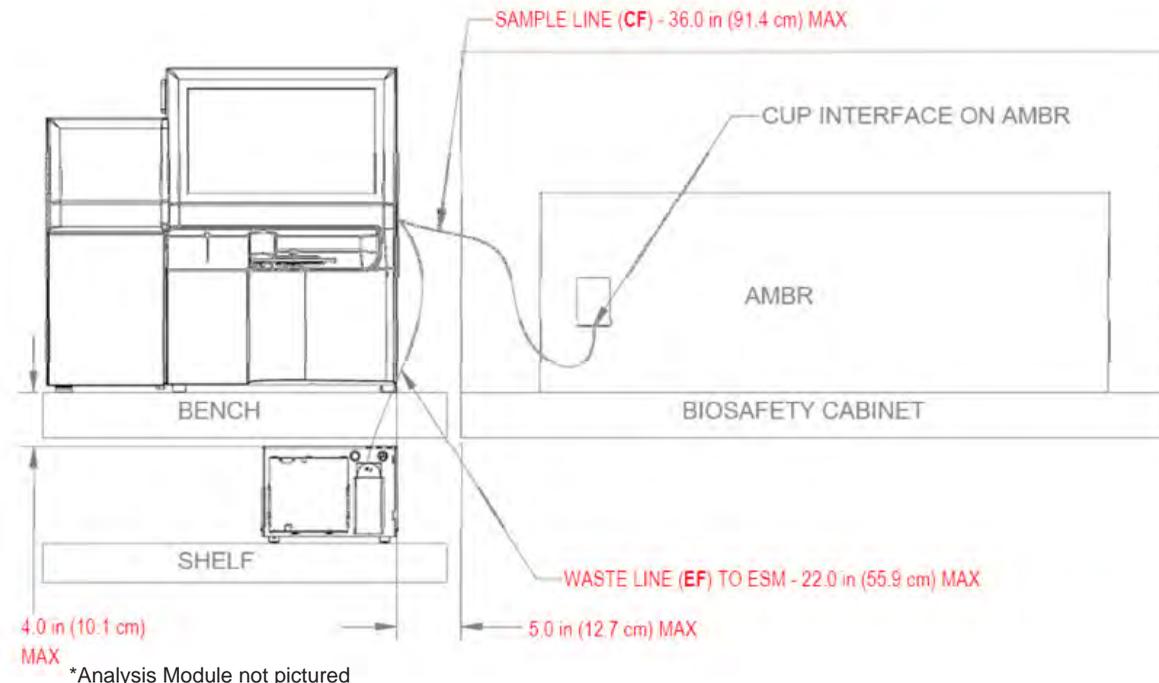


Figure 3.5 Option 2: ESM Below FLEX2

Table 3.4 Estimated Bench Space Required (Left of ambr® 15)- Option 2

Configuration	FLEX2 w/o Osmo	FLEX2 + Osmo
ESM below FLEX2	27.8W x 31.0D x 29.4H	36.2W x 31.0D x 29.4H
Inches (centimeters)	(70.6W x 78.8D x 74.7H)	(92.0W x 78.8D x 74.7H)

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## 3.2.3.1 Biosafety Cabinet Preparation (ambr® 15)

An existing sterile hood or biosafety cabinet may need preparation prior to installation of ambr® 15 and subsequent integration with FLEX2 + ESM, unlike ambr® 250 which is installed as a standalone biosafety cabinet. If an access opening on the left-side wall of the hood does not already exist, one must be drilled in order to route the ESM External Tubing Set (CF, CW, and PC lines) from the sample cup mounted on the ambr® 15 deck out to the FLEX2 + ESM.

Care should be taken to position the access hole so that it creates the shortest distance for the Sample (CF) line. As a general guideline, both the height and the depth of the access hole should be halfway between the inlet port on the right side of the FLEX2 and the sample cup on the ambr® 15 deck. Use the diagram below as an additional reference.

If desired, a split gland-nut assembly supplied by Nova (PN 60251) may be purchased separately to seal the access hole. The CF, CW, and PC lines can be routed together through a single gland-nut assembly.

**NOTE:** *It is the responsibility of the End User to prepare the FLEX2 + ESM bench space and Biosafety Cabinet access hole(s) prior to ESM installation.*

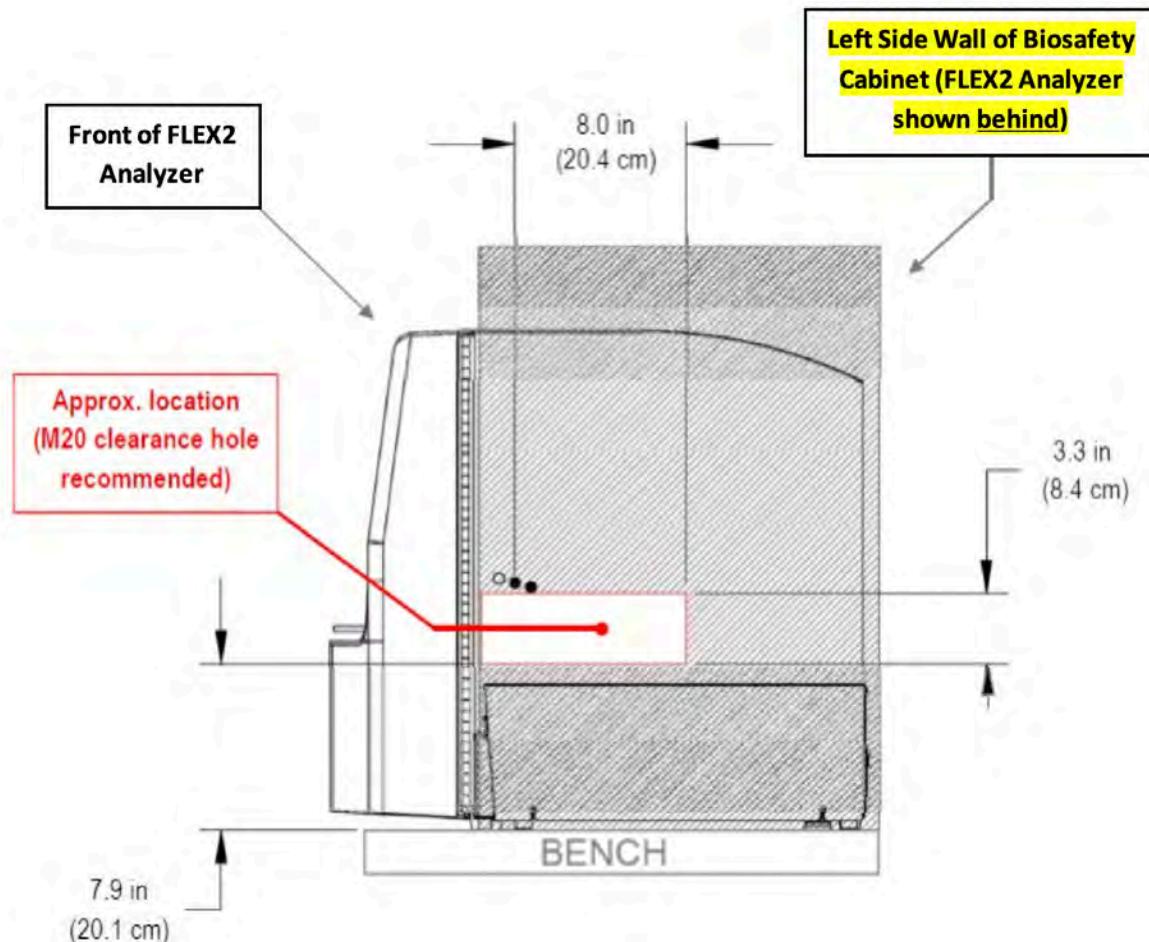


Figure 3.6 Left Side Wall of BioSafety Cabinet

## 3.2.4 ESM250/ambr® 250 Spatial Requirements

The FLEX2 ESM250 sits on a shelf mounted directly to the left wall of the ambr® 250 BSC. As with ambr® 15 integration, the FLEX2 must be placed on a table/bench to the left of the ambr® 250 BSC, and FLEX2 positioning is constrained by the length of external tubing lines listed in Table 3.2. The FLEX2 must be close enough to the BSC and ESM such that the Sample (CF) and Waste (EF) fluid lines are not stretched or crimped.

- Ensure that the right side of the FLEX2 resides no further than 20 in. from the left outer wall of the BSC.
- The FLEX2 should be positioned such that the main front panel door is not impeded by the BSC wall or ESM/shelf when opening or closing.

**NOTE:** The ESM will always sit on the shelf mounted to the left cover of the ambr® 250 regardless of whether an Analysis Module is present on the ambr® 250.

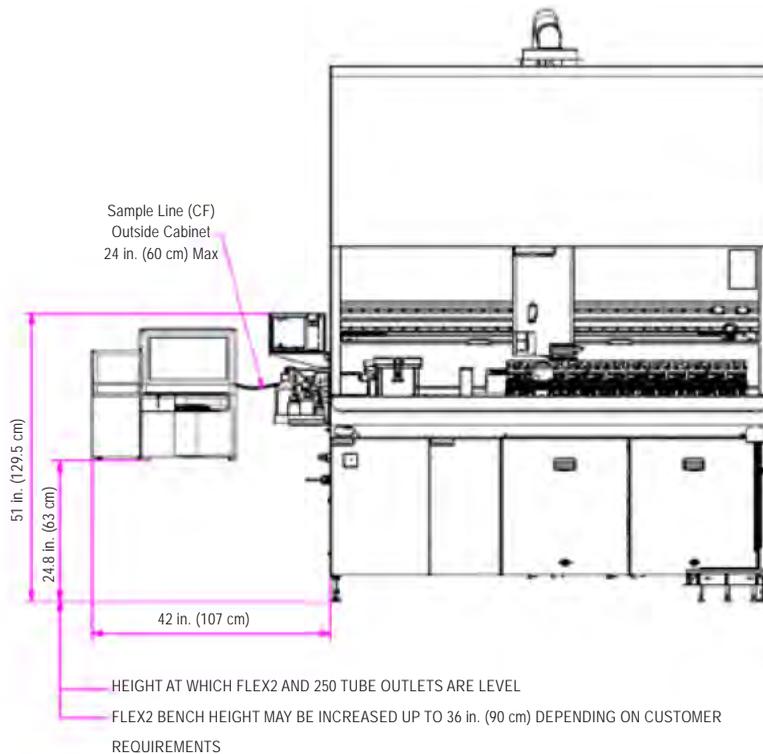


Figure 3.7 FLEX2 + Osmo + ESM integrated with ambr® 250 ht + AM

Table 3.5 Estimated Bench Space Needed to the Left Side of ambr250® Biosafety Cabinet				
ambr® configuration	No Analysis Module		With Analysis Module	
FLEX2 Configuration	FLEX2 w/o Osmo	FLEX2 + Osmo	FLEX2 w/o Osmo	FLEX2 + Osmo
ESM on shelf	29 in. (73.0 cm)	37 in. (94.0 cm)	34 in. (86.0 cm)	42 in. (107.0 cm)

## 3.3 Connectivity Requirements

The FLEX2 and ambr® control computer communicate over TCP/IP to allow for the FLEX2 + ESM to receive sampling instructions from and be managed remotely by the ambr® computer. This also enables the ambr® software to receive measured and calculated sample results from the FLEX2 following an analysis.

The ESM accessory pack includes a connectivity kit to network the FLEX2 + ESM with the ambr® computer and/or the End User's local area network. The components are listed below. Aside from at least one network cable, the remaining items are optional and may or may not be needed, depending on which configuration is desired.

### ESM Networking Kit:

- **Network cable- 20 ft. (6.1 m)**
- **Network cable- 6 ft. (1.8 m)**
- **Ethernet switch- 5 port**
- **C2G Ethernet-to-USB 3.0 adaptor**



Figure 3.8 NIC on rear of FLEX2 for Ethernet cable connection to ambr® computer or LAN.

The first possible arrangement involves a single network cable connecting the FLEX2 directly to the ambr® control PC, using the available NIC on the rear of the FLEX2 (Fig. 3.8). In this case, the FLEX2 is not connected to the corporate or laboratory network.



Figure 3.9 Option 1: Single network cable connecting FLEX2 to ambr®

To arrange the FLEX2 such that it is connected to the ambr® computer and a laboratory/corporate network, one of the following two arrangements must be implemented, since there is only one available NIC on the rear of the FLEX2.

One option involves connecting the ambr® control computer and FLEX2 to the corporate or laboratory network via a network switch. One Ethernet cable goes from the ambr® control computer to the network switch, a second Ethernet cable connects the FLEX2 to the network switch, and a third connects the switch to the laboratory or corporate network port.



Figure 3.10 Option 2: FLEX2 and ambr® connected to lab network via Ethernet switch

In the third arrangement, the FLEX2 and ambr® computer are connected to the network directly. In addition to the Ethernet cable connecting the FLEX2 and ambr® computer to each other, one Ethernet cable is required to connect the ambr® control computer to a laboratory/corporate network port, and another cable is needed to connect the FLEX2 to another available laboratory/corporate network port.

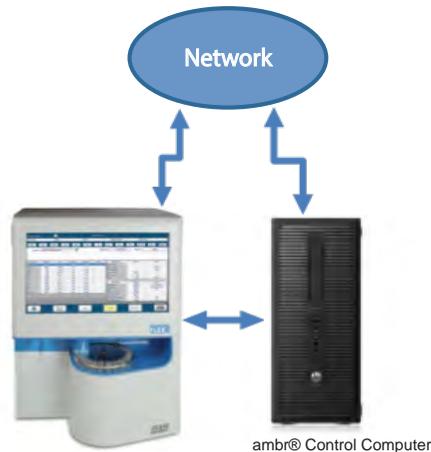


Figure 3.11 Option 3: FLEX2 and ambr® directly connected to network

**NOTE:** The Ethernet-to-USB adapter included in the ESM accessory pack should only be used to connect the FLEX2 to a local area network port. It should not be used for connecting the FLEX2 to ambr® computer.

In any of the three arrangements detailed above, the FLEX2 Bridge and ambr® computer must each be assigned a fixed IP address. During installation, the IP addresses will be configured as listed below, by default. If you wish to use an alternate address, please inform your Nova Service representative during the installation process.

- FLEX2 Bridge PC 192.168.7.4
- ambr® Control PC 192.168.7.1

### 3.4 Date and Time Settings

Communication between the ambr® and FLEX2 + ESM relies on proper synchronization between the ambr® computer and the FLEX2 Bridge/Host computers. The date, time, time-zone, and daylight savings settings on the FLEX2 Bridge computer must match those configured on the ambr® computer, and vice versa. It is important that the times set on each computer are configured to be as close together as possible (at least within one minute of each other). The FLEX2 Bridge computer can be set up to synchronize with the user's network time protocol if connected to a corporate/laboratory network.

# BioProfile FLEX2 ESM Instructions for Use Manual

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### 4 ESM System Operation

#### 4.1 ESM Setup & Initialization

This section details the steps and system checks that should be taken to bring the ESM to a “Ready” state in preparation to receive samples from an external sample handling device.

1. Ensure that the FLEX2 and ESM are each plugged into an appropriate power supply.
2. Verify the AD/Valve cable is secured to the ports on the rear of the ESM and the right cover of the FLEX2. The cable is secured with a locking connector sleeve labeled with a red marker. When installing the cable, align the red marker on the connector with the notch on either cable port and slide into place. To disconnect, slide the connector sleeve back and pull the cable straight out.

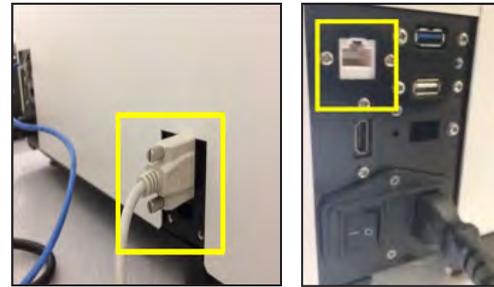


Figure 4.1 FLEX2 COM Port (left) and NIC (right)

**CAUTION:** Twisting the cable/cable connector may cause damage to the system.

3. Verify that the ESM COM cable is properly connected and secured to the COM port on the rear of the FLEX2 and that on the rear of the ESM (Fig.4.1 and 4.2, respectively).
4. Ensure that the Ethernet cable is securely plugged into a port on the ambr® computer and the network port on the rear of the FLEX2 (Fig. 4.1). See Section 3.3 for additional connectivity options.
5. Verify all segments of the ESM External Tubing set are secured in their respective positions (For more information on installing the ESM External Tubing set see Section 5.1.2)
6. To start up the ESM, flip the switch on the rear panel of the ESM to the On position (Fig. 4.2).
7. ESM operation requires the FLEX2 to be powered on. Power on the FLEX2 by flipping the rear switch to the On position. Once the FLEX2 startup sequence is complete, verify that the ESM status icon is present in the FLEX2 status bar. Select the ESM status icon to verify the Connected, Initialized, and Primed status read as "True." For more information regarding ESM System status, refer to Section 4.2.
  - If an ESM reagent cartridge is present when the system is powered on, the ESM syringe pump will begin to pump fluid and will automatically attempt to run an initialization sequence.
  - If no reagent cartridge is installed, the syringe pump will not move, and the ESM will remain in an idle and unready state.



Figure 4.2 ESM Rear View

# BioProfile FLEX2 ESM Instructions for Use Manual

## 4.1.1 ESM Reagent Cartridge Replacement

### Step 1: Septum and Pinch Valve Tubing Installation

A sample port septum and pinch valve tubing must be installed in the BioProfile FLEX2 for the ESM sample to be properly aspirated by the FLEX2 sample probe. A new septum and pinch valve tubing segment are included with each new ESM Reagent Cartridge. Nova Biomedical recommends that these components be installed with each ESM Reagent Cartridge replacement or after extended periods of ESM inactivity.

#### To install/replace septum:

1. Open the FLEX2 main door and locate the ESM sample port inside the FLEX2 Analytical Unit. The sample port is situated on the ESM top plate, in front of the clear, wash/waste well station.
2. Remove the septum cap (black upper portion of the sample port) from the lower amber portion by turning it counterclockwise to expose the septum.
3. Remove the old septum and install a new one.
4. Thread the cap of the sample port back on by turning clockwise until it is finger tight. Do not over-tighten.

**CAUTION:** Do not use pliers or a wrench when tightening the sample port. Over-tightening of the threaded portion may crack or break the assembly.

#### To install/replace pinch valve tubing:

1. Locate the white ESM pinch valve inside the FLEX2 Analytical Unit. The pinch valve is located to the right of the ESM Sample Port.
2. Remove the white tubing from the pinch valve and carefully disconnect the ends of the tubing from each port.
3. Connect each end of the new tubing to the open ports and seat the tubing fully inside the pinch valve.

**WARNING:** Cell culture samples are potential sources of infectious agents. Handle all sample and flow path components with care. Gloves and protective clothing are recommended.

**NOTE:** If the 1/4"-28 barbed fitting swivel adapter (the fitting to which the right end of the pinch tubing is connected) becomes damaged during pinch tubing replacement, Nova offers a Swivel Adapter Replacement Kit (PN 61326).

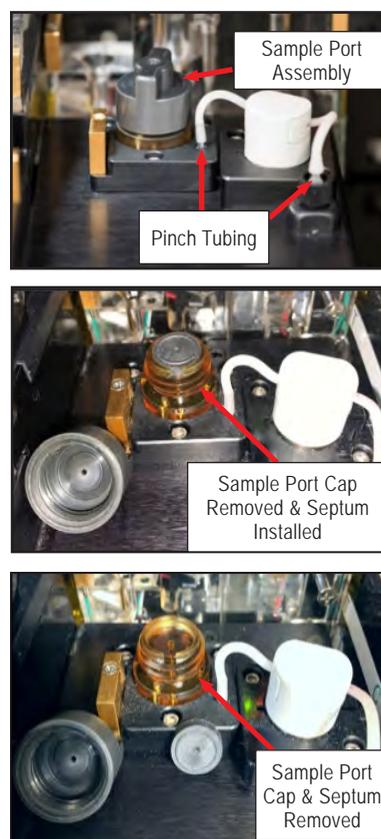


Figure 4.3 ESM Sample Port Assembly

### Step 2: Reagent Cartridge Installation

Inside the ESM reagent cartridge bay is an RFID reader that recognizes if a reagent cartridge is present. An ESM Reagent Cartridge must be installed and recognized by the FLEX2 for the system to be made ready for sampling. If a reagent cartridge is in place when the FLEX2 + ESM is powered on, the FLEX2 Smart Maintenance system will automatically recognize its RFID tag and install the pack and will attempt to run an initialization procedure. The Smart Maintenance system continuously monitors the status of the reagents to track fluid depletion and cartridge expiry.

The ESM Reagent Cartridge contains System Fluid solution for cleaning the sample cup and flow path between sampling. The reagent cartridge also contains Deproteinizing solution, Performance Check solution, and a waste collection pouch for System Fluid and residual sample waste. The reagent cartridge is sized for approximately 200 sample draws from the external liquid handling system and its on-board use life is 30 days.

***WARNING: The ESM Reagent Cartridge will contain waste cell culture material when removed from the system which is considered biohazardous. Use of protective equipment is recommended when handling the reagent cartridge, and waste should be disposed of in accordance with your facility's standard operating procedures***

The new ESM Reagent Cartridge packaging will include the aforementioned black septum and white pinch valve tubing segment, which should both be installed prior to the new reagent cartridge.

#### To install the ESM Reagent Cartridge:

1. Replace the septum and pinch valve tubing before removing the existing reagent cartridge (See Step 1).
2. Remove the used ESM Reagent Cartridge by pulling on the handle and discard.
3. Remove the new ESM Reagent Cartridge from its shipping packaging and gently invert a few times.
4. Point the fitments downward to remove air from inside the fitments, and gently slide the pack into the ESM pack bay until secure (Fig. 4.4). You should feel some resistance as the needle fitments at the back of the pack engage with the needle shrouds at the rear of the pack bay. Continue pushing until the face of the pack sits behind the bottom lip of the reagent pack bay. It should not sit flush with the front of the ESM.
5. The FLEX2 automatically employs Smart Maintenance to detect the new pack using an optical detection sensor in the ESM. Priming will occur automatically upon insertion, and updated pack information will appear in the FLEX2 User Interface Status Bar and ESM Status window (See Section 4.2 for more details).

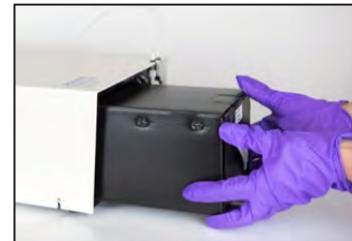


Figure 4.4 Proper reagent installation technique

***WARNING: The needle fitments inside the reagent pack bay are sharp. To avoid injury, never stick your hands inside the pack bay.***

## 4.2 ESM System Status

The ESM Status icon (i.e. ESM 80%) appears in the Status Bar which is always present at the top of the FLEX2 User Interface (Fig. 4.5). If the ESM is not enabled in the software, there will be no indication of its existence on the User Interface. Selecting the Module Status icon from the top right corner of the screen opens the ESM Status window (Fig. 4.6), where more detailed information is tracked including the ESM reagent cartridge lot number, install date, expiration date, samples remaining, Depro fluid remaining; and ESM version (i.e. 15 or 250). The ESM Status icon displays the status of the ESM at-a-glance using a system of color codes.



Figure 4.5 ESM in Status Bar

When the ESM Status icon appears in white  it is in Ready status, meaning it is initialized and available for external sampling. The percentage of volume remaining in the reagent cartridge will be visible on the icon. Within the module status window, Connected, Primed, and Initialized status will show as True. Additionally, the lot number, expiration date, install date, and samples remaining in the ESM Reagent Cartridge will be displayed.

When the ESM Module icon appears in red  the module is unavailable and not ready for external sampling. The ESM Module may be unavailable for a number of reasons: The ESM reagent cartridge is Empty, Expired, or Not Installed; the module is not connected (Connected=False); not primed (Primed=False); and/or not initialized (Initialized=False). If Primed or Initialized status shows as False, a user can initiate either sequence using the respective buttons at the bottom of the ESM Status window (Fig. 4.6).

When the ESM Status icon appears in yellow  the module is in alert status. It is still available for external sampling, but intervention will be needed soon to replace a reagent pack that is either within 24 hours of expiration or has reached <10% remaining volume.

### Performance Check Analysis

Performance Check (PC) analysis is built into the FLEX2 + ESM software so that ESM sample flow can be examined even if the ambr® is unavailable. A date-coded password is required to initiate PC analysis from the ESM Status window, so an operator should contact Nova Biomedical Technical Support if troubleshooting is required. During Performance Check (PC) analysis, the syringe pump draws PC solution from the ESM reagent cartridge and deposits it into the sample cup. The ESM then delivers the PC solution as it would a typical online sample for analysis by the FLEX2.



Figure 4.6 ESM Status Window

**NOTE:** Analyte concentrations in the Performance Check solution are not value-assigned, as it is intended solely to verify FLEX2 + ESM flow. To verify FLEX2 sensor performance, an operator should analyze onboard or external quality control material.

## 4.3 ESM Configuration

### 4.3.1 Configuring Sample Types

Once the ESM is in Ready status, a FLEX2 Sample Type must be selected before external sampling can occur. The End User can use an existing Sample Type or can program a Sample Type tailored for online sampling with customized parameter panels, module selections and configurations, a defined ESM sampling volume (ambr® 15, only), and specific key parameters. At least one selected analytical module must be available for sampling to proceed.

#### To Create a Sample Type:

1. From within any of the FLEX2 Sample Analysis screens, select **Create** next to the Sample Type dropdown.



Figure 4.7 Create Button

This will open the Sample Type Window:

Parameter	Offset Multiplier	Offset Intercept	Lower Limit	Upper Limit	Units	Key
pH	1.0000	0.000	5.000	8.000	-	False
PO2	1.00	0.0	3.0	500.0	mmHg	False
PCO2	1.00	0.0	3.0	200.0	mmHg	False
Gln	1.00	0.00	0.05	15.00	mmol / L	False
Glu	1.00	0.00	0.05	15.00	mmol / L	False
Gluc	1.00	0.00	0.05	40.00	g / L	False
Lac	1.00	0.00	0.05	20.00	g / L	False
NH4+	1.00	0.00	0.20	25.00	mmol / L	False
Na+	1.00	0.0	40.0	300.0	mmol / L	False
K+	1.00	0.00	1.00	100.00	mmol / L	False
Ca++	1.00	0.00	0.10	10.00	mmol / L	False
Osm	1.00	0	0	1500	mOsm / kg	False
CDV	1.00		1.0	800.0	x10 <sup>5</sup> Cells / mL	False

Figure 4.8 Create New Sample Type Menu

2. In the information field next to Sample Type, type the name of the new Sample Type.
3. On left side of the menu, configure the modules for the Sample Type. Selected module icons will turn blue.
4. If the Chemistry module is selected for this Sample Type, configure the desired dilution ratio from the dropdown menu (1:1, 1:2 or 1:4).
5. If the Cell Density (CDV) module is selected for this Sample Type, configure the desired dilution ratio (1:1, 1:2 or 1:6) and Cell Inspection Type from the dropdown menu.

**NOTE:** A new Sample Type cannot have the same name as another configured Sample Type (even one that has been deactivated).

6. To configure correlation factors for any individual parameter included in the Sample Type, set the Offset Multiplier and Offset Intercept to the desired values.

## BioProfile FLEX2 ESM Instructions for Use Manual

7. To configure a specified process range for each parameter, set the Lower Limit and Upper Limit to the desired values. The process ranges established in any given Sample Type function independently of the analytical range limits of the system.
  - If a parameter's value is outside of the analytical range, an error will be logged in the error log indicating [Parameter] Analytical Range High/Low.
  - If a parameter's value is outside the process range established in the Sample Type used for that analysis, no error will be logged in the error log. Instead, the status column for that parameter will be marked as Low or High on the sample results screen.

**NOTE:** When defining process limits or interpreting results flagged as Low/High, it is important to consider how each parameter's analytical range changes depending on the dilution ratio selected for that analysis.

8. Configure Key Parameters (see Section 4.3.1.2 for more information).
9. Save the Sample Type by selecting the green checkmark at the bottom right or select the red X to cancel the setup.

Parameter	Offset Multiplier	Offset Intercept	Lower Limit	Upper Limit	Units	Key
pH	1.0000	0.000	5.000	8.000	-	False
PO2	1.00	0.0	3.0	500.0	mmHg	False
PCO2	1.00	0.0	3.0	300.0	mmHg	False
Gln	1.00	0.00	0.05	12.00	mmol / L	False
Glu	1.00	0.00	0.05	12.00	mmol / L	False
Gluc	1.00	0.00	0.05	30.00	g / L	False
Lac	1.00	0.00	0.05	12.00	g / L	False
NH4+	1.00	0.00	0.20	25.00	mmol / L	False
Na+	1.00	0.0	40.0	300.0	mmol / L	False
K+	1.00	0.00	1.00	100.00	mmol / L	False
Ca++	1.00	0.00	0.10	10.00	mmol / L	False
Osm	1.00	0	0	2000	mOsm / kg	False
Total Density	1.00		1.00	800.00	x10 <sup>5</sup> Cells / mL	False
Viable Density	1.00		1.00	800.00	x10 <sup>5</sup> Cells / mL	False
Viability			0.0	100.0	%	False
Avg. Live Diameter			4.0	70.0	µm	False

Figure 4.9 Sample Type Configuration Menu

### To modify or to deactivate an existing Sample Type:

1. From within the Sample Analysis screen, select the desired Sample Type from the dropdown window (Fig. 4.7), then select Modify to open the Modify Sample Type Window (Fig. 4.9).
2. Modify the Sample Type to the desired configuration or select the Deactivate button, then save the modifications by selecting the green checkmark at the bottom right or cancel the modification by selecting the red X.

**NOTE:** A deactivated Sample Type cannot be re-activated and no two Sample Types can have the same name, even if one is deactivated.

## 4.3.1.1 Key Parameters

The Key Parameters function provides a means of suppressing analysis of online samples when specific "key" parameters are not available due to calibration failure or Quality Control failure (if QC Lockout is enabled). The Key Parameters function only applies to samples supplied to the FLEX2 by the External Sampling Module (ESM); it does not apply to the Manual or Load-N-Go carousel sampling modes. An operator has the option of identifying which parameters are "key" when creating or modifying a Sample Type.

Parameter	Offset Multiplier	Offset Intercept	Lower Limit	Upper Limit	Units	Key
pH	1.0000	0.000	5.000	8.000	-	False
PO2	1.00	0.0	3.0	500.0	mmHg	False
PCO2	1.00	0.0	3.0	300.0	mmHg	False
Gln	1.00	0.00	0.05	12.00	mmol / L	False
Glu	1.00	0.00	0.05	12.00	mmol / L	False
Gluc	1.00	0.00	0.05	30.00	g / L	False
Lac	1.00	0.00	0.05	12.00	g / L	False
NH4+	1.00	0.00	0.20	25.00	mmol / L	False
Na+	1.00	0.0	40.0	300.0	mmol / L	False
K+	1.00	0.00	1.00	100.00	mmol / L	False
Ca++	1.00	0.00	0.10	10.00	mmol / L	False
Osm	1.00	0	0	2000	mOsm / kg	False
Total Density	1.00		1.00	800.00	x10 <sup>5</sup> Cells / mL	False
Viable Density	1.00		1.00	800.00	x10 <sup>5</sup> Cells / mL	False
Viability			0.0	100.0	%	False
Avg. Live Diameter			4.0	70.0	µm	False

Figure 4.10 Configure Key Parameters

### To configure Key Parameters:

1. From the Sample Type menu (Fig. 4.10) select "True" from the drop down in the column labeled "Key" for the parameter of interest.
2. Press the green checkmark to save the Sample Type.

If an online sample is scheduled for analysis using a FLEX2 Sample Type that has Key Parameters enabled, all Key Parameters must be available for the analysis to proceed. If a Key Parameter is unavailable for any reason (QC Lockout included), any online sample analyses with that parameter included in the analysis panel will not run. This feature prevents the loss of sample material when there may be limited volume.

## 4.3.1.2 ESM Sampling Volume

In the bottom left corner of the Sample Type menu is a dropdown labeled "ESM Volume." For FLEX2 + ESM systems, this volume is the sample acquisition volume from the microbioreactor system. For FLEX2's integrated with ESM15, there is a choice of 3 different sample volumes: 400µL, 450µL or 500µL. For FLEX2's integrated with ESM250, there is only one default sampling volume: 675 µL.

Figure 4.11 ESM Sampling Volume

**NOTE:** To determine which volume is optimal for sampling from the ambr® 15, Nova Biomedical recommends performing a correlation study to test similarity of results between manual sampling and ESM15 sampling at the three different acquisition volumes.

# BioProfile FLEX2 ESM Instructions for Use Manual

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## 4.4 Online Sampling

### 4.4.1 Analysis Preparation

Prior to sampling, the ambr® queries the FLEX2 for ESM status along with the status of the analytical modules included in the selected Sample Type. The ESM must be connected, initialized, and primed. Further, all consumables must be installed and valid (non-expired), the selected FLEX2 modules must be available and primed, and key parameters must be calibrated and passing QC (if QC Lockout is enabled) for the analysis sequence to begin. The analysis time for an online sample is 6-7 minutes from the ambr® request to results reporting, depending on the configured analysis panel.

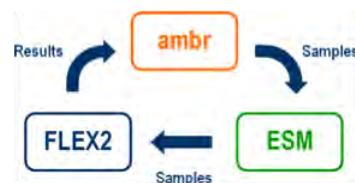


Figure 4.12 Sampling Sequence

### 4.4.2 Sampling Sequence

1. The ambr® computer sets the FLEX2 to a 'Busy' state, reserving it for analysis. The FLEX2 sequence countdown timer icon will appear in the Status Bar.
2. The ambr® robot arm obtains a full sample from a microculture vessel (400, 450, or 500µL from ambr® 15; 675µL from ambr® 250)
3. The ambr® robot arm moves above the ESM sample cup and dispenses 30 µL of sample into the cup for flow test.
4. The ESM syringe pump drains the test sample from the sample cup and draws it to the ESM air detector.
5. The ambr® robot arm dispenses the remainder of the sample volume into the sample cup.
6. The ESM syringe pump drains the cup and draws the sample through the sample (CF) line to the ESM air detector.
7. The air detector is queried for fluid to ensure there is sufficient sample volume.
8. If no fluid is detected at the air detector, the ESM syringe draws again and repeats Step 7.
9. If fluid is detected, FLEX2 sample acquisition proceeds.
10. The FLEX2 sample probe moves to the sample port septum and aspirates the sample.
11. FLEX2 sample analysis continues based on the selected modules configured in the Sample Type.
12. Analysis results appear on FLEX2 User Interface and are stored in FLEX2 Historical Results. They are automatically transmitted to the ambr® database.
13. The ESM flow path is purged, followed by an automated cleaning cycle.

## 5 Maintenance

The following sections provide detailed information and directions to operate and maintain the BioProfile FLEX2 ESM. It is important to perform and/or schedule routine maintenance as recommended.

**WARNING:** Cell culture samples are potential sources of infectious agents. Handle all sample and flow path components with care. Gloves and protective clothing are recommended.

### 5.1 ESM Maintenance Screen

From within the ESM Maintenance menu, an operator with the appropriate privilege level can perform the following maintenance procedures:

- ESM Depro
- Change ESM Tubing
- Change ESM Syringe
- Initialize ESM
- ESM Long-Term Shutdown

**To access the ESM Maintenance menu:**

1. From the FLEX2 Left Home Screen, select the Maintenance icon to view the system maintenance menu.
2. From within the maintenance menu, select ESM in the Command Bar to enter the ESM maintenance menu (Fig. 5.1).

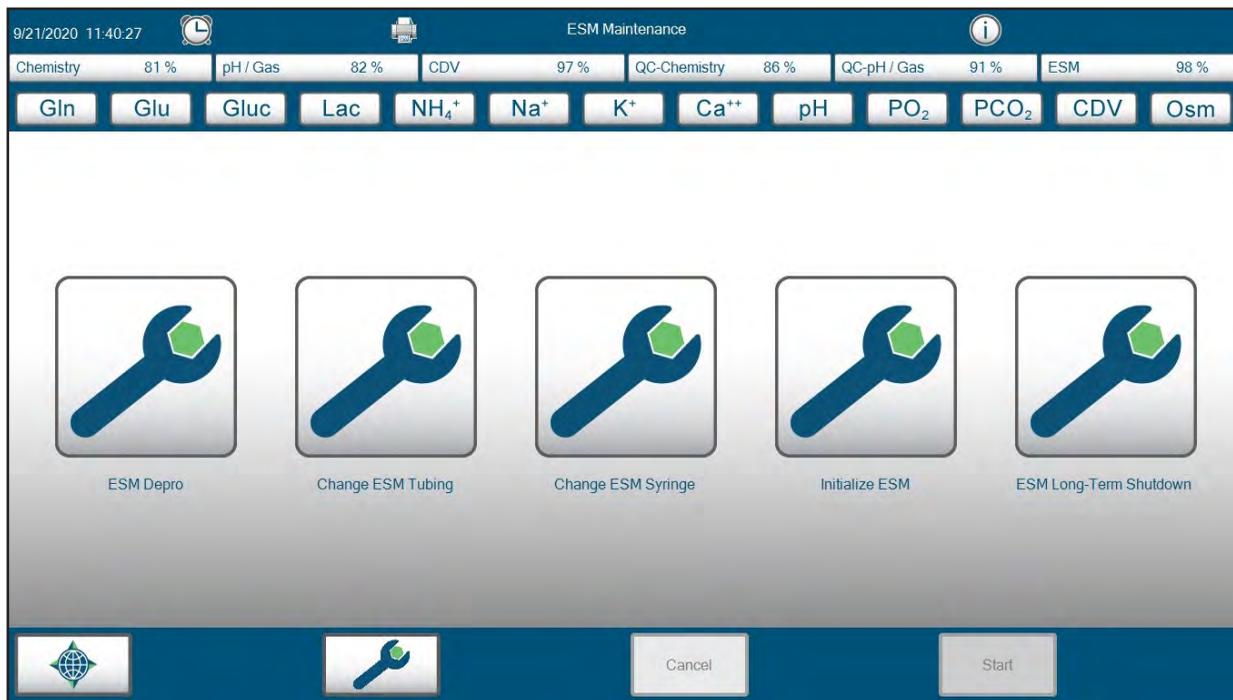


Figure 5.1 ESM Maintenance Menu

## BioProfile FLEX2 ESM Instructions for Use Manual

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### 5.1.1 ESM Depro

The ESM Depro function allows an operator with appropriate privileges to perform a Depro of the ESM sample flow path. During an ESM Depro, the ESM syringe pump draws Deproteinizing solution from the ESM Reagent Cartridge and deposits it into the ESM sample cup via the cup wash (CW) tubing line. The syringe pump then drains the Depro solution through the sample (CF) line and out the waste (EF) line to the self-contained waste receptacle in the ESM Reagent Cartridge.

The sequence utilizes onboard reagents and can be configured to run automatically at regular intervals to maintain optimal ESM performance. By default, the ESM Depro sequence is scheduled to occur daily, according to Nova Biomedical's recommendation for prevention of build-up and blockages of sample debris and protein in the ESM sample cup and sample tubing lines. The ESM Depro function cannot be disabled, but the End User can configure a start time and modify the frequency of occurrence to either weekly, bi-weekly, or monthly in the FLEX2 Settings menu under Scheduling. Additionally, ESM Depro can be scheduled to run after every sample acquisition through the ambr® User Interface to minimize crossover between samples.

**NOTE:** *The volume of Depro fluid remaining in the ESM Reagent Cartridge is tracked and monitored in ESM Module Status window. If Depro fluid runs out, the ESM Depro sequence will be disabled until a new reagent cartridge is installed. However, the pack will remain valid and online sample analysis functionality will remain unaffected.*

#### To perform an ESM Depro:

1. Select the ESM Depro icon from the ESM Maintenance screen.
2. Select the Start button in the Command Bar to begin the Depro Sequence.

#### To modify the automatic ESM Depro schedule:

1. Select the Settings icon from the Right Home Screen, then select the Scheduling button to open the Scheduling Menu. Press the ESM icon in the left column to display the ESM Depro Scheduler (Fig. 5.2).
2. Select a Start Date for the ESM Depro function from the dropdown.
3. Select a Start Time for the ESM Depro function.
4. Select a Frequency (i.e. Daily, Weekly, Bi-Monthly, Monthly) for the ESM Depro function.

**NOTE:** *The start date and start time must be set for a time in the future in order to save.*

ESM Depro
<input checked="" type="checkbox"/> Active
Start Date: 6/24/2019
Start Time: 2:42 PM
Frequency: Daily

Figure 5.2 ESM Scheduling Menu

5. Select the Save button in the Command Bar to save the scheduling configuration.

### 5.1.2 Change ESM Tubing

The Change ESM Tubing function allows an operator with appropriate privileges to change the ESM External Tubing Set. To prevent build-up and blockages of sample debris and protein in the ESM flow path and to preserve the integrity of the ESM External Tubing Set, this component should be replaced on a routine basis. The tubing set is comprised of four individual lines of tubing—Sample (CF), Waste (EF), Cup Wash (CW) and Performance Check (PC)—each of which is labeled with a white tag at either end.

**WARNING:** Cell culture samples are potential sources of infectious agents. Treat the Sample (CF) and Waste (EF) lines as biohazardous. Gloves and protective clothing are recommended.

**NOTE:** Nova Biomedical recommends that the pump tubing typically be changed every 12 months. This frequency may vary depending on system usage, and should be replaced as needed.

To replace the ESM tubing:

1. **Start the Change ESM Tubing Sequence:** Select the Change ESM Tubing icon from the ESM Maintenance screen, then select Start in the Command Bar. When prompted, enter the Lot/Part Number of the new tubing set and press the green checkmark to begin the sequence. The system will purge the ESM flowpath of fluid. Once the green Continue button appears in the Command Bar, proceed to Step 2.
2. **Replace the Sample (CF) Tubing Line:** Carefully disconnect the Sample (CF) line from the right-hand cover of the FLEX2 (Fig. 5.3) by turning the fitting counterclockwise until it releases. Disconnect the opposite end of the line from the base of the ESM sample cup (Fig. 5.5-5.6) in the same way. Discard the old tubing and secure the new CF line to the FLEX2 cover and sample cup by turning the fittings clockwise until finger-tight.

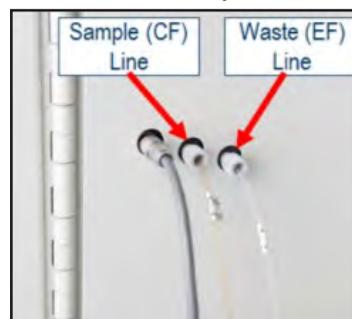


Figure 5.3 ESM Sample and Waste Tubing installed on FLEX2 cover

**NOTE:** The connectors on either end of the CF line are identical, therefore the orientation of the CF line is arbitrary.

**WARNING:** Exercise caution when replacing the tubing lines connected to the sample cup as to not alter cup position. A significant shift in cup position could require realignment of the ambr® liquid handling arm to the cup location

3. **Replace the Waste (EF) Tubing Line:** Carefully disconnect the Waste (EF) line from the right-hand cover of the FLEX2 (Fig. 5.3) by turning the fitting counterclockwise until it releases. Disconnect the other end of the tubing from the ESM syringe pump (Fig. 5.4) in the same way. Discard the old tubing and secure the new EF line to the FLEX2 cover and syringe pump by turning the fittings clockwise until finger-tight.

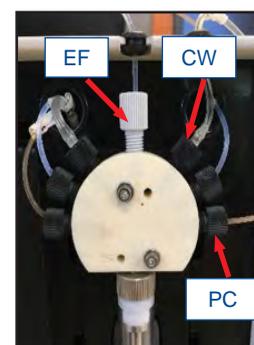


Figure 5.4 ESM Waste, Cup Wash, and Performance Check Tubing connections to ESM syringe pump

**NOTE:** The connectors on either end of the EF line are identical, therefore the orientation of the EF line is arbitrary.

4. **Replace the Cup Wash (CW) Line:** Carefully disconnect the CW line from the ESM syringe pump (Fig. 5.4) by turning the fitting counterclockwise until it releases. Carefully pull the other end of the CW line off the metal port on the sample cup to which it is attached (Fig. 5.5-5.6). Discard the old tubing. Secure the fitting at one end of the new CW line to the syringe pump by turning it clockwise until finger tight. Slide the free end of the new CW tubing securely onto either port on the sample cup
5. **Replace the Performance Check (PC) Line:** Carefully disconnect the PC line from the ESM syringe pump (Fig. 5.4) by turning the fitting counterclockwise until it releases. Carefully pull the other end of the PC line off the metal port on the sample cup to which it is attached (Fig. 5.5). Discard the old tubing. Secure the fitting at one end of the new PC line to the syringe pump by turning it clockwise until finger tight. Slide the free end of the new PC tubing securely onto either port on the sample cup.

**NOTE:** *The connection of the Cup Wash (CW) and Performance Check (PC) tubing lines to the metal ports on the sample cup is arbitrary—either line can be connected to either port.*

6. **Complete Change ESM Tubing Sequence:** Verify all new tubing connections, and then press the green Continue button on the Command Bar. The system will automatically prime the new fluid lines.

### 5.1.3 Change ESM Syringe

The Change ESM Syringe function allows an operator with appropriate privileges to replace the plunger and barrel of the syringe pump assembly on the ESM. Nova Biomedical recommends that the ESM syringe be replaced annually, or as needed.

#### To change the ESM syringe:

1. Select the Change ESM Syringe button from within the ESM Maintenance menu.
2. Select the Start button in the Command Bar to begin the Change ESM Syringe sequence. The analyzer will remove any remaining fluid from the syringe and will position the plunger all the way to the bottom of its axis (Fig. 5.7).
3. When the syringe plunger is all the way down and the Continue button appears, remove the syringe by loosening the thumb screw at the bottom, then unthread the syringe from the valve assembly to remove it (Fig 5.8).

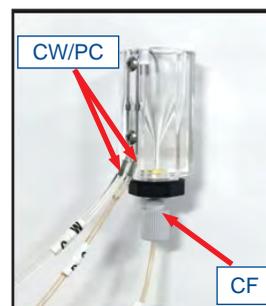


Figure 5.5 ESM Sample, Cup Wash, and Performance check tubing connections to Sample Cup for ambr® 15/250, PN 59975

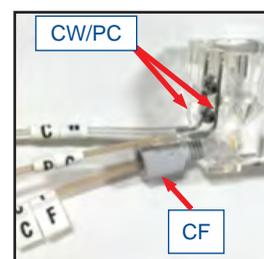


Figure 5.6 ESM Sample, Cup Wash, and Performance check tubing connections to Sample Cup for ambr® 15+Analysis Module PN 60094.

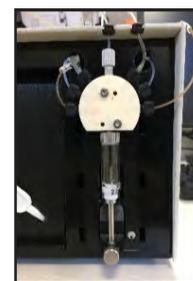


Figure 5.7 Syringe ready for replacement

4. Install the new syringe assembly making sure to secure the threaded connections.
5. When the new syringe is installed, select Continue to prime the syringe pump.

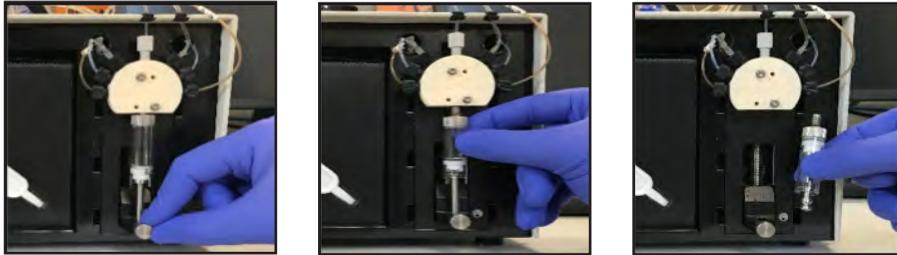


Figure 5.8 Syringe Plunger and Barrel Removal

### 5.1.4 Initialize ESM

The Initialize ESM function allows an operator with appropriate privileges to initialize the ESM when required. During an initialization procedure, the ESM primes the flowpath with System Fluid and attempts to calibrate the ESM air detector. The air detector is responsible for ensuring proper sample positioning at the FLEX2 sample port for aspiration by the FLEX2 sample probe. Therefore, the ESM must be initialized for external sampling to occur.

If a valid ESM Reagent Cartridge is installed when the ESM is powered on, the system will automatically validate the pack and attempt to run an initialization procedure. An initialize sequence may need to be run if the reagent cartridge is removed, if the air detector becomes uncalibrated, or if the ESM status shows Initialized=False.

#### To perform an initialization procedure:

1. Select the Initialize ESM icon from within the ESM maintenance menu.
2. Select Start in the Command Bar to begin.
3. If the ESM initializes properly, the ESM status from within the ESM Status window should read Initialized=True.

#### Alternatively, an initialization can be started through the ESM status window:

1. Select the ESM Module icon from the Status Bar to open the ESM Status window.
2. Select the Initialize button from the bottom of the ESM Status window (Fig. 5.9)

ESM Cartridge	
Lot Number	19176037
Expiration Date	3/19/2020
Install Date	2/18/2020
Samples Remaining	161
Connected	True
Primed	True
Initialized	True
DePro Fluid Remaining	80%
ESM Type	ESM 15
Initialize	Prime
	Performance Check

Figure 5.9 Initialize Icon in ESM Status Window

### 5.1.5 ESM Long-Term Shutdown

The ESM Long-Term Shutdown function is an important maintenance procedure that an operator should be familiar with performing. If the ESM will be powered off for a period greater than 72 hours, the system should be purged of all residual fluid in the internal tubing or flow paths. Failure to prepare the system for long periods of shutdown will result in reagent crystallization and internal tubing blockages, which may require service to correct.

**WARNING:** *Leaving the ESM in a powered-down state or without installed valid fluid cartridge for more than 3 days may result in analyzer damage. Any shutdown period lasting longer than 3 days should be preceded by purging the flow path in accordance with this procedure.*

#### Long-Term Shutdown Cartridge

FLEX2 analyzers operating with software version 4.0 or newer can utilize the ESM Shutdown Cartridge (PN 60612) to properly shutdown the system for extended periods of inactivity.

#### To perform a Long-Term Shutdown:

1. Remove the active ESM Reagent Cartridge from the pack bay, while keeping all other flow components installed.
2. Install the ESM Shutdown Cartridge into the ESM pack bay. Ensure the fitments on the rear of the pack engage with the needle shrouds in the back of the pack bay.
3. Once the pack is installed, the ESM shutdown sequence will automatically begin. Shutdown solution will be pumped from the cartridge into the ESM flowpath.
4. Once the sequence completes, power down the ESM using the power switch on the rear of the analyzer.

**NOTE:** *The Long-Term Shutdown procedure for the ESM can be run at the same time as the Long-Term Shutdown procedure for the FLEX2, but the ESM Shutdown Procedure must be started first.*

#### Long-Term Shutdown Flush Fixture

If the Long-Term Shutdown Cartridge function is not available, the 6-port Flush Fixture can always be utilized to perform the ESM Long-Term Shutdown sequence.

#### Purging the system requires the use of the following:

- PN 58355 Flush Fixture 6 Port
- Beaker with at least 250 mL of DI H<sub>2</sub>O
- Empty Beaker (capable of holding 250 mL of water waste)

### To perform a Long-Term Shutdown and ESM Flow Path Purge:

1. Remove the ESM Reagent Cartridge from the pack bay, while keeping all other flow components installed.
2. Install the 6-port Flush Fixture into the reagent pack bay. Make sure that the needle fittings at the rear of the fixture engage with the needle shrouds in the back of the pack bay.
3. Locate the length of tubing labeled “W” (Waste) on the fixture and place the end of this waste tubing into an empty waste collection beaker.
4. Gather all the remaining lengths of tubing on the fixture and place them into a beaker containing at least 250 mL of DI H<sub>2</sub>O. Make sure the ends of each line of tubing are submerged.
5. Select the ESM Long-Term Shutdown button and then select Start to begin the Flow Path Flush sequence.
6. The ESM will drain all residual reagent from its tubing and will flush the flowpath with the DI water for several minutes.
7. When the sequence is complete, remove each piece of flush fixture tubing from the DI water beaker; leave the “W” tubing in the waste collection beaker.
8. Select Start again to purge the residual DI H<sub>2</sub>O from the flow path and flush it with air.
9. When the second sequence is complete, remove the flush fixture and power down the ESM using the rear power switch.

**NOTE:** *The Long-Term Shutdown procedure for the ESM can be run at the same time as the Long-Term Shutdown procedure for the FLEX2, but the ESM Shutdown Procedure must be started first.*

### 5.1.6 Cleaning FLEX2 + ESM Surfaces

Nova Biomedical Corporation recommends using 70% Reagent Alcohol (V/V) or Isopropyl Alcohol (IPA) for cleaning the various FLEX2 + ESM surfaces or components when required. Use a lint-free cloth or Kimwipe® lightly dampened with the cleaning reagent to wipe down surfaces. Never spray or pour reagent directly onto or into the analyzer or ESM. Once wiped down, all residual fluid should be dried with a lint-free cloth or Kimwipe®.

**WARNING:** *Vapor from other cleaning reagents used within the lab may be corrosive to the BioProfile FLEX2 + ESM and could result in damage to the system. Use caution and protect the system, as needed, when using reagents that produce toxic vapors.*

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### 6 Troubleshooting

This section describes the error codes and associated troubleshooting procedures for the BioProfile FLEX2 ESM.

**WARNING:** *Cell culture samples are potential sources of infectious agents. Handle all sample and flow path components with care. Gloves and protective clothing are recommended.*

#### 6.1 Troubleshooting Procedures

The recommended troubleshooting procedures use the most logical and direct steps to resolve the error code. The solutions are setup in a format that lists groups of steps to perform in order to restore operation. These steps are also organized to prevent unnecessary consumable replacement.

It is important to recall the system flow path overview and sampling sequence when attempting to resolve an error that occurs during ESM operation or online sampling. Familiarizing oneself with the sequence of events that is supposed to take place can make it easier to identify the source of the issue. A useful approach is to inspect the performance of each subsystem: dispensation into the sample cup by the ambr®, sample transfer via the ESM fluidics, and aspiration at the sample port by the FLEX2 sample probe.

If the recommendations provided here do not resolve the problem, contact Nova Technical Services for troubleshooting assistance.

FOR TECHNICAL ASSISTANCE CALL:

Toll Free: USA 1-800-545-NOVA CANADA: 1-800-263-5999

OTHER COUNTRIES: Contact the local Nova Biomedical Sales Office or authorized Nova Biomedical Distributor.

#### 6.2 Error Codes

The following is a list of common potential errors related to the ESM that could appear in the FLEX2 Error Log menu, with error solutions.

##### **ESM Air Detector Calibration Failed**

During the last ESM analysis or maintenance sequence the ESM air detector failed to calibrate properly.

Recommended Solutions:

1. Verify the percent remaining in the ESM Reagent Cartridge. If the pack has less than 10% fluid remaining or is within 1 day of expiry, replace the cartridge, septum, and pinch valve tubing.
2. Run an Initialize sequence through the ESM status menu.
3. Contact Nova Biomedical Technical Support.

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### **ESM Line Not Primed**

The ESM is not primed properly.

Recommended Solutions

1. Run a Prime sequence through the ESM status menu.
2. Verify the percent remaining in the ESM Reagent Cartridge. If the pack has less than 10% fluid remaining or is within 1 day of expiry, replace the cartridge, septum, and pinch valve tubing.
3. Contact Nova Biomedical Technical Support.

### **ESM No Sample Detected**

During the last analysis sequence the ESM air detector did not detect the presence of a sample when expected.

Recommended Solutions:

1. Confirm delivery of the ambr® sample to the ESM cup.
2. Run an Initialize sequence through the ESM status menu.
3. Verify the percent remaining in the ESM Reagent Cartridge. If the pack has less than 10% fluid remaining or is within 1 day of expiry, replace the cartridge, septum, and pinch valve tubing.
4. Contact Nova Biomedical Technical Support.

### **ESM Issue**

During the last ESM analysis or maintenance procedure the ESM was unable to complete the sequence.

Recommended Solutions:

1. Run an Initialize sequence through the ESM status menu.
2. Verify the percent remaining in the ESM Reagent Cartridge. If the pack has less than 10% fluid remaining or is within 1 day of expiry, replace the cartridge, septum, and pinch valve tubing.
3. Contact Nova Biomedical Technical Support.

### **ESM Initial Dispense Timeout**

During the last attempted ESM sample analysis, the ESM failed to detect the initial sample dispense from the ambr®.

Recommended Solutions:

1. Rerun the analysis and confirm that the ambr® arm is dispensing the sample into the ESM cup and that the ESM syringe pump aspirates the sample into the FLEX2.
2. Ensure there is no obstruction inside the ESM sample cup.
3. Check and/or replace External ESM Tubing.
4. Contact Nova Biomedical Technical Support.

## A Appendix

### A.1 Spare Parts and Supplies List

You can order the following parts and supplies from Nova Biomedical and its Distributors.

Description	Part Number (PN)
Syringe, 2.5 mL, w/ Teflon Seal .....	60018
ESM Reagent Cartridge .....	56227
ESM External Tubing Set .....	59209
Pre-Pierced Septum (for ESM, Gas QC, and EOLS) 3/pack .....	59228
ESM Swivel Adapter Kit.....	61326
ESM Sample Cup (ambr® 15/250) .....	59975
ESM Sample Cup (ambr® 15+Analysis Module).....	60094
Split Gland-Nut Assembly (ambr® 15 BSC).....	60251
ESM Shutdown Cartridge .....	60612

### A.2 Warranty

Subject to the exclusions and upon the conditions specified below, Nova Biomedical or the authorized Nova Biomedical distributor warrants that he will correct free of all charges including labor, either by repair, or at his election, by replacement, any part of an instrument which fails within one (1) year after delivery to the customer because of defective material or workmanship. This warranty does not include normal wear from use and excludes: (A) Service or parts required for repair to damage caused by accident, neglect, misuse, altering the Nova equipment, unfavorable environmental conditions, electric current fluctuations, work performed by any party other than an authorized Nova representative or any force of nature; (B) Work which, in the sole and exclusive opinion of Nova, is impractical to perform because of location, alterations in the Nova equipment or connection of the Nova equipment to any other device; (C) Specification changes; (D) Service required to parts in the system contacted or otherwise affected by expendables or reagents not manufactured by Nova which cause shortened life, erratic behavior, damage or poor analytical performance; (E) Service required because of problems, which, in the sole and exclusive opinion of Nova, have been caused by any unauthorized third party; or (F) Instrument refurbishing for cosmetic purposes. All parts replaced under the original warranty will be warranted only until the end of the original instrument warranty. All requests for warranty replacement must be received by Nova or their authorized distributor within thirty (30) days after the component failure. Nova Biomedical reserves the right to change, alter, modify or improve any of its instruments without any obligation to make corresponding changes to any instrument previously sold or shipped. All service will be rendered during Nova's principal hours of operation. All requests for service outside Nova's principal hours of operation will be rendered at the prevailing weekend/holiday rates after receipt of an authorized purchase order. Contact Nova for specific information. The following exceptions apply:

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1. Consumable items, including the reagent cartridges and tubing are warranted to be free of defects at time of installation. The item must be placed into service prior to the expiration date printed on the packaging. All defects must be promptly reported to Nova Biomedical in writing. This warranty is invalid under the conditions specified after item 2.
2. Freight is paid by the customer.

The above warranties are invalid if:

1. The date printed on the package label has been exceeded.
2. Non-Nova Biomedical reagents or controls are used, as follows: Nova Biomedical will not be responsible for any warranties on sensor cards, tubing, probe, or other parts if these parts are used in conjunction with and are adversely affected by reagents, controls, or other material not manufactured by Nova but which contact or affect such parts. Reagent formulations not manufactured by Nova Biomedical may contain acids, concentrated salt solutions, and artificial preservatives that have been shown to cause problems such as shortened sensor life, electrode drift, erratic analytical results, and inaccurate instrument performance.

THE FOREGOING OBLIGATIONS ARE IN LIEU OF ALL OTHER OBLIGATIONS AND LIABILITIES INCLUDING NEGLIGENCE AND ALL WARRANTIES, OF MERCHANTABILITY OR OTHERWISE, EXPRESSED OR IMPLIED IN FACT BY LAW AND STATE OUR ENTIRE AND EXCLUSIVE LIABILITY AND BUYER'S EXCLUSIVE REMEDY FOR ANY CLAIM OF DAMAGES IN CONNECTION WITH THE SALE OR FURNISHING OF GOODS OR PARTS, THEIR DESIGN, SUITABILITY FOR USE, INSTALLATION OR OPERATION. NOVA BIOMEDICAL WILL IN NO EVENT BE LIABLE FOR ANY SPECIAL OR CONSEQUENTIAL DAMAGES WHATSOEVER, AND OUR LIABILITY UNDER NO CIRCUMSTANCES WILL EXCEED THE CONTRACT PRICE FOR THE GOODS FOR WHICH THE LIABILITY IS CLAIMED.

IN ORDER FOR THE WARRANTY TO BE EFFECTIVE, THE WARRANTY CARD MUST BE SENT TO NOVA BIOMEDICAL, 200 PROSPECT STREET, WALTHAM, MASSACHUSETTS, 02454, USA.

