BIOPROFILE®
FLEX2

On-Line Autosampler with Sample Retain Collector Instructions for Use Manual

nova® biomedical

Preface

BioProfile® FLEX2 OLS Instructions for Use Manual

Part Number and Ordering Information

The BioProfile® FLEX2 OLS Instructions for Use Manual (PN 63623) 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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Technical Assistance

For technical assistance inside the United States and Canada, call Nova Biomedical Technical Services at:

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1 Introduction

1.1 About This Manual

This manual provides all necessary instructions for the setup, routine operation, and maintenance of the BioProfile FLEX2 On-Line Autosampler (OLS). Please read this manual carefully. It has been prepared to help you attain optimum performance from your Autosampler. If the OLS 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 OLS is designed to maintain the sterility of the cell culture vessel(s) to which it is connected.

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.*

Table 1.1 Acronyms

Acronym	Definition
OLS	On-Line Autosampler
STM	Sample Transfer Module
RSM	Reactor Sampling Module
SOP	Standard Operating Procedures
QC	Quality Control
UI	User Interface
AU	Analytical Unit
CDV	Cell Density/Cell Viability Module
OSM	Osmolality Module
ESM	FLEX2 External Sampling Module
IFU	Instructions for Use (Manual)
BSC	Biosafety Cabinet
SRCS	Sample Retain Collector System

1.2 Safety

Personnel operating the BioProfile FLEX2 On-Line Autosampler must be proficient in the operation and maintenance procedures for the system. The following safety procedures must be followed:

General Safety

1. Read the safety and operating instructions before operating the Autosampler.
2. Retain all safety and operating instructions for future reference.
3. Observe all warnings on the Autosampler and in the operating instructions.
4. Follow all operating and usage instructions.
5. Do not install the system near water, for example, near a sink.
6. Use only on a bench or stand that is recommended by the manufacturer.

CAUTION: *The FLEX2 + OLS 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 systems.*

7. Place the system so that its location or position does not interfere with its proper ventilation.
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 device.
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 OLS should be serviced only by qualified service personnel.
15. Do not attempt to service the system beyond what is described in the operating instructions. All other servicing should be referred to qualified service personnel.
16. Do not attempt to remove the system covers.
17. The Retain Collector does not have a cover, exercise caution and keep hands clear of the probe arm while the system is in use.

Electrical Safety

1. To reduce the risk of electric shock, do not remove any of the system covers.
2. There are **NO** operator serviceable parts inside the OLS.
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 OLS is supplied with a main, non-rewireable plug for the intended country.
8. Ensure that the wall outlet receptacle used to power the Autosampler 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 RSM Fluid Pack 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.

7. The STM Waste Bottle collects biological material. This bottle is considered a biohazard and should be disposed of accordingly.
8. The optional SRCS Waste Bottle collects biological material. This bottle is considered a biohazard and should be disposed of accordingly.

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

1.3 System Requirements

Table 1.2 **Specifications**

		FLEX2 w/o Osmo	FLEX2 w/ Osmo
Physical	Weight	< 125 lbs (56.7 kg)*	< 145 lbs (65.8 kg)*
	Dimensions (H x W x D)	23.5 x 16.8 x 25.0 in (59.7 x 42.6 x 63.5 cm)	23.5 x 24.8 x 25.0 in (59.7 x 62.9 x 63.5 cm)
Electrical	Operating Voltage	90-264 VAC	90-264 VAC
	Operating Frequency	50-60 Hz	50-60 Hz
	Nominal Power Consumption	480 W Maximum	590 W Maximum
	Fuse Rating	Slo-Blo (SB) 8A or T8A-250V	Slo-Blo (SB) 8A or T8A-250V
	Recommended Power Supply	1100 – 1200 VA Universal Power Supply (UPS)	1100 – 1200 VA Universal Power Supply (UPS)
Environmental	Ambient Operating Temperature	59°F to 86°F (15°C to 30°C)	59°F to 86°F (15°C to 30°C)
	Humidity	20-85% Non-condensing	20-85% Non-condensing
	Operating Altitude	Up to 8202 ft (2500 m)	Up to 8202 ft (2500 m)
	Safety Standards	Installation Category II, Pollution degree 2	Installation Category II, Pollution degree 2
		For indoor use only. Keep working area free of dirt, debris, corrosive fumes, and excessive temperature changes.	

*weight with applicable fluid cartridge(s) installed

Table 1.2 **Specifications**

		STM (1 per FLEX2)	RSM (up to 10 per FLEX2)	SRCS ASX-7200 (1 per FLEX2)	
Physical	Weight	8.45 lbs (3.84 kg)	6.5 lbs (2.95 kg)*	45 lbs (20.4 kg)	50 lbs (22.7 kg)
	Dimensions (H x W x D)	11.4 x 7.3 x 11.5 in (29.2 x 18.5 x 28.9 cm)	16.5 x 4.5 x 16.1 in (41.9 x 11.4 x 40.9 cm)	22 x 15 x 22 in (55.9 x 38.1 x 55.9 cm)	22 x 24 x 22 in (55.9 x 61 x 55.9 cm)
Electrical	Operating Voltage	120-240 VAC	120-240 VAC	85-265 VAC	85-265 VAC
	Operating Frequency	50-60 Hz	50-60 Hz	47-63 Hz	47-63 Hz
	Nominal Power Consumption	180 W Maximum	65 W Maximum	353 W Maximum	705 W Maximum
	Fuse Rating	Slo-Blo (SB) 2A or T2A-250V	Slo-Blo (SB) 2A or T2A-250V	Slo-Blo 5A	Slo-Blo 10A
	Recommended Power Supply	1100 – 1200 VA Universal Power Supply (UPS)	1100 – 1200 VA Universal Power Supply (UPS)	N/A	N/A
Environmental	Ambient Operating Temperature	59°F to 86°F (15°C to 30°C)	59°F to 86°F (15°C to 30°C)	41°F to 104°F (5°C to 40°C)	41°F to 104°F (5°C to 40°C)
	Humidity	20-85% Non-condensing	20-85% Non-condensing	0-80% non-condensing up to 31°C, decreasing linearly to 50% at 40°C	0-80% non-condensing up to 31°C, decreasing linearly to 50% at 40°C
	Operating Altitude	Up to 8202 ft (2500 m)	Up to 8202 ft (2500 m)	Up to 6,562 ft (2000 m)	Up to 6,562 ft (2000 m)
	Safety Standards	Installation Category II, Pollution degree 2	Installation Category II, Pollution degree 2	Pollution degree 2	Pollution degree 2
		For indoor use only. Keep working area free of dirt, debris, corrosive fumes, and excessive temperature changes.			

*weight with applicable fluid cartridge(s) installed

1.4 Intended Use and Tests Performed

The BioProfile FLEX2 On-Line Autosampler is an accessory sample delivery system designed to integrate the BioProfile FLEX2 Analyzer with as many as 10 cell culture vessels for the quantitative determination of 16 key cell culture and media parameters, including (depending on FLEX2 module configuration): **pH**, **pO_2**, **pCO_2**, glutamine (**Gln**), glutamate (**Glu**), glucose (**Gluc**), lactate (**Lac**), ammonium (**NH_4^+**), sodium (**Na^+**), potassium (**K^+**), calcium (**Ca^{2+}**), Total Cell Density (**TCD**), Viable Cell Density (**VCD**), Viability (%), Live Cell Diameter, and Osmolality (**Osm**).

Using the directly measured results and the vessel temperature configured by the operator, the BioProfile FLEX2 + OLS offers the following calculated parameters:

- Temperature-Corrected pH (for entered values other than 37°C)
- Temperature-Corrected pO_2 (for entered values other than 37°C)
- Temperature-Corrected pCO_2 (for entered values other than 37°C)
- Air Saturation
- CO_2 Saturation
- HCO_3^- (Bicarbonate) Concentration

NOTE: *This manual covers the On-Line Autosampler. For information on the FLEX2 Analyzer, such as the applicable analytical ranges for each test module, a list of known interfering substances, or other sample information, refer to the BioProfile FLEX2 Instructions for Use Manual, PN 57960.*

2 System Details

2.1 Features

The BioProfile FLEX2 On-Line Autosampler (OLS) is robust and highly flexible, with a modular design to streamline system set-up and operating features to accommodate varying laboratory needs. The FLEX2 OLS features a small sample volume and fast analysis time, using individual syringe pumps at each Reactor Sampling Module (RSM), and two independent sample flow paths between the STM and FLEX2 for increased productivity through interleaved sampling and cleanup of reactors. The OLS ensures reactor sterility by maintaining an aseptic air gap between the RSM and bioreactor. Further, the OLS sampling sequence and use of onboard reagents and disinfectants eliminate sample-to-sample carryover and cross contamination between cell cultures. With proven data comparability to manual sampling methods, the OLS can be connected to OPC-compliant control systems for fully automated sampling, analysis, and feedback control. The Sample Retain Collector System (SRCS) is an optional module that saves a duplicate sample after an analysis is run. It connects to the STM and pulls a second sample of specified volume immediately after the analysis sample enters the FLEX2. The SRCS stores the samples in individual capped tubes that are chilled, with options for different tube volume size.

2.2 Main Components

The FLEX2 On-Line Autosampler's main components consist of the following:

- **Sample Transfer Module (STM)**- 1 per FLEX2 analyzer
- **Reactor Sampling Module (RSM)**- 1 per cell culture vessel (up to 10)
- **Fluidic Interface Ports** on the right side FLEX2 cover for sample inlet and waste outlet
- **Sample Ports** within the FLEX2 Analytical Unit for sample aspiration by the FLEX2 sample probe
- **Sample Retain Collector (SRCS)**- 1 per FLEX2 analyzer (optional)

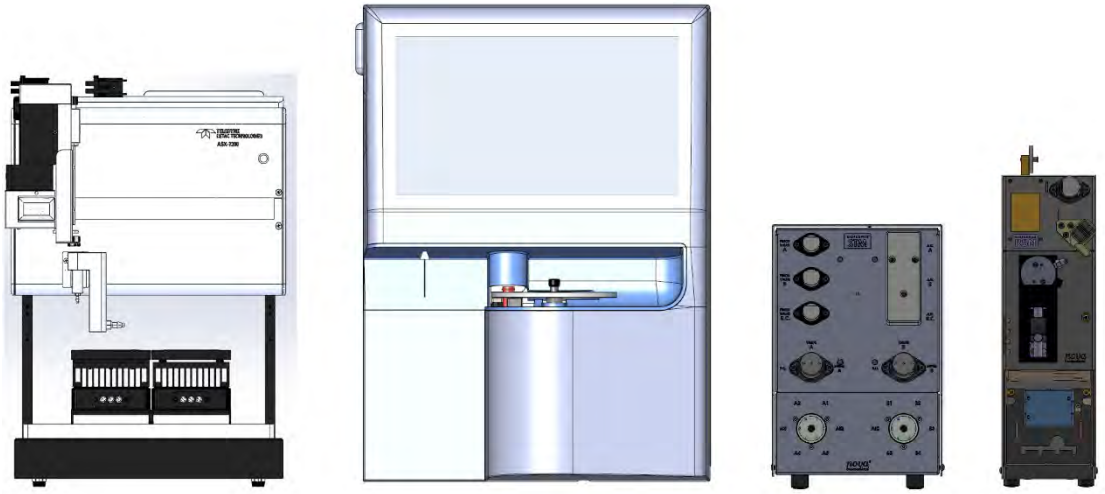


Figure 2.1 SRCS (left), FLEX2 (left center), STM+ (right center), and RSM (right)

The OLS is shipped with accessory packs containing additional parts necessary for system installation and operation. The core accessories consist of the following:

- **RSM Fluid Pack** (1 per RSM) to supply reagents to the OLS flow path and collect local waste
- **External Tubing Sets** to supply bioreactor sample material and Fluid Pack reagents to the RSM, from the RSM to the STM, from the STM to the FLEX2 for analysis, and from the FLEX2 to waste
- **Power and communication cables** for the STM and each RSM
- An external **Waste Container** for sample waste (optional)

SRCS-specific accessories consist of the following:

- **Peltier Rack Cooling Modules (PRMs)** x2 or x4 depending on the SRCS model
- **Tubing Racks** (size and quantity depend on order)
- **SRCS Probe**
- **Pump/Wash Tubing** to clean the SRCS probe and dispose of excess sample material
- **Power and communication cables** for the SRCS and its power supply
- **SRCS accessory pack** including a waste container, wash container, and the sample line connecting the STM to the SRCS

2.2.1 STM

The **Sample Transfer Module (STM)** is designed to sit directly to the right of the FLEX2 Analytical Unit. With fluid lines and communication cables connecting to the FLEX2, RSM(s), and the optional SRCS, the STM operates as the central hub of the OLS system by linking each RSM to the FLEX2 and the Retain Collector.

The STM is divided into two identical banks— "A" and "B"—each with its own air detector, pinch valve, rotary valve, and external tubing for control of sample/reagent flow from the RSM(s) to the FLEX2, and waste flow from the FLEX2 to an external receptacle. The two STM banks function independently of each other allowing for simultaneous sampling and cleanup of "A" and "B" RSMs.

The two **rotary valves** (1) are designed to connect up to five RSMs each, for a total of ten potential bioreactor connections (the sixth port on each valve is reserved for air).

The two **3-way valves** (2) are linked to the rotary valves, and control the flow of sample from either bank to the FLEX2 or the SRCS (the third port on each valve is reserved for air).

During sampling, the rotary valve opens to the location of the RSM being sampled (i.e. A1-A5 or B1-B5), and works in conjunction with the **pinch valve** (3) and **air detector** (4) on the same bank to position the sample for aspiration and analysis by the FLEX2. If retain collection is configured, the 3-way valve diverts the first portion of the sample to the FLEX2, then switches positions and works in conjunction with the SRCS pinch valve and air detector to divert the required retain(s) to the retain collector.

A small **indicator LED** (5) on the front of the STM illuminates in green to indicate connection to the FLEX2. On the rear of the STM is a **power entry module** (6) with an On/Off power switch for independent power supply to the STM, and multiple **COM ports** for communication with the FLEX2 (7), Retain Collector (8), and RSM(s) (9) and control of hardware components.

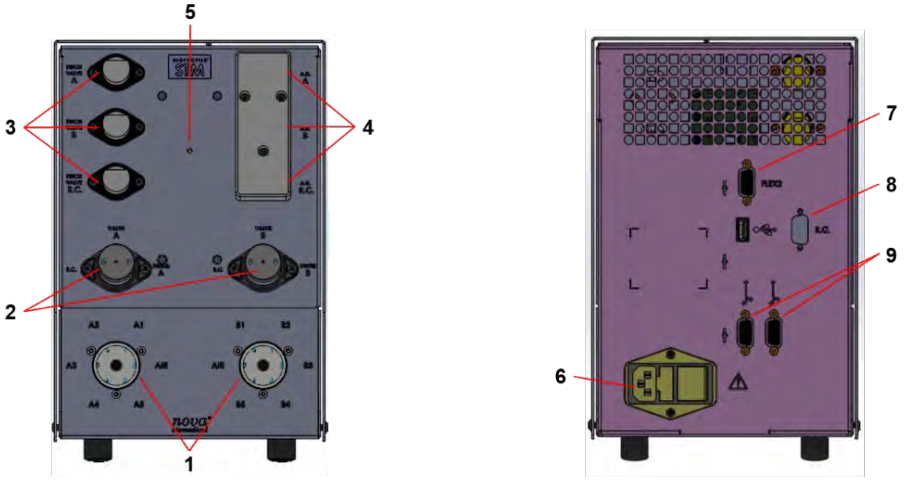


Figure 2.2 STM View Front (left) and Rear (right)

2.2.2 RSM

The **Reactor Sampling Module (RSM)** is designed to sit directly adjacent to its cell culture vessel and within 20 ft of the STM. The RSM is responsible for sample acquisition, sample delivery, and post-analysis cleanup, making it the core liquid handling component of the OLS system. Up to 10 RSMs can be interfaced with the FLEX2; and each RSM is fluidically independent of the others with its own syringe pump, internal/external tubing, Fluid Pack, and air detectors. The RSM components include the following:

An **LCD touchscreen** (1) displays RSM configuration/status and enables analysis/priming sequences.

A **syringe pump** (2) draws bioreactor samples and Fluid Pack reagents and delivers them to the STM.

A **Fluid Pack bay** (3) with needle fixtures and internal tubing supplies reagents from and local waste to the Fluid Pack.

A 3-way **pinch valve** (4) controls sample flow from the reactor and maintains a sterile boundary between the RSM and reactor.

A built-in **RSM Air Detector** (5) detects sample material during sample acquisition and Depro solution during Reactor Line cleaning.

Rear **COM ports** (6) enable communication between the RSM(s) and STM and control of hardware components.

A rear **power entry module** (7) with an On/Off power switch supplies independent power to the RSM.

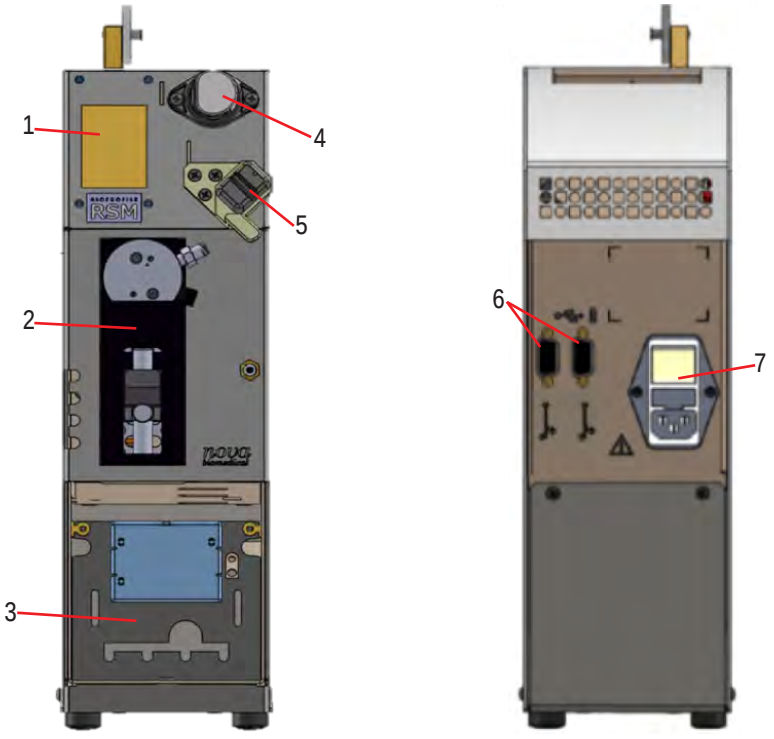


Figure 2.3 RSM View Front (left) and Rear (right)

2.2.2.1 RSM User Interface

Each RSM touchscreen displays various icons pertaining to RSM operation. The icon functions are as follows:

ADDRESS	A1	Unique indicator based on the RSM Sample Line connection to the STM rotary valve (i.e. A1-A5 or B1-B5) that determines the rotary valve position during sample and flow path maintenance.
AUTO-TERMINATION		Present at the top of only 1 or 2 RSM screens, and may be used to troubleshoot hardware function.
HEARTBEAT		Flashes green when the RSM is powered on and may be used to troubleshoot hardware function.
STATUS		Flashes with arrows when an analysis or maintenance sequence is in progress.
ERROR		Appears only when there is an issue with the system; select icon for error details.
ANALYZE	Analyze	Select to open Confirmation screen. Select ✓ to sample the respective bioreactor or ✗ to return Home.
PRIME REACTOR	Prime Reactor	Select to open Confirmation screen. Select ✓ to prime the respective bioreactor or ✗ to return Home.
CONFIGURE	Configure	Select to modify the RSM address once the Sample Line is installed.

NOTE: *The Analyze and Prime Reactor sequences can be initiated locally from the RSM UI or from the FLEX2. These sequences are discussed in greater detail in the following sections: Section 4 Operation and Section 5 Maintenance, respectively.*

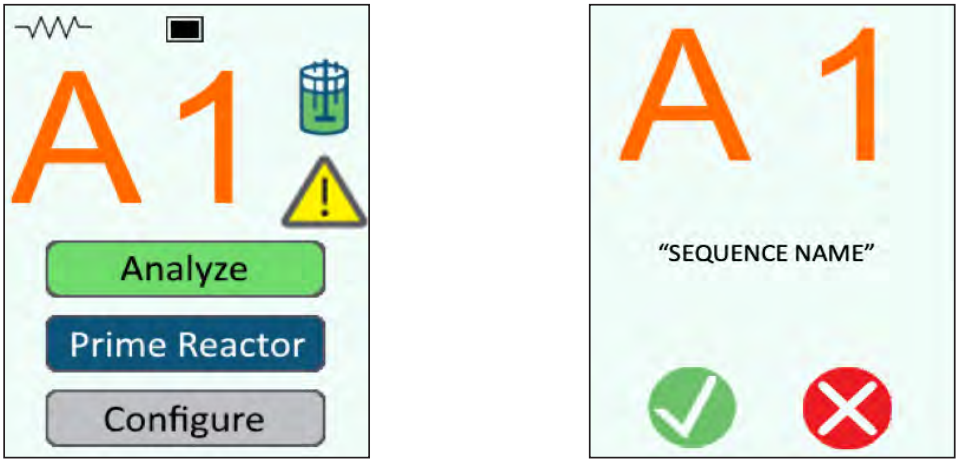


Figure 2.4 RSM User Interface Home Screen (Left) and Confirmation Screen (Right)

2.2.2.2 RSM Fluid Pack

Each RSM requires its own Fluid Pack for operation, containing 3 fluids for OLS flow path maintenance: **System Fluid** for priming, **Shutdown Solution** for end-of-sequence cleanup and long-term shutdown, and **Deproteinizing Solution (Depro)** which is a dilute bleach solution for intensive cleaning. The Fluid Pack also contains a **Waste Pouch** for collection of local reactor and reagent waste.

Like other FLEX2 module reagent cartridges, the RSM Fluid Pack has an **RFID tag** and fitments on the rear of the pack that engage with the needle fixtures in the pack bay when installed. When a new Fluid Pack is installed, FLEX2 Smart Maintenance automatically detects, installs, and primes the pack. On the FLEX2 UI, pack information such as the percentage of fluid remaining and expiration is displayed and updated in real-time.

The fluid packs are sold in a set of 2 that comes with replacement septa for the OLS sample ports located within the FLEX2 Analytical Unit. The proper installation of these septa is necessary for on-line sampling, and Nova Biomedical recommends these septa are replaced monthly for optimal performance. The Fluid Pack has a shelf-life of 12 months and a use-life of 90 days, after which it must be replaced by the End User.

NOTE: *The sample capacity for the RSM pack is approximately 60 samples with the default settings. The pack use-life will vary based on RSM configuration, sample throughput, and retain collection.*

WARNING: *Once installed, the RSM Fluid Pack waste bag will contain cell culture material which is considered biohazardous. Use of protective equipment is recommended when handling the Fluid Pack, and waste should be disposed of in accordance with your facility's standard operating procedure.*

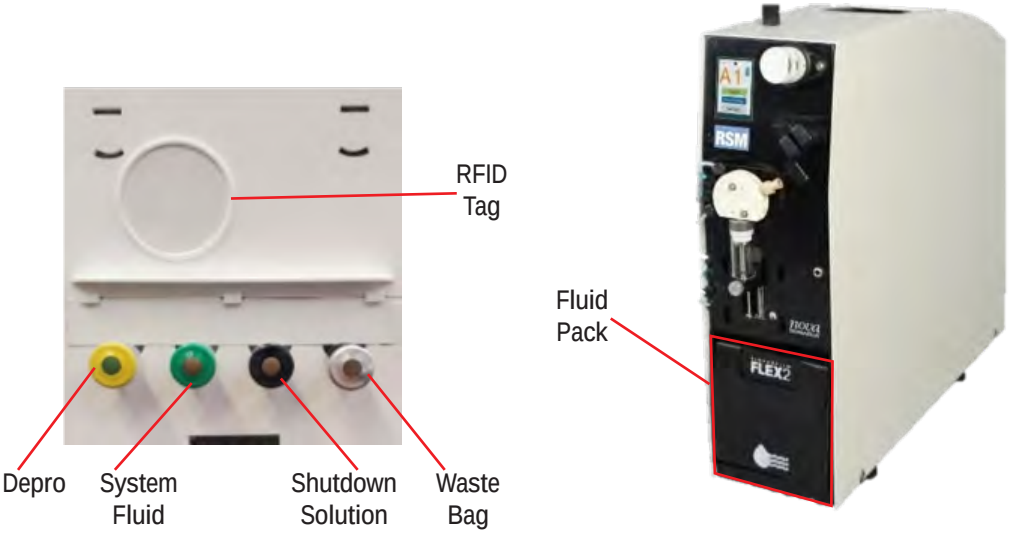


Figure 2.5 RSM Fluid Pack Rear View (Left) and Front View- Installed on RSM (Right)

2.2.3 Sample Retain Collector

The Sample Retain Collector System (SRCS) is designed to sit next to the STM while connected to both the STM and FLEX2 via COM cable. The SRCS is an optional module that is responsible for dispensing additional retain samples of specified volumes into chilled tubes as part of the sample analysis process. A single SRCS can be interfaced with the FLEX2 and accepts retains directly from the STM through its dedicated sample line. The SRCS has its own waste/wash bottles, waste/wash tubing, and waste/wash peristaltic pump that serve to clean the SRCS probe and dispose of any extra sample material. The SRCS components include the following:

A **Z-Drive Assembly, Sample Probe** (1) allows the sample probe to position above the sample racks and dispense into the sample tubes.

A **Rinse Station** (2) that cleans the probe with the wash fluid and collects the extra sample material as waste.

Two or Four **Sample Racks** (3) that hold the sample tubes.

A **Power Indicator** (4) shows green while the unit is powered on.

The **Peltier Rack Modules** (5) sit below the sample racks and chill the retains inside to 4°C.

A **COM port** (COM1) (6) allows the SRCS to communicate with the STM.

A **Peristaltic Pump** (7) pulls the wash and waste fluids through the rinse station to the waste bottle.

A **Power Supply** (8) distributes power to the cooling modules and to the SRCS itself.

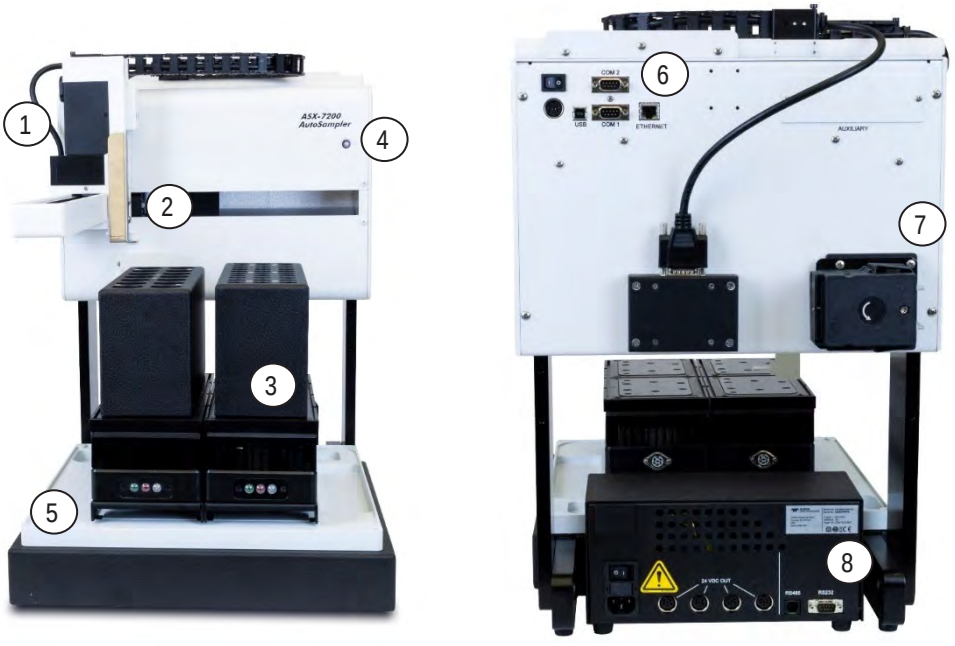


Figure 2.6 Sample Retain Collector Front/Rear View

2.2.4 FLEX2

The BioProfile FLEX2 Analyzer must be in OLS-Ready configuration for the OLS (i.e. STM and RSM(s)) to be installed. The FLEX2 can either be purchased as OLS-Ready or upgraded to such configuration in the field by a trained Nova Field Service Specialist (FSS). Installation of the FLEX2 OLS also requires that the FLEX2 analyzer is operating on software version 4.0 or newer.

NOTE: *The On-Line Autosampler requires an OLS top plate in place of the 96-well plate sampling deck within the FLEX2 User Domain. Once installed, the FLEX2 will no longer offer 96-well plate sampling, but Manual and Load-and-Go carousel sampling will still be available.*

2.2.4.1 OLS Interface Ports

The On-Line Autosampler requires a 4-port interface plate on the right side of the FLEX2 cover. The STM Tubing Harness is installed on these **fluidic interface ports** for sample delivery to and waste evacuation from the FLEX2 at each STM bank. The interface ports are plumbed directly to the OLS sample ports.

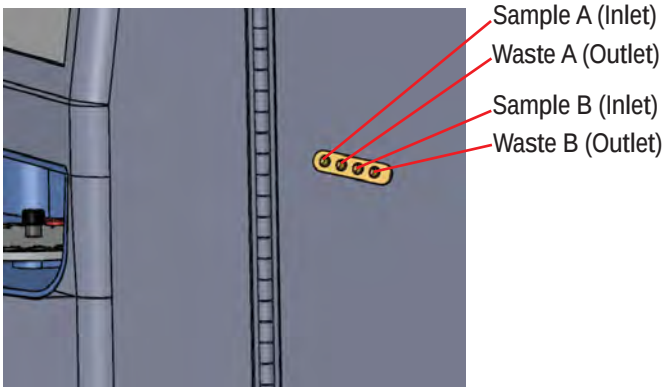


Figure 2.7 OLS Interface Ports (Right FLEX2 Cover)

2.2.4.2 OLS Sample Ports

Within the FLEX2 User Domain there is a sample port for each STM bank. During on-line sampling, the FLEX2 sample probe inserts into the respective port to aspirate the sample for analysis. Each **sample port** assembly consists of a lower threaded portion, a cap and an internal rubber septum to keep the connection airtight. The rubber septa are initially installed by Nova, but spare septa are supplied with each RSM Fluid Pack set and must be replaced by the End User regularly.

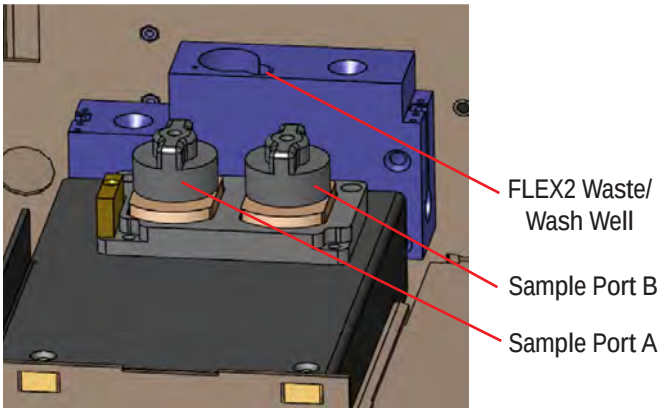


Figure 2.8 OLS Sample Ports (FLEX2 User Domain)

2.2.4.3 FLEX2 User Interface

OLS Status Indicator

When the Autosampler is enabled in the FLEX2 software, the **Autosampler (OLS) Status Indicator** appears in the Status Bar at the top of the FLEX2 User Interface to allow for at-a-glance system monitoring. The Autosampler Status Indicator consists of two rows of five RSM status squares. The top row represents STM Bank A and its interfaced RSMs (A1-A5, from left to right), while the bottom row represents STM Bank B and its associated RSMs (B1-B5, from left to right). The background color of a square at a given time indicates the status of the respective RSM. Each row of status squares is backed by an STM status panel, and the color of the panel indicates the status of the respective STM bank.

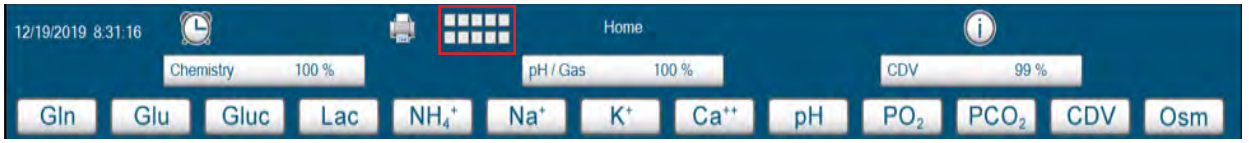


Figure 2.9 OLS Status Indicator on FLEX2 User Interface

RSM Status Squares Indicators:

TRANSPARENT (blue) indicates the RSM is Not Connected.

DARK GRAY indicates the RSM is connected but *Not Configured*. Each RSM must be configured from the FLEX2 User Interface prior to analysis (See Section 4.1 for more information).

RED indicates the RSM is connected and configured but *Not Ready* for analysis. The RSM may be *Not Ready* for any of the following reasons: The RSM Pack Status is *Empty*, *Expired*, or *Not Installed*; the RSM Reactor Primed or Initialized status is *False*.

WHITE indicates the RSM is *Ready* for sampling, meaning the RSM is initialized, a valid Fluid Pack is installed and primed, and the reactor is primed.

GREEN indicates the RSM is *Busy*, meaning an analysis or other sequence is in progress.

YELLOW indicates the RSM is in *Alert* status. The RSM is still ready for sampling, but intervention will be needed soon to replace the Fluid Pack that is either within three days of expiration or has less than 10% remaining volume.

STM Status Panel (Row) Indicators:

TRANSPARENT (blue) indicates the STM Bank is *Ready*.

YELLOW indicates the STM is *Not Ready* or *Not Connected*.

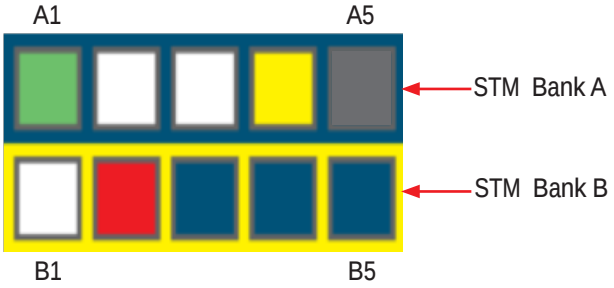


Figure 2.10 OLS Status Indicator (enlarged)

OLS Status Window

Select the **OLS Status Indicator** to open the OLS Status Window, where OLS system details are displayed for the STM (upper table) and each RSM (lower table). Each row represents an individual STM Bank or RSM, and the text color of each row indicates the status of the respective STM bank or RSM. Blue text indicates Ready status, red text indicates Not Ready, and gray text indicates Not Connected or Not Configured (RSM only). Table 2.1 outlines all possible RSM and STM statuses and their implications.

From the Autosampler Status Window, an operator with the applicable privileges can also use the icons at the bottom of the window to navigate to the OLS **Configuration**, **Maintenance**, **Scheduling**, and **Management** menu screens.

NOTE: *The user-configured name for each RSM will be displayed alongside its address (i.e., RSM-A1) in the RSM column.*

STM	Status	Initialized	Flow Path
STM-A	Ready	True	Ready
STM-B	Ready	True	Ready

RSM	Status	Initialized	Pack Status	Fluid Remaining	Sample Line Status	Reactor Primed	Expiration Date	Sample Volume (µL)
RSM-A1 - RSM-A1	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-A2 - RSM-A2	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-A3 - RSM-A3	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-A4 - RSM-A4	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-A5 - RSM-A5	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-B1 - RSM-B1	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-B2 - RSM-B2	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-B3 - RSM-B3	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-B4 - RSM-B4	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800
RSM-B5 - RSM-B5	Ready	True	Ready	97 %	Ready	True	12/30/2019	3800

Configuration | Maintenance | Scheduling | Management

Figure 2.11 Autosampler Status Window

STM and RSM Status

Table 2.1 STM and RSM Status

STM Status	STM Initialized	STM Flow Path
Shows the status of STM banks A and B: **Ready** indicates the STM is initialized and ready for sampling. **Not Ready** indicates the STM is connected but requires attention. **Not Connected** indicates the STM is not connected to the FLEX2.	Indicates if the STM is properly initialized. The STM hardware must be initialized to be ready for analysis. If the STM is initialized, the status shows **True**. If the STM is connected but not initialized, the status shows **False**.	Indicates the status of the respective STM flow path: **Not Ready** indicates the flow path is not washed. **Ready** indicates the flow path is washed and ready for analysis.
RSM Status	**RSM Initialized**	**Pack Status, Fluid Remaining, & Expiration Date**
Shows the status of each RSM (A1-A5 and B1-B5): **Ready** indicates the RSM is initialized and available for on-line sampling, with a valid Fluid Pack installed and primed; and the reactor is primed. **Not Ready** indicates the RSM is connected and configured but is not ready for analysis. **Not Configured** indicates the RSM is connected, but its settings have not been configured, and **Not Connected** indicates the RSM is not communicating with the STM/FLEX2.	Indicates if each RSM is properly initialized. The RSM hardware must be initialized to be ready for on-line sampling. If the RSM is initialized, the initialized status will read **True**. If the RSM is connected but not initialized, the status will read **False**.	Shows the Pack Status (**Not Installed**, **Expired**, **Not Primed**, **Ready**, **Empty**), Fluid Remaining (% Volume), and Expiration Date of the installed RSM Fluid Pack. Once the fluid remaining reaches <10% or the expiration date is within 3 days, the RSM will enter alert status (yellow indicator square). Once the pack reaches less than 10% volume remaining, the Fluid Remaining will show as <10% until the pack is Empty or Expired.
Reactor Primed	**Sample Line Status**	**Sample Volume**
Indicates the status of each Reactor Line. **True** indicates the reactor line is clean and ready. **False** indicates the reactor line is not ready.	Indicates if the Sample Line is **Ready** or **Not Ready** for on-line sampling based on whether it is clean or not and overall system status.	Shows the configured Sample Volume (µL) for each reactor as programmed on the FLEX2 OLS Configuration Menu screen.

Retain Collector Status Indicator

When the SRCS is installed, the Retain Collector Status Indicator appears in the FLEX2 Status Bar adjacent to the OLS Status Indicator. The Retain Collector Status Indicator consists of two rows of either two or four squares. Each vertical column represents a tray slot (up to four trays supported depending on RC configuration). The top row displays the number of available vials remaining in the respective tray. The bottom row displays the volume of the vials in the tray.

WHITE indicates 3 or more vials are available in the tray.

YELLOW indicates 2 or fewer vials are available in the tray

RED indicates 0 vials are available in the tray.

GRAY indicates the tray is not configured but available.

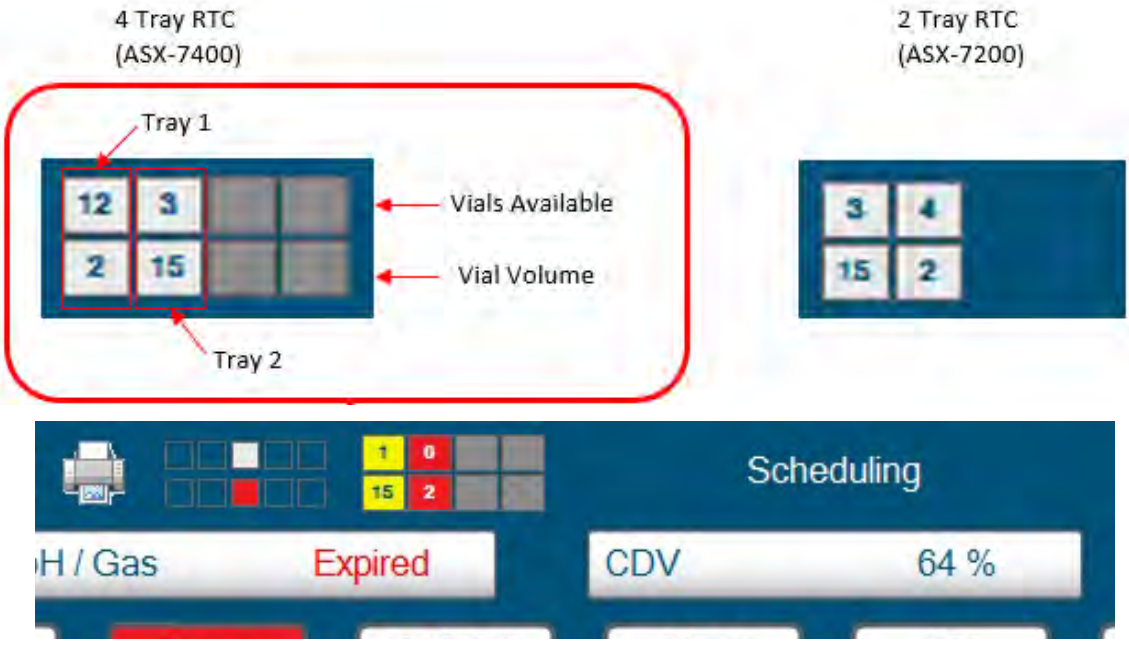


Figure 2.12 Retain Collector Status Indicator

Retain Collector Status Window

Select the Retain Collector Status Indicator to open the Retain Collector Status Window, where retain collector status, tray configurations, and sample information is displayed. There are icons for each available tray (2 or 4 depending on how many trays are available) that can have their type changed to match the tray type installed using the dropdown box at the top of the screen. Once the tray size is selected, it will populate the icon with all available vial positions. Vials can be loaded by selecting them individually and pressing "Load Selected" or selecting a vial and pressing "Load All" at the bottom of the screen to load that entire tray. Vials can be unloaded in the same way by selecting "Unload Selected" or "Unload All."

The window on the right side of the screen will display the sample information for any filled vials and includes its tray location, time it was filled, sample ID, and which RSM it came from.

GRAY is not loaded.

GREEN is loaded and available.

GREEN with LOCK indicates that vial is being used for the current sequence.

YELLOW is filled and unavailable.

RED with X indicates an error occurred during the retain.

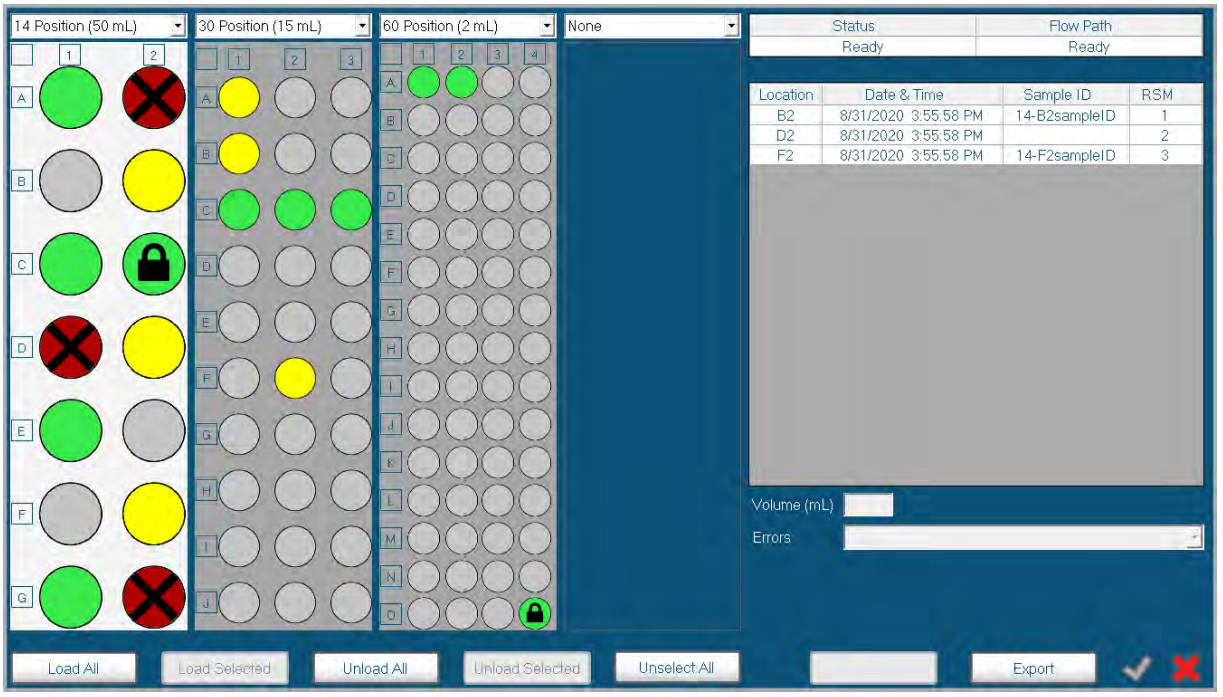


Figure 2.13 Retain Collector Status Window

2.2.5 External Tubing

There are several external tubing sets that comprise the OLS flow path, all of which are provided and installed by Nova personnel. The flow path must be maintained by the End User through routine cleaning and replacement of the tubing lines at regular intervals. These tubing sets include the STM Tubing Harness **(1)**, STM Waste Tubing Harness **(2)**, Sample Line **(3)**, and Reactor Line **(4)**. If the Retain Collector is included, the Retain Collector Line **(5)** and External Waste Line Kit **(6)** are also required.

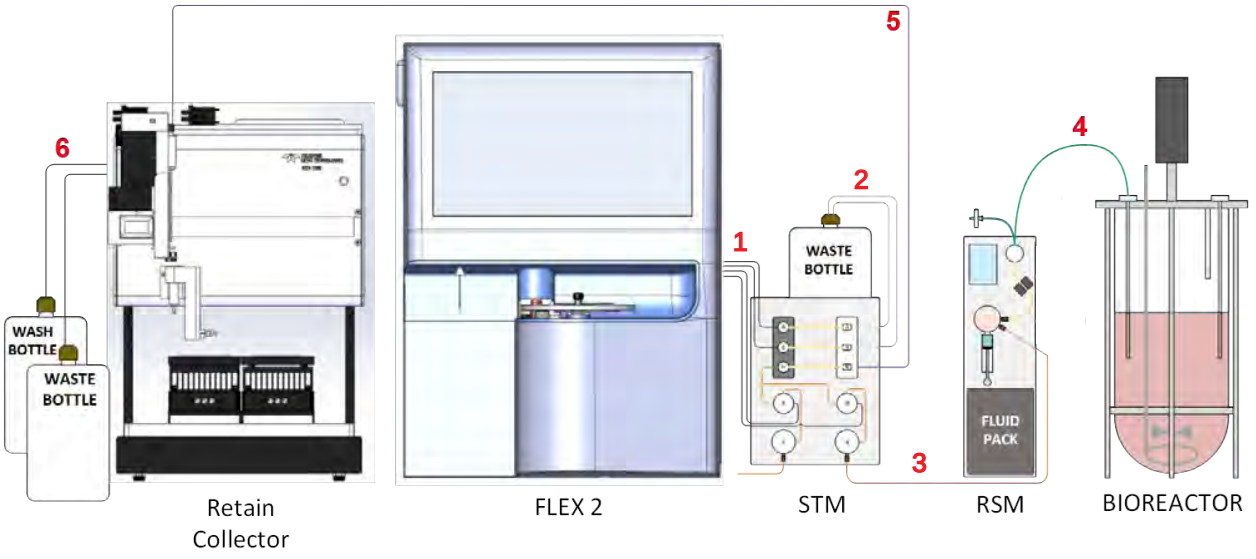


Figure 2.14 External Tubing Overview

2.2.5.1 STM Tubing Harness

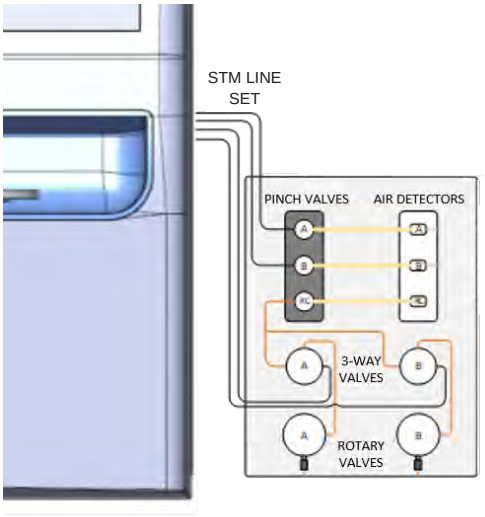


Figure 2.15 STM Tubing Harness

The **STM Tubing Harness** consists of four lengths of tubing—a sample inlet and waste outlet line for each STM bank, A and B. The two inlet lines deliver sample from the STM to the FLEX2 for analysis. Each connects to the right port on the respective STM 3-way rotary valve, and a corresponding interface port on the right cover of the FLEX2 analytical unit. The two waste outlets evacuate sample waste from the FLEX2 AU back to the STM. Each outlet line connects to an interface port on the FLEX2 cover and is then seated in the STM pinch valve and fastened to the STM air detector of the respective bank. The STM must be placed directly to the right of the FLEX2 so that the lines of the STM Tubing Harness are not under stress.

2.2.5.2 Waste Tubing

The **Waste Tubing Harness** consists of two **Waste Lines** for routing of sample waste from each STM bank to the Waste Bottle provided by Nova. The two waste lines attach to a **Waste Bottle Cap** that is installed on the Waste Bottle.

An **External Waste Line Kit** (not pictured) is available for routing of waste from the STM to a remote waste bag or carboy, if the waste container provided by Nova is not preferred. Both sets of waste tubing are provided at installation to accommodate either preference.

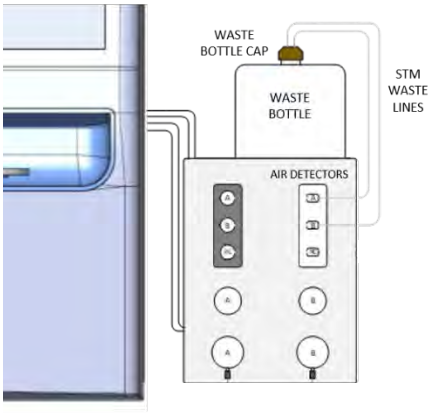


Figure 2.16 Waste Tubing Harness

2.2.5.3 Sample Line

The **Sample Line** connects the RSM to the STM. It is installed on the RSM Syringe Pump via the Sample Line Air Detector, and the other end is plumbed to one of the two STM rotary valves. The RSM can be placed to either side of the FLEX2 so long as the 20-ft Sample Line is not under strain. Once the Sample Line is installed, the RSM must be configured on the RSM UI by assigning an alphanumerical address based on its STM Rotary Valve connection (i.e. A1-A5 or B1-B5).

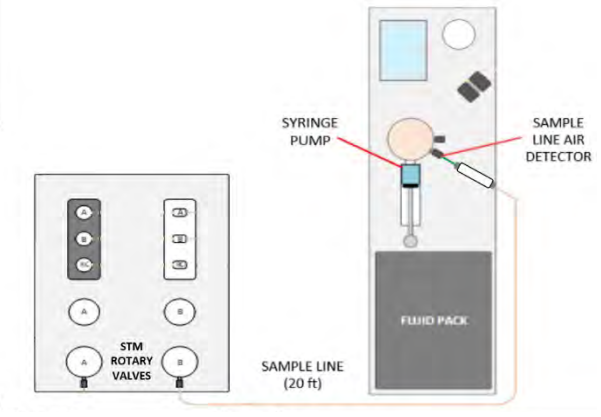


Figure 2.17 Sample Line and Sample Line Air Detector

The **Sample Line Air Detector** is a replaceable sensor that is installed along the Sample Line and connected to the RSM syringe pump. It serves to verify RSM Fluid Pack priming and the delivery of System Fluid and Depro solution during cleanup sequences.

2.2.5.4 Reactor Line

In the Biopharmaceutical industry, there are many different vessels available from a variety of vendors for cell culture and/or fermentation-based processes. The BioProfile FLEX2 On-line Autosampler is best suited for integration with a bench-scale, stirred tank, glass bioreactor with a 1.0-10.0 L working volume, and can be customized for other vessel types like single-use bench-scale reactors, single-use bags (SUBs), rocker bags, and the ambr® 250 modular system by Sartorius Stedim Biotech.

The **Reactor Line** is a 2-ft-length of autoclavable tubing that branches into two segments: a vent line, equipped with an overpressure valve and sterile air filter for insertion of an air gap between the RSM and reactor, and a reactor line that connects the bioreactor to the RSM for sample acquisition. At the RSM, both segments are seated in the pinch valve, and then the reactor line is seated in the RSM air detector and installed on the syringe pump.

The Reactor Line features a ¼”-28 threaded fitting for connection to a dip tube installed on the reactor headplate. Nova Biomedical has various dip tube assemblies available for 10 mm standard (M10 thread) stainless steel reactor headplate ports, including 7", 9", and 15" threaded dip tube assemblies; and 5.5-15” options for hose barbed dip tube assemblies. For 12 mm standard (PG 13.5 thread) headplate ports, Nova offers 7.75" and 8.88" barbed dip tube assemblies. (See Appendix A or contact your local sales representative for a comprehensive Autosampler Parts List).

The Reactor Line can be customized for connection to other vessel types using the **Reactor Line Adapter**. The adapter is an autoclavable, Y-shaped tubing assembly that enables the standard Reactor Line to be customized for other applications, such as connection to a barbed dip tube, the ambr® 250 modular system by Sartorius Stedim Biotech, or a variety of other single-use vessels.

NOTE: *If you have questions regarding vessel connections, please contact Nova Biomedical Technical Support and ask to speak with a BioProfile Applications representative. For customers outside of the USA or Canada, please contact your local Nova Biomedical Subsidiary or Distributor.*

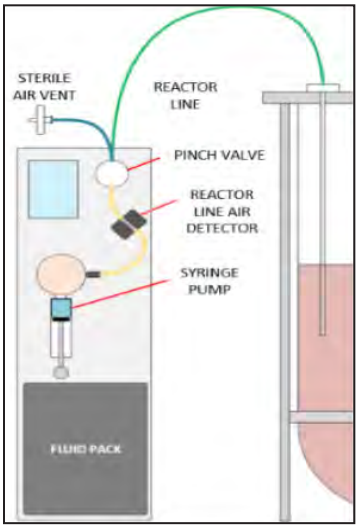


Figure 2.18 Reactor Line

2.2.5.5 Retain Collector Lines

The Retain Collector has several sections of tubing. The **SRCS Sample Line** connects the STM Retain Collector air detector to the top of the probe arm on the SRCS. The **SRCS Waste/Wash** tubing set connects the rinse station to the peristaltic pump and pumps wash fluid from the wash bottle and waste to the waste bottle.

2.3 Overview

The on-line sampling sequence entails sample acquisition by the RSM, sample delivery from the RSM to FLEX2 via the STM, sample analysis by the FLEX2, optional retain collection, and cleanup of the OLS sample flow path. Once a scheduled or manual Autosampler analysis is initiated, sampling proceeds as follows:

1. The RSM checks to verify that no fluid is present in the Reactor Line.

NOTE: *The RSM pinch valve isolates the reactor from the rest of the system by remaining closed to the reactor in the idle state and maintaining a sanitized air gap between the pinch valve and syringe pump.*

2. The Reactor Line is purged with fresh culture from the reactor and cycled to the Fluid Pack Waste Bag.
3. Additional sample is drawn to prime the vent line and provide the leading edge of the sample.
4. The sample is drawn by the RSM syringe pump as specified in the FLEX2 Configuration menu.
5. Once the sample is acquired by the RSM, it is pushed to the STM by the RSM syringe pump.
6. The STM Rotary Valve opens to the address of the selected RSM.
7. The Sample line is partially primed using a fixed volume, then the remainder of sample is delivered through the sample inlet line until fluid is detected at the STM Air Detector.

8. Once sample is detected at the STM Air Detector, the STM pinch valve closes and the Rotary Valve opens to air.
9. FLEX2 analysis is initiated.
10. The FLEX2 sample probe inserts into the sample port septum and aspirates the sample for analysis.
11. After analysis, the residual sample flows out the waste outlet line to the external waste bottle or a remote waste receptacle.
12. As soon as the sample is delivered to the FLEX2 AU, the RSM can start Smart Cleanup or retain collection.
13. Once the FLEX2 is available again following analysis, sampling can immediately begin on the alternate STM bank if no retain is scheduled on that bank.

Retain Collection

14. If a retain is being taken, after the sample is delivered to the FLEX2, the 3-way valve on the respective STM bank switches positions to open to the retain collector.
15. The sample line is partially primed using a fixed volume, then the remainder of sample is delivered until fluid is detected at the RC Air Detector on the STM.
16. Once the RC Air Detector detects fluid, a fixed amount of sample is pumped to ensure the sample reaches the end of the SRCS probe.
17. The Retain Collector probe moves and pumps each retain into the allotted tubes.
18. Smart Maintenance is run for the Retain Collector.
19. Once the FLEX2 and Retain Collector are available following sample analysis, sampling can resume.

NOTE: *Retains of 5mL or more require multiple RSM syringes worth of sample. If a retain is scheduled on both banks, the Retain Collector will be cleaned before the second bank is sampled and retained.*

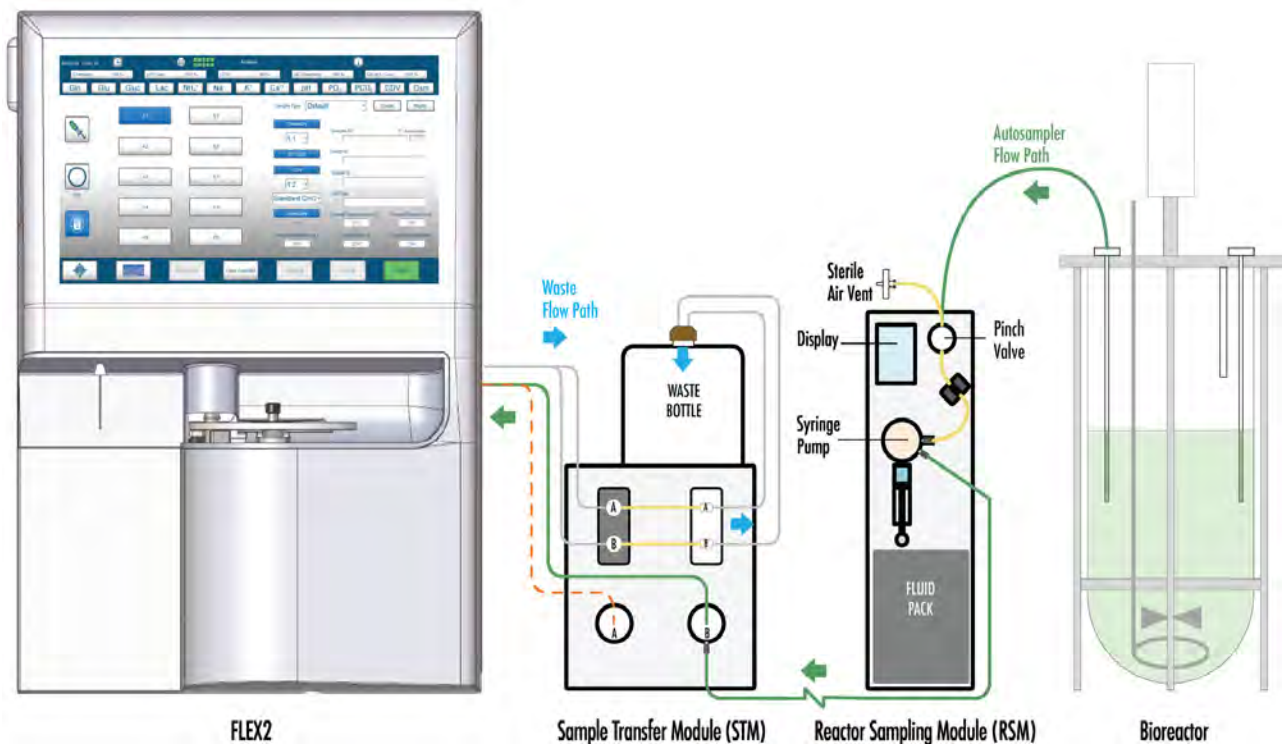


Figure 2.19 FLEX2 On-Line Autosampler Flow Overview

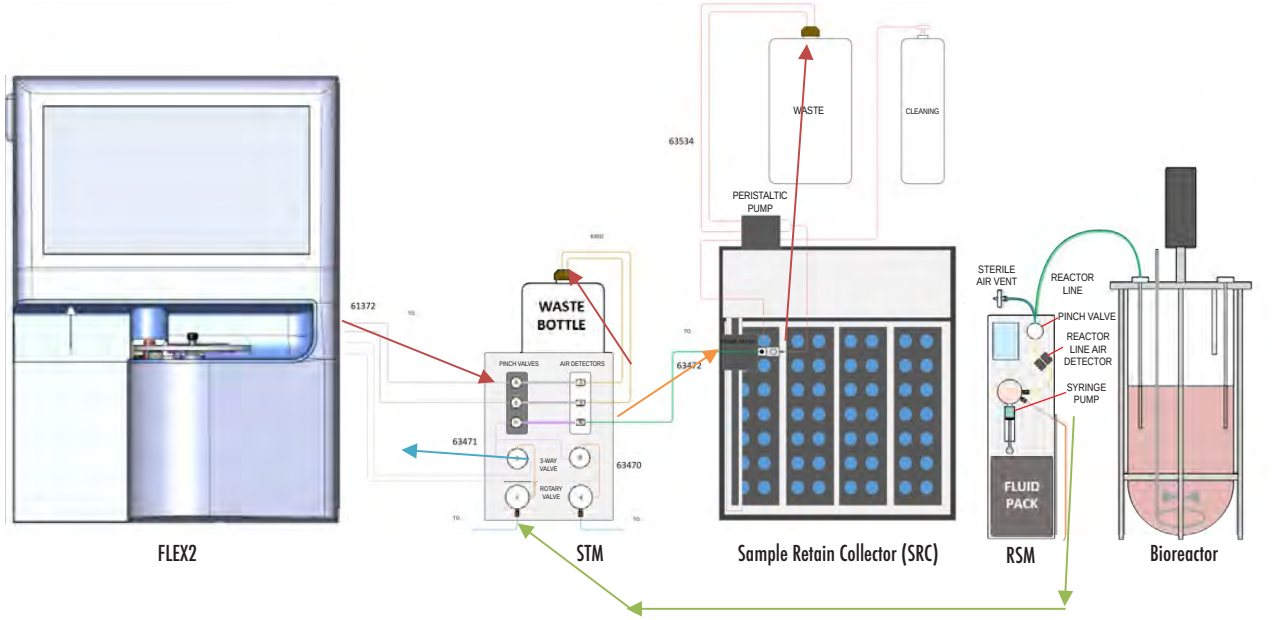


Figure 2.20 Sample Retain Collector Flow Overview

3 Installation

3.1 Installing the OLS

This section serves as an implementation guide for the End User when setting up a BioProfile FLEX2 OLS system. The On-Line Autosampler should only be installed by a trained and qualified Nova Field Support Specialist (FSS). During installation, the FSS will unpack the STM, RSM(s), and Retain Collector (if applicable), place them in position, make the necessary electrical connections, install all fluidic lines, and verify system flow.

The End User is responsible for site preparation leading up to OLS installation, which may involve ensuring adequate bench space for the FLEX2, STM, RSM(s), optional Retain Collector, and cell culture vessel(s); confirming availability of a power supply for all components; and communicating with Nova Biomedical BioProfile Applications personnel to determine the optimal vessel connection(s). The End User will also be responsible for installing the reactor dip tube, autoclaving the reactor line (and optional adapter), and making the RSM reactor line connection to the vessel during final system setup.

Setting up the BioProfile FLEX2 OLS for on-line sampling involves 4 main steps that are outlined in the following sections:

1. Position the STM and RSM(s) on the lab bench, and position the Retain Collector if applicable, see Section 3.2 Spatial Considerations.
2. Connect power and communications cables to the OLS components, see Section 3.3 Electrical Connections.
3. Connect external tubing lines to the components, see Section 3.4 Flow Connections.
4. Configure RSM(s) for analysis, see Section 4.1 RSM Configuration.

3.2 Spatial Considerations

The operator should use the information outlined in Tables 1.2 & 3.1 when preparing the lab space for system installation and when setting up the system. An additional 6 inches of clearance should be left on any sides of the system components containing air vents.

The STM sits directly to the right of the FLEX2. The STM should be positioned within 1.5 feet of the FLEX2 so that the STM Tubing Harness is not under strain. It should also be positioned such that the FLEX2 main door can open freely.

The RSM(s) can be placed to either side of the FLEX2 + STM. The distance from the RSM to the STM should not exceed 20 feet, and the distance from the RSM to the bioreactor should not exceed 2 feet.

The Retain Collector can be placed also to the right of the FLEX2 and cannot be further than 5 feet away from the STM. The required bench space will depend on the Retain Collector model.

NOTE: *When installing multiple RSMs, it is preferable to balance the system by having some RSMs on each side of the FLEX2, if the physical layout of the lab permits.*

3.3 Electrical Connections

Parts Required	Quantity	P/N
Power Cable	1/ FLEX2, 1/STM, 1/RSM, 1/SRCS	N/A
COM Cable	1/STM, 1-2/RSM, 1/SRCS	62563

The independent power cords required for each component are supplied by Nova Biomedical. The STM, RSM(s), and Retain Collector must communicate with the FLEX2 and each other to enable control of hardware components and coordination of reactor sampling and cleanup. Identical, 10-ft DB-9 cables are included in the STM (Qty: 3), RSM (Qty: 1), and SRCS (Qty: 1) accessory packs. The male/female connectors on these cables and the dual COM ports on the rear of the STM and RSM(s) allow for flexibility in RSM wiring (daisy chain) in either direction from the FLEX2/STM. If an RSM is put into long-term shutdown, two RSM cables can be connected to one another to prevent loss of communication to the rest of the system. A grounded, 3-wire receptacle is required within 5 ft of the FLEX2, STM, and each RSM for system operation. At installation, all cables are installed as follows:

NOTE: *Nova recommends using the provided COM cables for optimal performance, the provided cable clamps to neatly dress all power and COM cables.*

1. **Connect the power cables** for the STM and each RSM to the rear power connector on each component and the appropriate power supply. Ensure the power switch on the rear of each component remains OFF.
2. **Connect the STM COM cable** by securing one plug to the COM port on the rear of the FLEX2 and the other plug to the designated rear port on the STM labeled *FLEX2*.

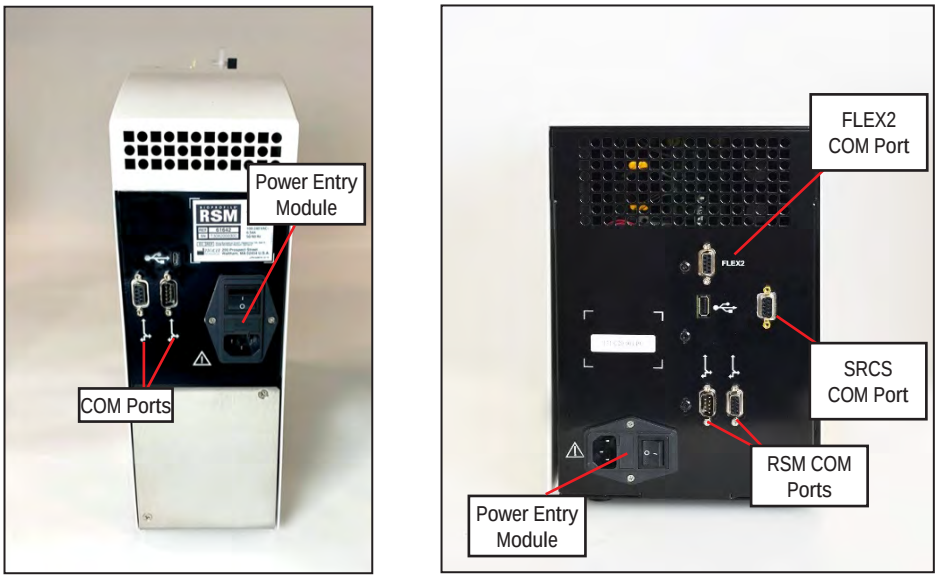


Figure 3.1 System Component Rear View- RSM (left) and STM (right)

Figure 3.2 Designated rear COM ports for STM cable on FLEX2 (left) and STM (right)

3. Connect the RSM COM cable(s) based on RSM quantity and location:
   a. If installing one RSM, connect one end of the cable to a rear COM port on the RSM, and the other end to a lower COM port on the rear of the STM.
   b. If installing two RSMs, connect the cable for the second RSM according to one option below before installing it on the RSM:
      i. Connect to the remaining port on the first RSM (i.e. daisy chain) if the two RSMs are positioned to the same side of the FLEX2.
      ii. Connect to the remaining port on the STM if the two RSMs are positioned to opposite sides of the FLEX2.

NOTE: *The STM accessory pack includes two spare COM cables. If an RSM directly connected to the STM must be placed 10-20 ft from the STM, daisy chain that RSM's COM cable with a spare cable.*

Figure 3.3 COM cable configuration

c. If installing more than two RSMs, daisy-chain additional RSMs together in sequence by connecting the cable for a given RSM to the preceding RSM.

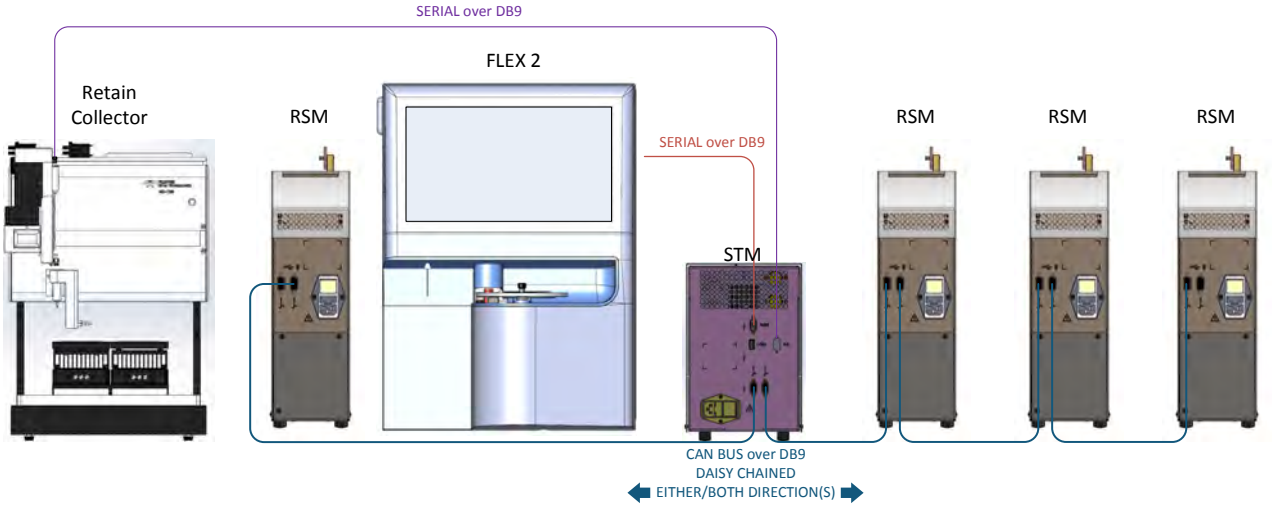


Figure 3.4 RSM COM cable daisy chain configuration

4. If installing a Retain Collector, position the external power supply under the Retain Collector and connect the power cable. Connect the power distribution cables from the power supply outputs to the SRCS and each Peltier Cooling Module (1 cable for each).
5. Use the provided cable clamps to neatly dress all power and communication cables.
6. If the FLEX2 is not already running, power it on now.
7. Once the FLEX2 is fully powered on and initialized, power on the STM by flipping the rear switch to the ON position.
   a. On startup, the STM pinch valves initialize and the status LED on the face of the STM illuminates solid green to indicate active connection to the FLEX2.
8. In the FLEX2 Status Bar, select the **Autosampler Status Indicator** to open the Autosampler Status Window.
   a. Verify that STM Initialized status is *True*, then proceed to the fluidics setup procedure.
   b. If STM Initialized Status is *False*, select **Maintenance** from the bottom of the OLS Status Window to open the Autosampler Maintenance menu, then select **Initialize STM** in the Command Bar.

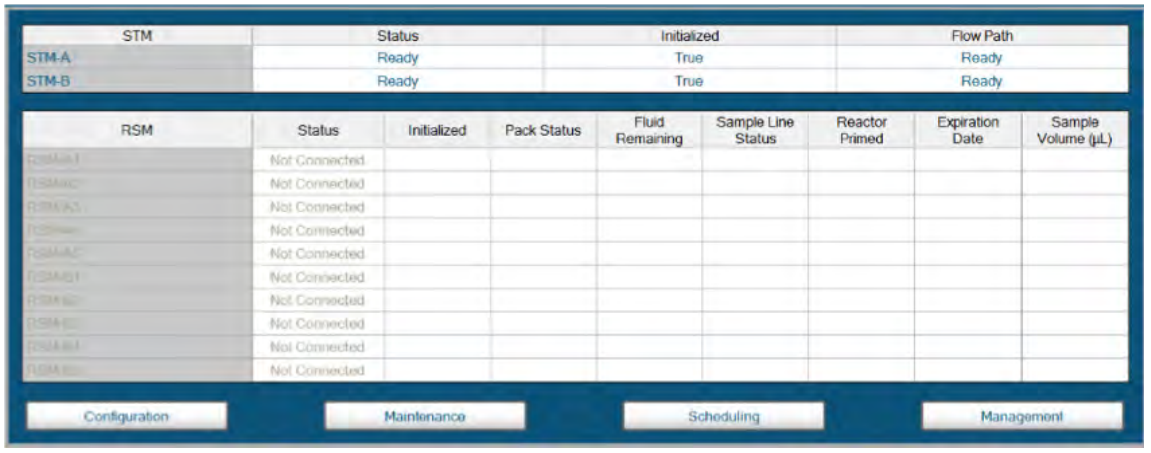


Figure 3.5 OLS Status Window- STM Initialized

3.4 Flow Connections

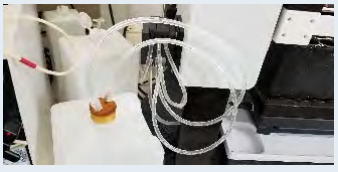
All external tubing sets are included in the OLS accessory packs and initially installed by Nova personnel. For ease of installation and replacement, a wrench-like tightening tool is provided with each RSM to assist in releasing/securing the ¼"-28 threaded connectors featured on most tubing lines. Table 3.1 details the fluidic components required for system setup. The operator should use the specifications provided in Table 1.2 and Table 3.1 to prepare the lab space for system installation.

The instructions detailed in this section assume all components have been positioned on the bench and that all electrical connections have been made according to Section 3.3.

Table 3.1 Flow Component Details

Flow Component		Connection/Details	Length	P/N
STM Tubing Harness		• 2 sample inlet lines from STM rotary valves to FLEX2 cover • 2 waste outlet lines from FLEX2 cover to STM air detectors	30 in (0.76 m)	61372
Waste Tubing Harness		• Waste Cap (PN 63033) & Waste Lines (PN 63032) to connect STM to Nova Waste Bottle	2 ft (0.61 m)	61371
External Waste Line Kit (Optional)		• 2 waste lines connect STM to remote waste receptacle • Alternative to the Waste Tubing Harness, no bottle connections	4 ft (1.22 m)	62878
Sample Line		• Connects to RSM syringe pump valve (via Sample Line Air Detector) and STM rotary valve	20 ft (6.1 m)	61376
Reactor Line		• Connects Bioreactor dip tube to RSM (1/4"-28 Connection) • Includes 2x 0.22 µm sterile air filters	24 in (0.60 m)	61370

Table 3.1 Flow Component Details

Flow Component		Connection/Details	Length	P/N
Reactor Line Adapter (Optional)		• Accessory for reactor line connection to vessel, if needed. • Includes 0.22 µm sterile air filter	4 in (10.2 cm)	63274
Sample Line Air Detector		• Installed on RSM syringe pump valve • Connects to Sample Line	N/A	62849
Wrench/ Tightening Tool		• Use to secure and release ¼"-28 threaded fluidic connections	N/A	N/A
SRCS Sample Line		• Connects STM RC air detector to SRCS Probe	5 ft (1.52 m)	63472
SRCS Wash/Waste Tubing Set		• Connects wash well to the peristaltic pump • 1 single line connects wash bottle to pump 3 input, second line connects pump 3 output to rinse station	18 in (0.46 m) 16 in (0.41 m)	63534
		• 1 Y-tubing connects single wash well output to pump 1 and 2, two additional tubings both connect to shared waste bottle	21.5 in (0.53 m) 24 in (0.61 m)	
STM + Tubing Sets: Rotary Valve to 3-Way Valve and 3-Way Valve to RC AD		• Rotary Valve to 3-Way Valve tubing set consists of 2 separate lines that each connect the center of the rotary valve to the top port of the 3-way valve to the top port of the 3-way valve for both A and B bank	7 in (20.3 cm)	63470
		• 3-Way Valve to RC AD tubing set consists of 1 Y-tubing that connects both of the right-side ports for the 3-way valves for both A and B banks to the STM RC air detector	11 in (27.9 cm)	63471

3.4.1 Installing the STM Tubing Harness

The four lengths of tubing that comprise the STM Harness are sheathed for ease of installation. Follow the steps below and refer to the labels on the ends of each tubing line, the FLEX2 cover interface ports, and the STM chassis to install the harness.

1. Install the STM harness on the FLEX2. Secure Sample Inlet A (SA), Waste Outlet A (WA), Sample Inlet B (SB), and Waste Outlet B (WB) to the first (front-most), second, third and fourth interface ports, respectively.

Figure 3.6 Sample Inlets and Outlets

2. Ensure the STM is positioned such that the STM Harness will not be under strain, then secure the other ends of Sample Inlets A and B (VA and VB) to the right-side ports on STM 3-Way Valves A and B, respectively.

Figure 3.7 Sample Inlet A (VA)

3. Secure the other ends of Waste Outlets A and B (A and B) to the ports left of Air Detectors A and B, respectively.

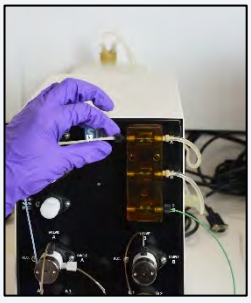


Figure 3.8 Waste Outlet A

4. Seat the outlet pinch tubing segments fully inside Pinch Valves A and B.

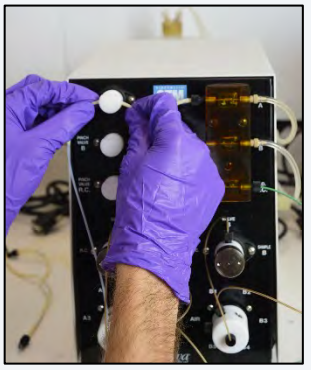


Figure 3.9 Pinch Segment A

5. If a retain Collector is installed, follow steps 5-7. Connect the Rotary Valve to 3-Way Valve tubing to the center of the rotary valve to the top port of the 3-way valve for both A and B bank.

6. Secure the 3-Way Valve to RC AD tubing to both of the left-side ports for the 3-way valves for both A and B banks.

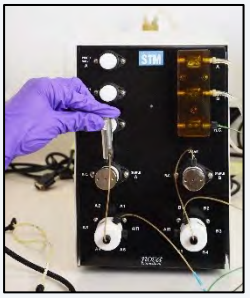


Figure 3.10 3-Way Valve Sample A

Figure 3.11 3-Way Valve A Bank to RC AD

7. Seat the 3-Way Valve to RC AD tubing in the pinch valve in the same manner as steps 3 and 4.

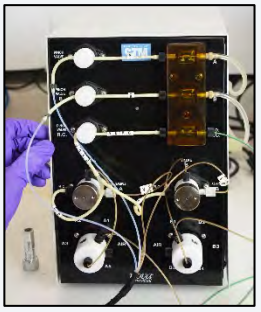


Figure 3.12 RC AD tubing in Pinch Valve

8. Use the wrench to tighten up all connections an additional ¼-turn.

Figure 3.13 Tighten the Connections

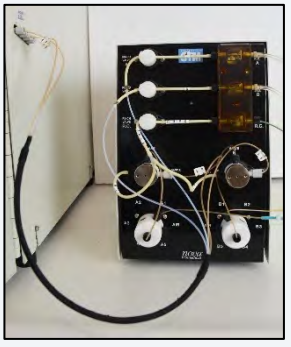


Figure 3.14 STM Tubing Harness Installed

3.4.2 Waste Tubing

If using the OLS Waste Bottle provided by Nova, install the Waste Tubing Harness, PN 61371. Otherwise, install the External Waste Line Kit, PN 62878, if using a remote waste receptacle.

3.4.2.1 Installing the Waste Tubing Harness

1. Remove the white cap that comes installed on the Waste Bottle.

Figure 3.15 Waste Bottle

2. Remove the Waste Tubing Harness from its packaging and disconnect each of the two waste lines from the beige cap to avoid twisting of the lines during installation.
3. Install the beige cap on the Waste Bottle.

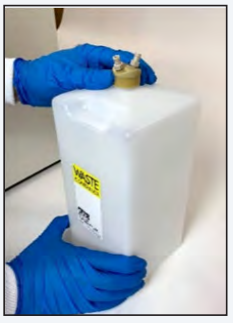


Figure 3.16 Waste Bottle and Beige Cap

4. Place the Waste Bottle in a stable position within 2 feet of the STM.
5. Fasten the ¼"-28 fitting at the end of one waste line to the right-side port of STM Air Detector A, and the other line to Air Detector B.

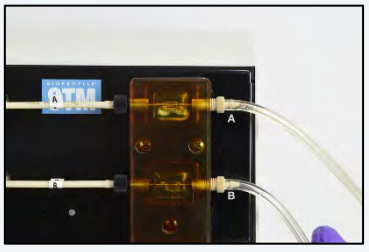


Figure 3.17 Waste Line A

6. Secure the Luer connection at the other end of each waste line to a port on the Waste Bottle cap.

Figure 3.18 Luer Connection on Cap

Figure 3.19 Waste Tubing Harness Installed on Nova Bottle

3.4.2.2 Installing the External Waste Line Kit

1. Fasten the fitting at the end of one waste line to the right-side port of STM Air Detector A, and the other line to the right-side port of STM Air Detector B.

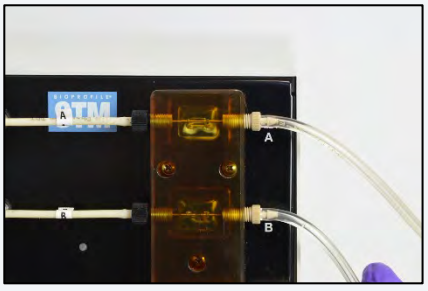


Figure 3.20 Waste Line A

2. Secure the free ends of both tubing lines to the remote waste receptacle.
3. Use the provided rip-ties and/or clamps to neatly dress the waste lines.

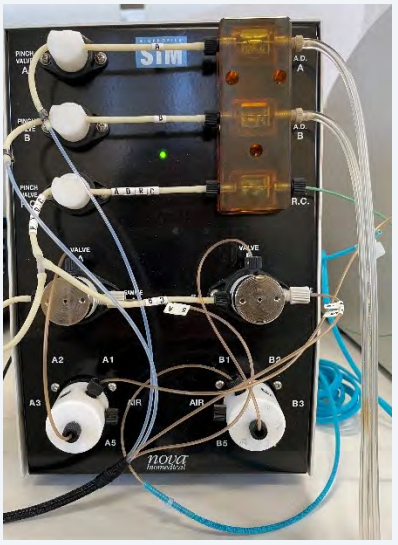


Figure 3.21 External Waste Line Kit Installed

3.4.3 Installing the Sample Line(s) & Sample Line Air Detector(s)

The Sample Line is 20 ft long. Ensure the RSM is within 20 ft of the STM as to not strain the tubing.

The Sample Line connects the RSM to STM Rotary Valve A or B. STM Banks A and B are independent flow paths that enable interleaved sampling and cleanup of A and B RSM's.

1. To avoid confusion when installing multiple RSMs, label the tabs on both ends of each Sample Line with its intended, unique address. Alternate STM Banks (i.e. A1, B1, A2, B2,...,A5, B5).

   Do this for all Sample Lines. Then, install the Sample Line for one RSM at a time.

2. Secure the Sample Line to an available port on the perimeter of either STM rotary valve. This valve location will determine the RSM address, which will be configured in Step 8.

Figure 3.22 Sample Line on Rotary Valve A

3. Use the wrench to tighten the Sample Line an additional ¼-turn on the STM rotary valve.

Figure 3.23 Rotary Valve A

4. Install the Sample Line Air Detector Assembly:

NOTE: *Refer to the label on the front of the RSM when installing the Air Detector on the syringe pump valve.*

CAUTION: *The Sample Line air detector is required for proper function of the system. Do not install the Sample Line directly to the RSM syringe pump.*

a. Fasten the connector on the short length of green tubing to the *FLEX2* port on the RSM syringe pump valve.

Figure 3.24 FLEX2 Port

b. Tighten the connection using the wrench.

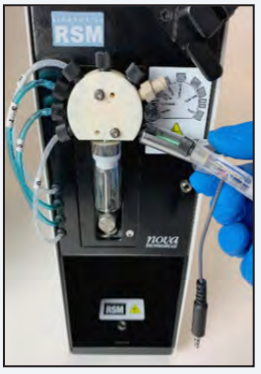


Figure 3.25 Tighten Air Detector

c. Plug in the air detector cable to the jack on the front of the RSM.

Figure 3.26 Air Detector Cable Jack

5. Run the Sample Line tubing from the STM rotary valve to the RSM and secure it to the air detector body.
6. Use the provided rip-ties to neatly dress the Sample Line.

NOTE: *The fittings at either end of the Sample Line tubing are identical, therefore its orientation is arbitrary.*

Figure 3.27 Sample Line and Sample Line Air Detector

Figure 3.28 Sample Line Dressed with Rip-Ties

7. Power on the RSM by flipping the rear switch. The pinch valve initializes, the LED on the built-in RSM air detector illuminates in red, and the User Interface displays the Home Screen.
8. Assign the RSM address according to which STM rotary valve and port the Sample Line was installed on in Step 1 (or refer to the Sample Line tab labeled in Step 1). The STM uses this address to determine which RSM to open the rotary valve to during sampling and other maintenance sequences.
   a. On the RSM User Interface, select **Configure** to open the address configuration screen.
   b. Use the two left arrows to toggle between the two STM bank rotary valves (A or B). Use the two right arrows to set the RSM number (1-5).
   c. Select ✓ to save changes, or ✗ to cancel.
9. Repeat Steps 2-8 for any remaining RSMs.

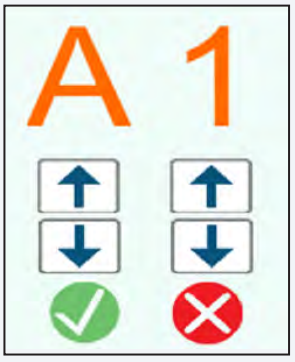


Figure 3.29 RSM UI Configuration Screen

3.4.4 Installing the RSM Fluid Pack(s)

1. Remove a Fluid Pack from its shipping packaging. Included in the package is a small plastic bag containing replacement black rubber septa.
2. Invert the pack a few times. Point the fitments on the rear of the pack downwards and gently slide the pack into the RSM pack bay, ensuring the needle fitments pierce the rubber septa on the rear of the pack.
3. Ensure the face of the pack sits flush with the RSM chassis.
4. Repeat Steps 1-3 for any remaining RSMs.

Figure 3.30 Installing Fluid Pack

Figure 3.31 Pack Fully Installed

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

5. Install the OLS Sample port septa:
   a. Open the FLEX2 main door and remove the threaded caps from both OLS Sample Port assemblies. Then remove and discard the existing septa.
   b. Install two black, replacement septa from the RSM Fluid Pack packaging.
   c. Reinstall both caps until finger tight. Do not overtighten.

Figure 3.32 Cap B Removed to Show Septa

Figure 3.33 Both Caps Installed

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*

NOTE: *The OLS Sample Port septa caps are made of a different material than the pH/Gas Auto-QC septa caps and should not be interchanged.*

3.4.5 Initializing the STM

1. Once all RSM addresses have been assigned, and all RSM Fluid Packs have been installed, Initialize the STM:

   a. On the FLEX2 User Interface, select Maintenance > Autosampler, or the OLS Status Indicator > Maintenance to open the Autosampler Maintenance screen.

   b. Select Initialize STM in the Command Bar. This will initialize the OLS hardware components and query the RSM address(es) assigned in step 8 of Section 3.4.3.

   NOTE: *At this point, the RSM(s) may automatically install and prime the pack(s).*

2. Check the OLS Status Indicator. The status square for each RSM setup so far should show in dark gray indicating the RSM is Connected but Not Configured.

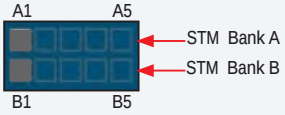


Figure 3.34 RSMs A1 and B1 Not Configured

3.4.6 Connecting to the Bioreactor

Connecting to any vessel, regardless of the type and style of bioreactor, will require the Reactor Line that connects the bioreactor to the RSM for sample acquisition, and maintains the sterile barrier between the vessel and the rest of the non-sterile OLS system. Depending on the vessel type, the Reactor Line Adapter may be required as an addition to the Reactor Line.

During installation, the Nova Field Service Specialist will install the Reactor Line on the RSM and verify system flow with a test solution before the vessel connection is made. Prior to installation and between experiments, the reactor line (and optional adapter) should be autoclaved to ensure complete sterilization. In between runs, the reactor line can be cleaned by manually flushing the tubing or implementing the FLEX2 Intensive Clean Reactor Line maintenance sequence. Follow the steps below to install the reactor line (and reactor line adapter, if applicable) on the reactor and autoclave per your lab's SOP.

3.4.6.1 Installing the Dip Tube(s)

Nova Biomedical has various dip tube assemblies available for standard stirred-tank, glass benchtop style vessels with available headplate ports. The available dip tubes allow for proper headplate port size selection and can accommodate the desired sampling depth from within the reactor. See Appendix A for a comprehensive Autosampler Parts List.

The 10mm threaded dip tube assemblies include a ¼ - 28" fitting at the headplate end that allows for direct connection to the Reactor Line. The barbed dip tube assemblies require the Reactor Line Adapter to make the connection with the Reactor Line Assembly.

Installing the 10 mm Dip Tube Assembly

The custom Nova 10 mm Dip Tube Assemblies are made from 316 L electropolished stainless steel and fit any M10 port in a glass vessel headplate. These assemblies are not pre-sterilized at the factory so they should be thoroughly cleaned using your laboratory's SOPs, prior to use.

1. Remove the selected Dip Tube Assembly from its packaging and remove the protective cap from the sample end of the tube. If working with a barbed dip tube, be sure to also remove the protective cap from the barbed end of the tube.
2. Thoroughly clean the dip tube as you would normally clean the other bioreactor headplate components following your lab's SOP.
3. Each dip tube comes with an orange O-ring for providing an airtight seal between the dip tube and the reactor headplate.

   Verify that the O-ring is properly installed and seated on the dip tube. Inspect the O-ring for any rips or deformities and replace the O-ring if needed (especially for repeated use).

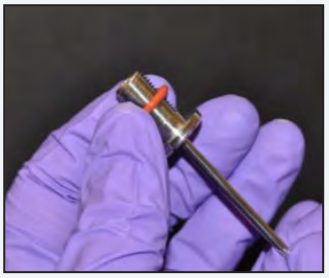


Figure 3.35 O-Ring on Threaded Dip Tube

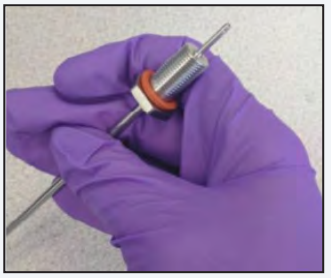


Figure 3.36 O-Ring on Barbed Dip Tube

4. Install the Dip Tube Assembly on the headplate. The 10mm dip tubes are installed upwards from the underside of the headplate and through the M10 port. Install the headplate nut downward onto the dip tube so that it is finger tight, then use a wrench to tighten.

Figure 3.37 Threaded Dip Tube on Headplate

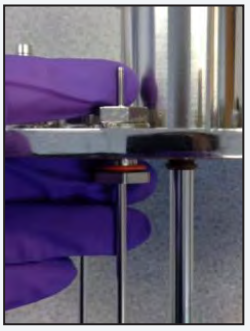


Figure 3.38 Barbed Dip Tube on Headplate.

CAUTION: *If tightening the nut with a wrench, be careful not to over-tighten as this can deform the O-ring and compromise the seal.*

Installing the 12 mm Dip Tube Assembly

The custom Nova 12 mm Dip Tube Assemblies are made from 316 L electropolished stainless steel and fit any PG13.5 port in a glass vessel headplate. These assemblies are not pre-sterilized at the factory so they should be thoroughly cleaned using your laboratory's SOPs, prior to use.

1. Remove the selected Dip Tube Assembly from its packaging and remove the protective cap from the sample end of the tube. Be sure to also remove the protective cap from the barbed end of the tube.
2. Thoroughly clean the dip tube as you would normally clean the other bioreactor headplate components following your lab's SOP.
3. Each dip tube comes with and black O-ring and a white spacer for providing an airtight seal between the dip tube and the reactor headplate.

   Verify that the O-ring and spacer are properly installed and seated on the dip tube. Inspect the O-ring for any rips or deformities and replace the O-ring if needed (especially for repeated use).

Figure 3.39 Black O-Ring and White Spacer

4. Install the Dip Tube Assembly on the headplate. The dip tube should be threaded downward into the PG13.5 port until it is finger tight, then use a wrench to tighten.

Figure 3.40 Dip Tube on Headplate

CAUTION: *If tightening the nut with a wrench, be careful not to over-tighten as this can deform the O-ring and compromise the seal.*

3.4.6.2 Connecting the Reactor Line to the Reactor

The Reactor Line includes a length of green rigid tubing with a ¼"-28 threaded end that connects to the dip tube. The opposite end of the Reactor Line includes flexible tubing that is split with a Y-fitting into two separate lengths. The length with the larger 0.22 µm syringe filter and over-pressure valve is the sterile air vent that mounts atop the RSM. The other end with the smaller 0.22 µm syringe filter is the RSM end which connects directly to the sample inlet (*Reactor*) port of the RSM syringe pump.

NOTE: *The reactor line comes packaged in an autoclavable pouch for purposes of consolidating the various tubing segments.*

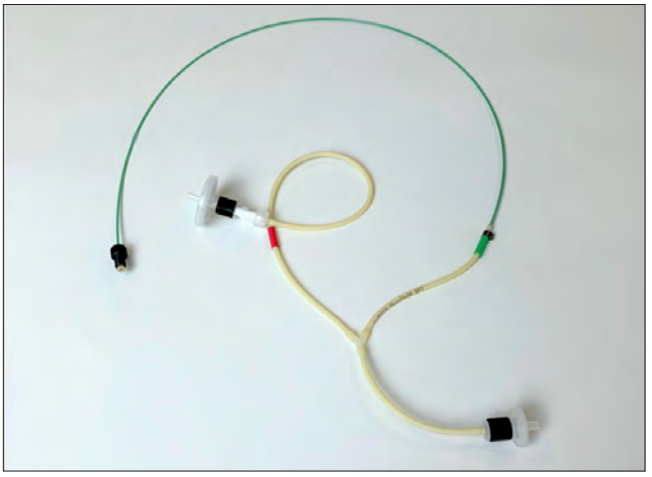


Figure 3.41 Reactor Line Assembly

Standard Vessel Connection (Threaded Dip Tube Assembly)

When using the 10 mm, ¼"-28 threaded dip tubes, the Reactor Line is installed directly on the reactor dip tube. The Reactor Line must be connected to the reactor dip tube before the vessel is sterilized in an autoclave. The Reactor Line is not pre-sterilized and should be thoroughly cleaned using your laboratory's SOPs, prior to use.

NOTE: *Nova recommends that the Reactor Line only be reused for maximum of five (5) autoclave cycles before it is replaced.*

The numbered tubing segments referenced in the steps below are displayed in Figure 3.42.

1. Verify that clean, dry autoclavable 0.22 µm syringe filters are securely installed on the *RSM End (3)* and the *Air Vent End (2)* of the Reactor Line.
2. Fasten the ¼"-28 threaded fitting on the green *Vessel End (1)* to the dip tube installed on the reactor headplate until finger-tight.
3. Using the ¼"-28 Wrench included with each RSM, make sure that the threaded fitting is fully tightened and seated in the dip tube.

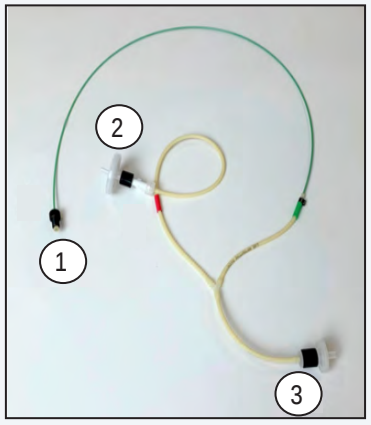


Figure 3.42 Reactor Line Assembly Segments: (1) Vessel End, (2) Air Vent Line, and (3) RSM End.

Figure 3.43 Threaded Headplate Connection

Figure 3.44 Reactor Line Tightened

4. Autoclave the reactor line and vessel per your lab's SOP:

   a. To prevent damage to the Reactor Line during sterilization and pooling of condensate at the filters, it is recommended to loop up the line and tie it back to the reactor with a cable tie. The Reactor Line should only be secured loosely with the cable tie. Take care not to tighten the cable tie down so much that it bends, pinches, or kinks the tubing.

   b. Follow your SOP for autoclaving the size and type of vessel with an additional 15 minutes added to the time of the cycle. Nova also recommends that the dip tube assembly not be submersed in fluid during the autoclaving to allow steam to pass through the lines.

   NOTE: *Additional time is recommended to allow for sufficient steam to move through the small internal diameter of the dip tube and Reactor Line for sterilization.*

   c. Once steam sterilization is complete and the vessel is removed from the autoclave and allowed to cool, check each air filter and threaded connection to make sure that they are still tightly secured.

Custom Vessel Connection with Reactor Line Adapter

Connecting the Reactor Line to a glass, benchtop vessel that either uses a barbed dip tube assembly or has no additional port available for installation of a dip tube, requires the use of the Reactor Line Adapter. Further, the Reactor Line Adapter provides a means of connecting the OLS to single-use bioreactors (SUBs) such as disposable bench-scale vessels, wave or rocker bag-type vessels, or any vessel with which the standard dip tube assemblies cannot be used.

At one end, the Reactor Line Adapter is connected to the barbed dip tube assembly or existing manual sampling port that comes factory installed on these types of single-use reactors, and one arm of the "Y" becomes the new manual sampling port while the other arm is connected to the OLS Reactor Line Assembly. The Reactor Line Adapter is not pre-sterilized at the factory and should be thoroughly cleaned using your laboratory's SOPs, prior to use.

NOTE: *Nova recommends that the Reactor Line Adapter only be reused for maximum of five (5) autoclave cycles before it is replaced.*

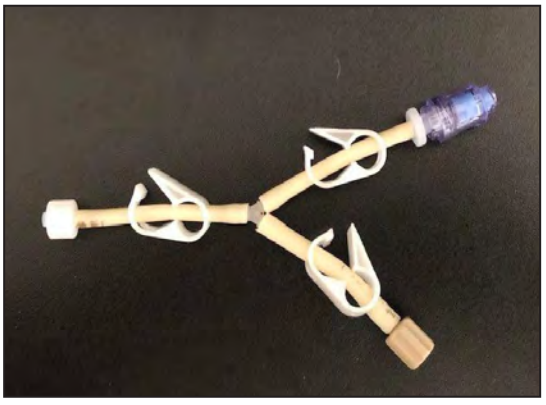


Figure 3.45 Reactor Line Adapter

Connecting to a Barbed Dip Tube Assembly

The numbered tubing segments referenced in the steps below are displayed in Figures 3.42 and 3.46.

1. Verify that clean, dry autoclavable 0.22 µm syringe filters are securely installed on the *RSM End (3)* and the *Air Vent End (2)* of the Reactor Line Assembly.

2. Ensure the sterile filter is attached to the *Vessel End (4)* of the Reactor Line Adapter, and ensure all three tubing clamps are open.

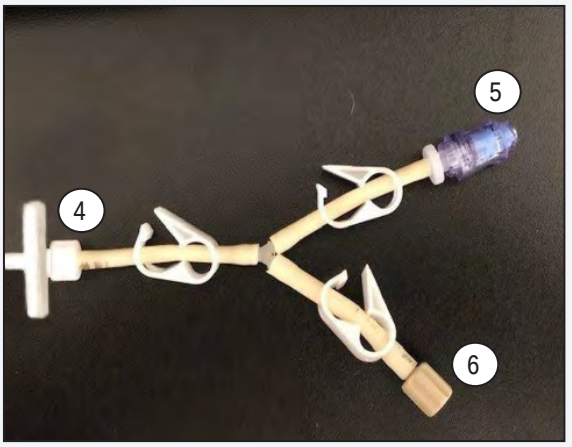


Figure 3.46 Reactor Line Adapter with Filter: (4) Vessel End, (5) Auxiliary Manual Sampling Port, and (6) Reactor Line End

3. Connect the adapter to the Reactor Line. Fasten the ¼"-28 threaded fitting at the *Vessel End (1)* of the Reactor Line to the threaded *Reactor Line End (6)* of the Adapter until finger-tight.

Figure 3.47 Adapter (left) and Reactor Line (right)

4. Connect the Reactor Line Adapter to the vessel.

   a. Remove the sterile filter and the barbed Luer fitting from the *Vessel End (4)* of the Reactor Line Adapter.

Figure 3.48 Sterile Filter/Fitting

   b. Push the open end of the tubing down onto the barbed end of the reactor dip tube until it is fully secured.

Figure 3.49 Adapter on Barbed Dip Tube

5. Autoclave the reactor line + adapter and vessel per your lab's SOP:
   a. To prevent damage to the Reactor Line during sterilization and pooling of condensate at the filters, it is recommended to loop up the line and tie it back to the reactor with a cable tie. Take care not to tighten the cable tie down so much that it bends, pinches, or kinks the tubing.
   b. Follow your SOP for autoclaving the size and type of vessel with an additional 15 minutes added to the time of the cycle. Nova also recommends that the dip tube assembly not be submersed in fluid during the autoclaving to allow steam to pass through the lines.

   NOTE: *The additional time recommendation is due to allow sufficient steam to move through the small internal diameter of the dip tube and Reactor Line + Adapter for sterilization.*

   c. Once steam sterilization is complete and the vessel is removed from the autoclave and allowed to cool, check each air filter and threaded connection to make sure that they are still tightly secured.

6. Manually prime the Auxiliary Manual Sampling Port of the Reactor Line Adapter assembly.
   a. Once the reactor is batched with media, close the *Reactor Line (6)* tubing clamp, and open the *Auxiliary Manual Sample Port (5)* and *Vessel (4)* tubing clamps on the Adapter.
   b. Connect a syringe to the *Auxiliary Manual Sampling Port (5)* and draw back until all the air has been expelled from the dip tube and the Reactor Line Adapter.
   c. Close the tubing clamp on the *Auxiliary Manual Sampling Port (5)* and leave the tubing clamp on the *Reactor Line End (6)* of the Adapter closed until the Reactor Line has been properly installed and connected to the RSM.
   d. Once the *Reactor Line* is connected to the RSM (See Section 3.4.6.3), the tubing clamps on the *Reactor Line End (6)* and *Vessel End (4)* of the adapter should be opened and remain open.

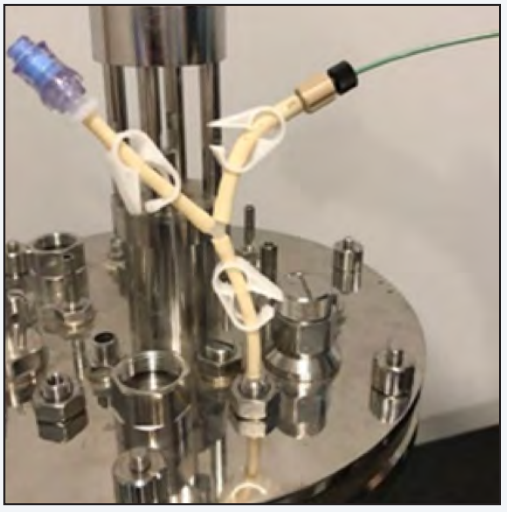


Figure 3.50 Reactor Line Adapter on Barbed Dip Tube Assembly

Connecting to an Existing Manual Sampling Port or Single Use Bioreactor (SUB)

The numbered tubing segments referenced in the steps below are displayed in Figures 3.42 and 3.46.

1. Verify that clean, dry autoclavable 0.22 µm syringe filters are securely installed on the *RSM End (3)* and the *Air Vent End (2)* of the Reactor Line Assembly.
2. Ensure the sterile filter is attached to the *Vessel End (4)* of the Reactor Line Adapter and ensure all three tubing clamps are open.
3. Connect the adapter to the Reactor Line. Fasten the ¼"-28 threaded fitting at the *Vessel End (1)* of the Reactor Line to the threaded *Reactor Line End (6)* of the Adapter until finger-tight.

Figure 3.51 Adapter (left) and Reactor Line (right)

4. Autoclave the reactor line + adapter per your lab's SOP:

   a. Loop up the reactor line + adapter assembly and place it inside the autoclavable pouch. Seal the pouch.

   b. Follow your SOP for autoclaving the assembly in the pouch.

   c. Once the sterilization cycle is complete, move the pouch and bioreactor into the BSC to cool.

5. Connect the Reactor Line Adapter to the vessel. When the pouch and its contents have cooled down to room temperature, connect the reactor line and adapter assembly to the existing manual sampling port of the bioreactor inside the BSC using proper aseptic technique.

   a. Remove the syringe filter from the male Luer fitting at the *Vessel End (4)* of the reactor line adapter.

   b. Connect the sampling port of the bioreactor to the Luer fitting at the *Vessel End (4)* of the adapter until it is securely fastened and make sure that all tubing connections are tight.

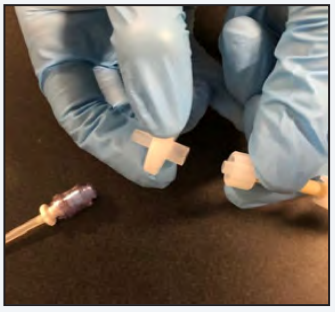


Figure 3.52 Filter Removed from Luer Fitting

Figure 3.53 Existing reactor Manual Sampling Port (Left) and Reactor Line Adapter (Right)

c. Manually prime the Auxiliary Manual Sampling Port of the Reactor Line Adapter assembly.

i. Once the reactor is batched with media, close the *Reactor Line (6)* tubing clamp, and open the *Auxiliary Manual Sample Port (5)* and *Vessel End (4)* tubing clamps on the Adapter.

ii. Connect a syringe to the *Auxiliary Manual Sampling Port (5)* and draw back until all the air has been expelled from the dip tube and the Reactor Line Adapter.

iii. Close the tubing clamp on the *Auxiliary Manual Sampling Port (5)* tubing and leave the tubing clamp on the *Reactor Line End (6)* of the Adapter closed until the Reactor Line has been properly installed and connected to the RSM.

iv. Once the Reactor Line is connected to the RSM (see Section 3.4.6.3), the tubing clamps on the *Reactor Line (6)* and *Vessel End (4)* lines of the adapter should be opened and remain open.

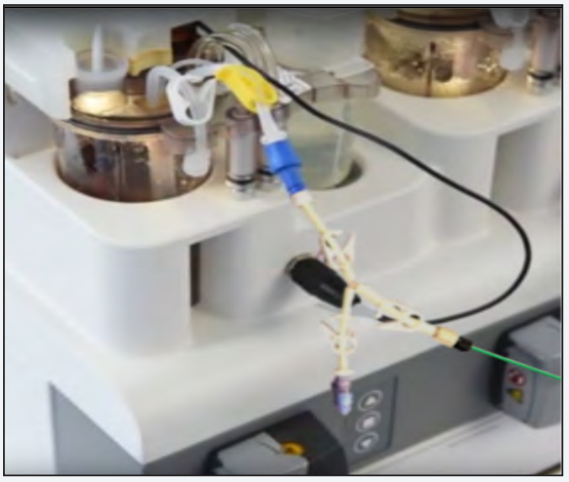


Figure 3.54 Reactor Line Adapter on ambr® 250 Modular Bioreactor Vessel

3.4.6.3 Connecting the Reactor Line to the RSM

Once the reactor line (and adapter, if applicable) have been autoclaved and attached to the reactor, follow the steps below and refer to the syringe pump valve label on the front of the RSM to install the reactor line on the RSM.

The numbered tubing segments referenced in the steps below are displayed in Figures 3.42 and 3.46.

1. Remove the Reactor Line from its autoclavable pouch and seat the tubing segments in the RSM pinch valve according to the colored labels on the valve and each line of tubing:

   d. Seat the *Air Vent Line (2)* in the rear pinch valve position (red) by pulling on the tubing to either side of the valve until it is seated fully inside.

   e. Seat the sterile air vent filter in the designated mounting bracket atop the RSM.

   f. Seat the beige *Vessel (1)* segment fully in the front valve position (green) by pulling on the tubing to either side of the valve until it is seated fully inside.

Figure 3.55 Vent Line in Pinch Valve

Figure 3.56 Filter Mount

Figure 3.57 Reactor Line in Pinch Valve

CAUTION: *The Reactor Line pinch valve remains closed to isolate the bioreactor from the rest of the OLS flow path. It is important to seat the Reactor line pinch tubing in the valve before removing the sterile filter from the bottom of the tubing line RSM segment for sterility.*

2. Remove the sterile air filter from the bottom *RSM End (3)* of the reactor line tubing and secure the connector to the beige Luer fitting installed on the *Reactor* port of the syringe pump valve

Figure 3.58 Air Filter Removed

Figure 3.59 Reactor Port Luer Fitting

3. Seat the Reactor Line inside the RSM air detector by sliding it into place. Avoid stretching the line tubing while performing this step.

Figure 3.60 RSM Air Detector

4. Adjust the tubing so the Y-fitting is as close to the pinch valve as possible, and ensure the tubing is not twisted or crimped between the pinch valve and air detector, or between the air detector and syringe pump.

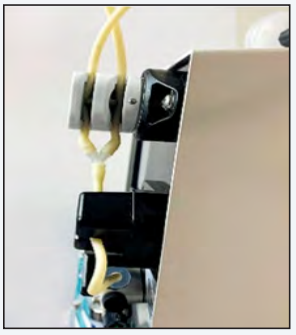


Figure 3.61 Reactor Line Y-fitting

Figure 3.62 Reactor Line Fully Installed on RSM and Bioreactor

3.4.7 SRCS Tubing Connections

1. Connect SRCS Sample Line to the STM RC Air Detector.

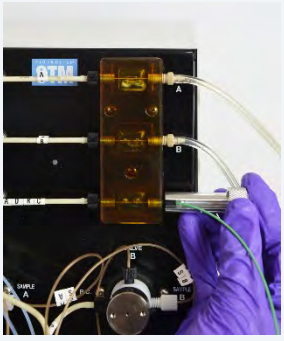


Figure 3.63 SRCS Sample Line STM side

2. Open SRCS Sample Line gates with gate tool and thread the SRCS Sample Line through.

Figure 3.64 Gate Tool

3. Connect the SRCS Sample Line to the top of the probe arm.

Figure 3.65 SRCS Probe Arm

4. Connect the Wash/Waste tubing to the peristaltic pump as shown, (1) From Wash Bottle Straw Cap, (2) To Wash Station Cup, (3) 2 lines Y-tubing from bottom of Wash Station Waste, (4) 2 lines to the Waste Bottle Cap.

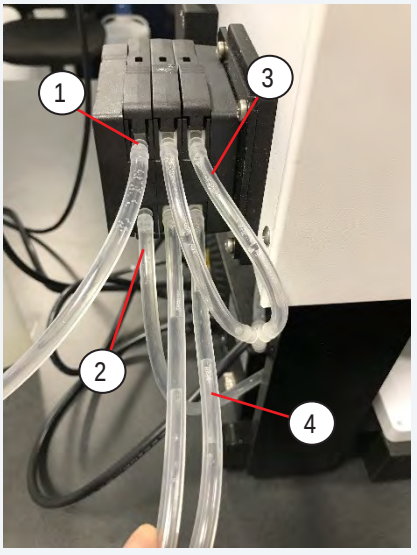


Figure 3.66 SRCS Peristaltic Pump

5. Connect the Wash/Waste tubing to the Wash Station and to their designated bottles as shown, (1) Wash Bottle Cap, (2) Wash Station Cup, (3) Wash Station Waste, (4) Waste Bottle Cap.

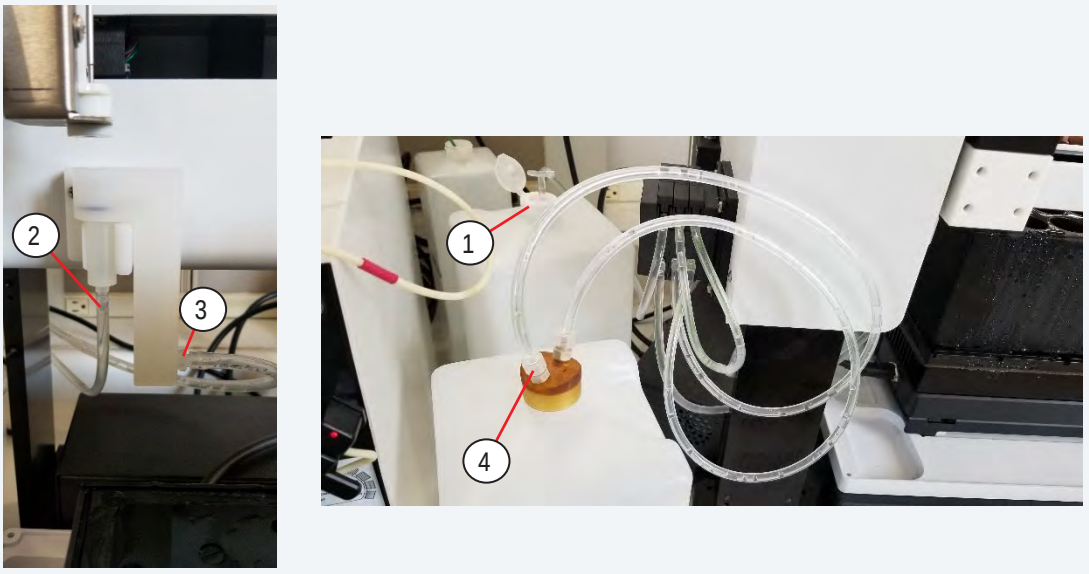


Figure 3.67 Wash Station and Wash/Waste Bottles

3.5 Configuring and Initializing the RSM(s)

Once the electrical and flow connections have been made, the RSM(s) must be configured on the FLEX2 User Interface prior to sampling.

To configure the RSM(s), refer to Section 4.1 RSM Configuration.

3.6 Configuring the Retain Collector

Once the electrical and flow connections have been made, the Retain Collector must be configured on the FLEX2 User Interface prior to taking retains. Refer to section 4.3 SRCS Configuration.

4 Operation

This section details how to operate the BioProfile FLEX2 On-line Autosampler for auto-sampling of up to 10 reactors.

Some OLS operations can be performed from both the RSM and the FLEX2 UIs, while others are unique to only the RSM or FLEX2 screen. When operating the Autosampler from the FLEX2 UI, see Table 4.1 below for a summary of the privilege level required for each function.

NOTE: *There is no log-in required to access the RSM User Interface.*

Table 4.1 FLEX2 OLS User Privileges

Menu	Privilege	Basic	Intermediate	Advanced	Admin
Analysis Menu	Run Autosampler Sample Analysis (with/without retain)	✓	✓	✓	✓
	Cancel Autosampler Sample Analysis	✓	✓	✓	✓
Autosampler Scheduling	Access Autosampler Scheduling	✗	✓	✓	✓
Autosampler Configuration	Access Autosampler Configuration	✗	✗	✓	✓
Autosampler Maintenance	Prime Reactor	✓	✓	✓	✓
	Prime Pack	✓	✓	✓	✓
	Initialize RSM	✓	✓	✓	✓
	Change Syringe	✓	✓	✓	✓
	Clean Sample Line	✓	✓	✓	✓
	Intensive Clean Sample Line	✗	✓	✓	✓
	Intensive Clean Reactor Line	✗	✓	✓	✓
	Long Term Shutdown	✗	✓	✓	✓
	Depro System	✓	✓	✓	✓
	Initialize STM	✓	✓	✓	✓
Historical Results Menu	View, Export, and Print Historical Results	✓	✓	✓	✓
Retain Collector Configuration	Teach SRCS Probe Tray Positions	✗	✗	✓	✓

4.1 RSM Configuration

Once an RSM is connected it must be configured prior to sampling. An operator with a minimum of Advanced privilege can access the Autosampler Configuration screen on the FLEX2 UI to select and customize various analysis parameters and sample information for each RSM prior to analysis, which will be applied to all scheduled and manually initiated autosampler analyses.

Configuring an RSM for analysis involves 3 main steps:

1. Select the RSM(s)
2. Modify configuration parameters as needed:
   a. RSM Name
   b. Line Length
   c. Sample Volume
   d. Dip Tube Length
   e. Additional Purge Volume
   f. Sample Positioning Offset
   g. Reactor Draw Rate
   h. Sample Pump Rate
3. Select FLEX2 Sample Type/Enter Sample Information

These steps are detailed in Section 4.1.3 Configuring the RSM(s).

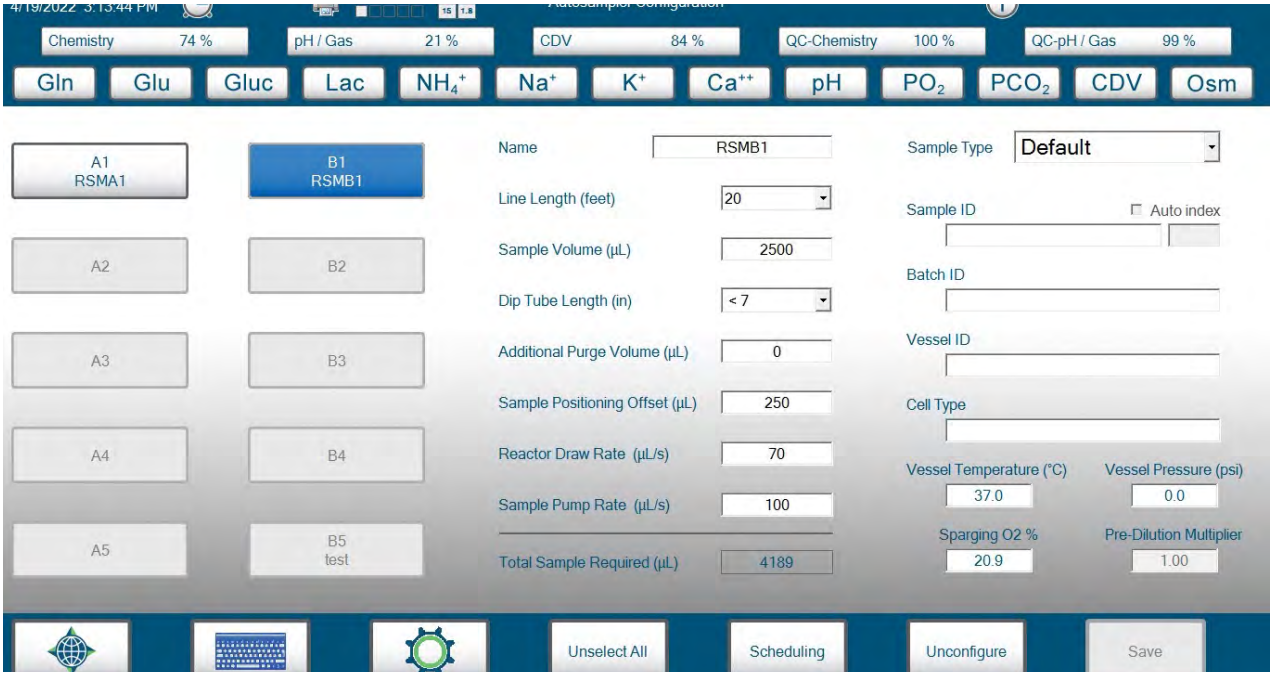


Figure 4.1 RSM Configuration menu screen

4.1.1 Accessing the FLEX2 Autosampler Configuration Menu

To access the Autosampler Configuration Menu screen follow either step below:

- Select the **Autosampler Status Indicator** from the FLEX2 Status Bar to open the Autosampler Status Window. Select **Configuration** from the bottom of the OLS Status Window.
- Select **Settings** from the FLEX2 Right Home Screen. Then, select **Autosampler** from the Settings menu.

On the Autosampler Configuration menu screen, the indicators for all connected RSMs will appear available for selection. From this screen an operator can also navigate to the FLEX2 Settings and OLS Scheduling menus using the icons in the Command Bar.

4.1.2 Default RSM Configuration

The default RSM configurations were determined by Nova during development and may be subject to change depending on the specific reactor connection and process specifications. The default configurations will populate on screen for all RSMs until changes are made by the operator. An operator can restore the default configuration for an RSM at any given time by following the steps below. Table 4.2 summarizes the default configuration values and provides the lower and upper limits for each parameter.

To Restore Default RSM Configuration:

1. Select one or more available and configured RSMs so they are highlighted in blue.
2. Select **Unconfigure** in the Command Bar, then press **Save**.

Table 4.2 Default RSM Configuration Values and Limits

Parameter	Unit	Default Value	Lower Limit	Upper Limit
Line Length	ft	20	20	20
Sample Volume	µL	3800	2000	5000
Dip Tube Length	in	<15	<6	<15
Additional Purge Volume	µL	0	0	8500
Sample Positioning Offset	µL	250	0	1000
Reactor Draw Rate	µL/s	100	40	300
Sample Pump Rate	µL/s	50	40	200

NOTE: *For assistance in optimizing these RSM configurations for your process, contact Nova Biomedical Technical Support and ask to speak with a BioProfile Applications representative.*

4.1.3 Configuring the RSM(s)

Refer to the steps below when configuring an RSM for analysis. Use the touchscreen or external keyboard mouse assembly to enter in values when required. Once configuration changes are made, press **Save** in the Command Bar to save the settings. If there are defined limits for a parameter and the entered value falls outside these limits, a red exclamation point will appear next to the entry field, and any changes will not be saved until the acceptance criteria are met.

1. Select the RSM(s) to be configured from the indicators on the left side of the OLS Configuration Menu screen.
   a. A selected RSM turns blue while an unselected RSM appears white. An RSM that is not connected will be unavailable for selection.
   b. All RSMs can be selected or deselected at once by pressing **Select All/Unselect All** in the Command Bar.
   c. If settings have been configured for an RSM, they will be displayed upon selection. Otherwise, the default configuration will be displayed.
   d. If multiple RSMs are selected, the configuration for the first will be displayed (The Name and Sample Information fields will be blank).

Figure 4.2 Configure RSM Selection

2. Modify the Configuration parameters:
   a. Enter a **Name** for the RSM at the top of the screen if it is preferred to have a unique identifier aside from its alphanumerical address (i.e. A1-A5 or B1-B5). The Name field can only be modified for a single RSM at a time
   b. The **Line Length** value reflects the length of the RSM-to-STM Sample Line. This value is fixed at 20 ft as the line length is not variable at this time.
   c. **Sample Volume** refers to the baseline volume of sample that is drawn from the reactor by the RSM.

d. **Dip Tube Length** refers to the length (in) of the reactor headplate dip tube to which the RSM is connected and defines the required purge volume. Use the dropdown to select the appropriate option: <6, <7, <10, or <15. i.e. For a 7" dip tube, select <7. For a dip tube between 7-10" select <10.
e. **Additional Purge Volume** is an optional field that allows a user to configure additional Reactor Line purge volume (µL) prior to sampling.

NOTE: *The **Total Sample Required** is calculated and displayed on the Configuration and Analysis screens based on the Reactor Line length (fixed) and the inputs for Dip Tube Length, Sample Volume, and Additional Purge Volume. Retains will also affect Total Sample Required.*

f. The **Sample Positioning Offset** is the volume of sample that is pushed past the STM air detector. This allows the user to control which portion of the sample is aspirated by the FLEX2.
g. Adjust the **Reactor Draw Rate** (µL/s) as needed based on sample viscosity. This is the volumetric flow rate of the RSM syringe pump while aspirating a sample from the reactor.
h. Adjust the **Sample Pump Rate** (µL/s) as needed. This is the volumetric flow rate of the RSM syringe pump while delivering (pushing) sample to the STM/FLEX2.

NOTE: *The inputs for Sample Volume, Sample Draw Rate, and Sample Pump Rate define the sample acquisition and delivery sequences. The draw and pump rates can be adjusted to help minimize sheer stress on cells.*

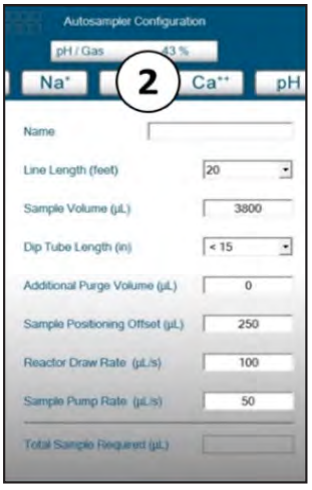


Figure 4.3 RSM Configuration Parameters

3. Configure FLEX2 Sample Information
   a. Select a FLEX2 **Sample Type** from the dropdown menu.
      i. The Sample Type determines which FLEX2 modules are included in the analysis panel (See Section 4.1.3.2 for more information).
      ii. A Sample Type can be created/modified within the Analysis screen.
   b. Configure the FLEX2 Sample Information for analysis. Sample Information can only be modified when a single RSM is selected.
      i. Enter in Sample ID, Batch ID, Vessel ID, and Cell Type, if desired.
      ii. Modify the Vessel Temperature, Vessel Pressure, and Sparging 0_2% if different from the defaults: 37°C, 1 atm, and 20.9%, respectively.

NOTE: *The configured Sample Type and Sample Information are automatically applied to scheduled analyses and automatically populate on the analysis screen for manual on-line samples.*

   c. Press **Save** in the Command Bar before navigating away or selecting a different RSM indicator to ensure all changes are saved. Once an RSM is configured (assuming a valid Fluid Pack is installed and there are no errors) its status square displays in white, indicating it is ready for analysis.

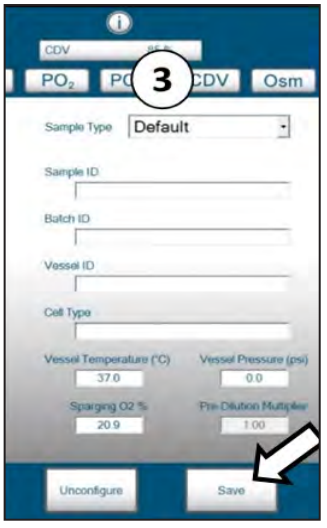


Figure 4.4 Configure and Save Sample Information

4.1.3.1 Required Sample Volume

The total sample volume drawn from the reactor by the RSM is dependent upon the length of the reactor line (fixed) and dip tube, as well as the configurations for Sample Volume and Additional Purge Volume (optional) as defined on the FLEX2 OLS Configuration screen. The Total Sample Required is calculated and displayed on the Configuration and Analysis screens based on the saved inputs for these parameters. The Sample Positioning Offset and FLEX2 Sample Type (i.e. selected modules and dilution ratios) do not impact the required sample volume. Table 4.3 provides some examples of the required sample volume based on RSM configuration. If a retain is added to the sequence, the total volume increases by the volume of the retain(s) plus an additional 3.9 mL for the SRCS purge.

Table 4.3 Total Sample Volume Required (µL) Based on RSM Configuration & Dip Tube Length

Sample Volume (µL)	Additional Purge Volume (µL):					
	0	700	1400	2100	2800	8500
	Dip Tube Length (in.): <6					
2000	*3666*	*4366*	*5066*	*5766*	*6466*	*7166*
2600	*4266*	*4966*	*5666*	*6366*	*7066*	*7766*
3200	*4866*	*5566*	*6266*	*6966*	*7666*	*8366*
3800	*5466*	*6166*	*6866*	*7566*	*8266*	*8966*
4400	*6066*	*6766*	*7466*	*8166*	*8866*	*9566*
5000	*6666*	*7366*	*8066*	*8766*	*9466*	*10166*
	Dip Tube Length (in.): <7					
2000	*3689*	*4389*	*5089*	*5789*	*6489*	*7189*
2600	*4289*	*4989*	*5689*	*6389*	*7089*	*7789*
3200	*4889*	*5589*	*6289*	*6989*	*7689*	*8389*
3800	*5489*	*6189*	*6889*	*7589*	*8289*	*8989*
4400	*6089*	*6789*	*7489*	*8189*	*8889*	*9589*
5000	*6689*	*7389*	*8089*	*8789*	*9489*	*10189*
	Dip Tube Length (in.): <10					
2000	*3758*	*4458*	*5158*	*5858*	*6558*	*7258*
2600	*4358*	*5058*	*5758*	*6458*	*7158*	*7858*
3200	*4958*	*5658*	*6358*	*7058*	*7758*	*8458*
3800	*5558*	*6258*	*6958*	*7658*	*8358*	*9058*
4400	*6158*	*6858*	*7558*	*8258*	*8958*	*9658*
5000	*6758*	*7458*	*8158*	*8858*	*9558*	*10258*
	Dip Tube Length (in.): <15					
2000	*3873*	*4573*	*5273*	*5973*	*6673*	*7373*
2600	*4473*	*5173*	*5873*	*6573*	*7273*	*7973*
3200	*5073*	*5773*	*6473*	*7173*	*7873*	*8573*
3800	*5673+*	*6373*	*7073*	*7773*	*8473*	*9173*
4400	*6273*	*6973*	*7673*	*8373*	*9073*	*9773*
5000	*6873*	*7573*	*8273*	*8973*	*9673*	*10373*

+ Total required sample volume using default RSM configuration

4.1.3.2 FLEX2 Sample Types

A FLEX2 Sample Type must be selected before on-line sampling can occur, as the Sample Type determines the FLEX2 modules to be included in the analysis panel. The Default Sample Type (All Modules, 1:2 Chemistry Dilution Ratio, 1:2 CDV Dilution Ratio) can be utilized, or an operator with a minimum of Advanced privilege can create a new Sample Type tailored to on-line sampling with customized module selections, dilution ratios, and key parameter selections. Once a Sample Type is created, it is made available for selection on the Autosampler Configuration screen and any FLEX2 Analysis screen.

Creating New Sample Types:

Figure 4.5 Create and Modify Sample Type icons

1. From any FLEX2 Sample Analysis screen, select **Create** next to the Sample Type dropdown menu. This opens the Create New Sample Type Window.
2. In the entry box next to Sample Type, type the name of the new Sample Type.

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

3. Configure the **module selections** for the Sample Type. Selected module icons turn blue.
   a. If the Chemistry module is selected for this Sample Type, configure the desired dilution ratio from the drop-down menu (1:1, 1:2 or 1:4).
   b. If the Cell Density 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 drop-down menu.
4. To configure correlation factors for any parameter included in the Sample Type, set the **Offset Multiplier** and **Offset Intercept** to the desired values.
5. 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 from the analytical range limits of the system.
   a. If a parameter recovers outside of the analytical range during a sample analysis, an error is logged in the error log indicating [Parameter] Analytical Range High/Low.
   b. If a parameter recovers outside the process range established in the Sample Type used for that analysis, no error is logged in the error log. Instead, the status column for that parameter is 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. See the BioProfile FLEX2 Instructions for Use Manual for more information*

6. Configure **Key Parameters** (see next section, Key Parameters, for more information).
7. Select ✓ in the bottom right corner to save changes or select ✕ to cancel.

Figure 4.6 FLEX2 Sample Type Window

Modifying Existing Sample Types:

1. From any Analysis screen, select the desired Sample Type from the dropdown window then select **Modify** to open the Modify Sample Type Window.
2. Modify the Sample Type to the desired configuration following steps 2-7 of the above procedure, or select **Deactivate,** then save the modifications by selecting ✓ or cancel the modification by selecting ✗ .

NOTE: *A deactivated Sample Type cannot be re-activated.*

Configuring Key Parameters

The Key Parameters function provides a means of suppressing analysis of on-line samples when specified parameters are not available due to calibration or QC failure (if QC Lockout is enabled). This function only applies to samples supplied to the FLEX2 by the Autosampler or an ESM; it does not apply to the Manual or Load-N-Go carousel sampling modes. An operator has the option to identify which parameters are "key" when creating or modifying a Sample Type:

1. From the Sample Type Window select **True** from the dropdown in the **Key** column for the parameter of interest.
2. Select ✓ to save the Sample Type.

If an on-line 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.

4.1.4 Configuring the Sample Retain Collector

Refer to the steps below when configuring the Sample Retain Collector. The first section instructs how to configure the Retain Collector trays when adding/removing tubes or changing rack sizes. The second section is on the Retain Collector probe calibration which only needs to be done once for each tray size on installation or if poor alignment is observed.

Tray Configuration:

1. Navigate to the RC Configuration Screen using the RC overlay.

Figure 4.7 RC Overlay Icon

2. Select the tray type that matches the trays installed on the Retain Collector using the dropdown menu.

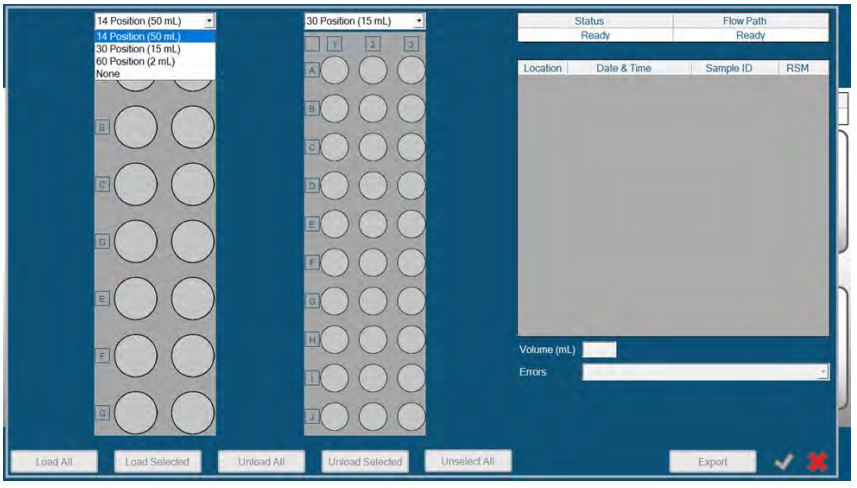


Figure 4.8 RC Tray Size Selection

3. Load tubes by selecting the icons individually or by choosing individual rows and columns to highlight multiple tubes at a time and press the "Load Selected" button. Loaded tubes will be colored green.

Figure 4.9 RC Tray Tubes Loaded

4. Repeat steps for each tray installed. When trays are emptied/tubes removed, repeat steps to match the current configuration by selecting tubes and pressing "Unload Selected/Unload All."

RC Probe Calibration:

1. Go to the Service Menu and select the AutoSampler button to bring up the SRCS service screen. Select the Retain Collector icon.

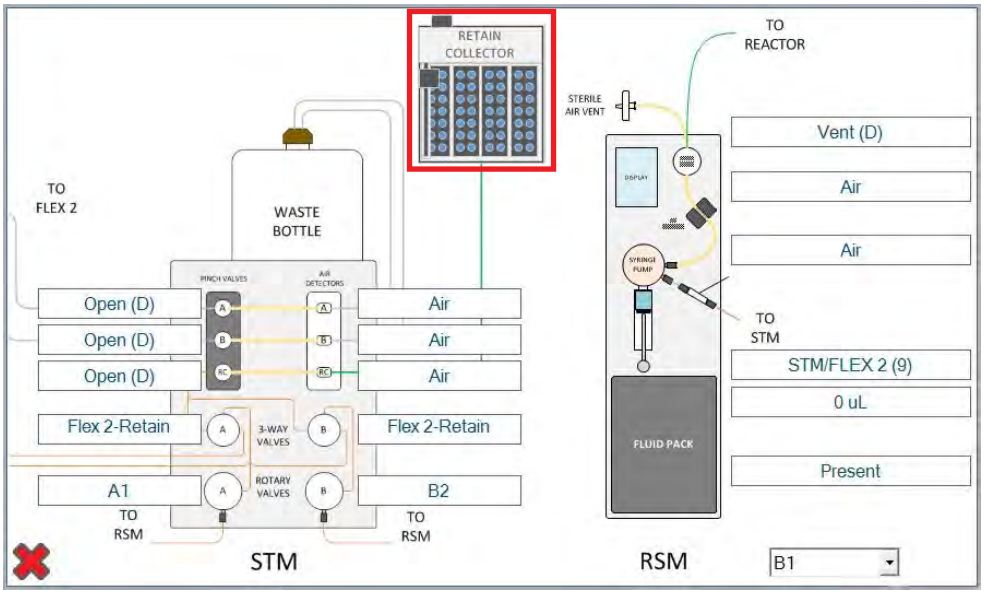


Figure 4.10 SRCS Service Screen

2. Follow this sequence to teach the position for tube A1 (top left tube) of Rack 1. Ensure rack size is properly configured before teaching positions using the previous section. It is not necessary to have a tube installed in the A1 position to teach the positions, but it may be easier to identify the center point.

   a. Select Home in the dropdown and press "Initialize Probe" (Probe moves to back left corner, X and Y coordinates are 0,0).

   b. Select Rack 1 and press "Initialize Probe."

   c. Enter **20** into the Depth field and press "Lower."

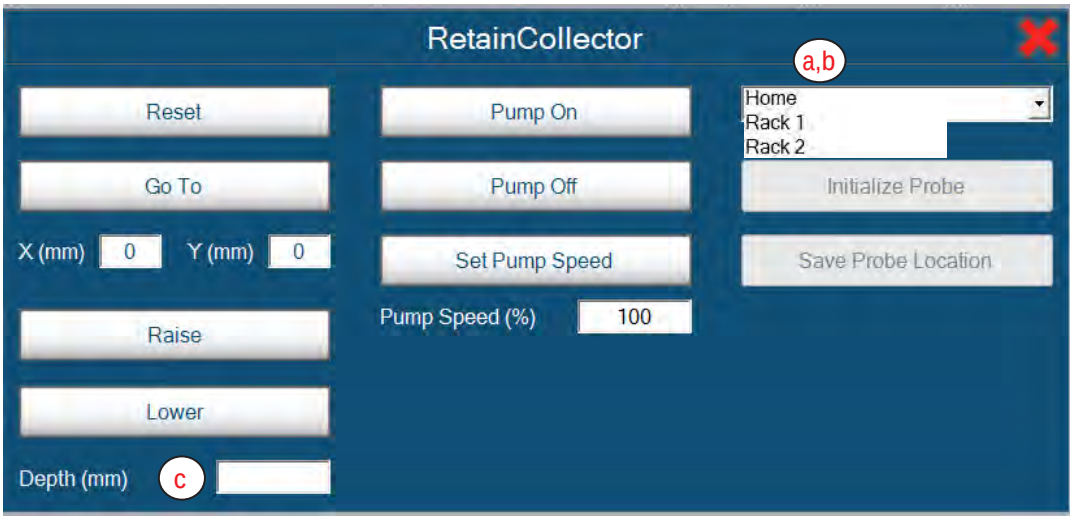


Figure 4.11 Retain Collector Probe Calibration Screen

   d. The probe will protrude. Visually center the probe over Tube 1's position by entering X and Y coordinates and pressing "Go To" to move the probe. Repeat until probe is centered.

WARNING: *Only enter small increments (1-2mm) for the X and Y coordinates each time the probe is moved to avoid crashing the probe and causing system damage.*

   e. Press "Raise" and then press "Save Probe Location."

Swap the dropdown to select Rack 2 and repeat steps c-e for tube A1 on the second rack. Follow the same procedure for Racks 3 and 4 if applicable.

4.2 OLS Analysis

The OLS is designed to enable fast and simple sample analysis through manually initiated auto-samples or scheduling of recurrent analyses for walkway, automated sampling of up to 10 vessels. Once the STM and RSM(s) are in Ready status (i.e. the RSM(s) have been configured and the reactor is primed) the system can run either scheduled or manually initiated on-line samples.

4.2.1 Analysis Preparation

Prior to sampling, the statuses of the STM, RSM(s), and all FLEX2 analytical modules included in the selected Sample Type are checked by the system. The STM and RSM must be Initialized and Ready, and the bioreactor of interest must be primed prior to sampling.

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.

4.2.2 Interleaved Sampling and Cleanup

As discussed in Section 4.1, sample acquisition and delivery are dependent upon the user-defined Sample Volume, Sample Draw Rate, and Sample Pump Rate configured for each RSM. Using the default RSM configuration outlined in Table 4.2, automated sample acquisition and delivery takes approximately 4 minutes.

Once the sample is delivered to the FLEX2, the RSM begins its automated cleanup sequence. The two, independent STM banks (A & B) enable simultaneous sampling and cleanup of A and B reactors. As soon as the FLEX2 becomes available following analysis, sampling can begin from a reactor on the alternate STM bank while cleanup is in progress on the first bank.

The sampling sequence (i.e. sample acquisition, delivery, and FLEX2 analysis) takes between 6-8.5 minutes before results are available and sampling can begin on the alternate bank. The entire sequence (including smart cleanup) takes approximately 12 minutes before sampling can begin on the same STM bank.

NOTE: *The FLEX2 analysis time varies based on modules configured in the selected Sample Type, and settings such as CDV Settling Time.*

4.2.3 Retain Collection

Retain Collection can be enabled for an analysis and will take place after delivering the analysis sample to the FLEX2. The sequence is as follows:

The 3-way valve switches positions to direct flow towards the STM RC air detector. The sample line is partially primed using a fixed volume, the remainder is delivered until the air to fluid transition is detected at the STM RC air detector. Once it detects fluid, a fixed amount of sample is pumped to ensure that sample arrives at the end of the SRCS probe. The probe then moves to the designated tube's position in the tray and dispenses the retain.

NOTE: *Samples over 5 mL require multiple syringes worth of sample.*

4.2.4 Scheduled OLS Analysis

From within the Autosampler Scheduling menu, an operator with a minimum of intermediate privileges can schedule analysis for all configured RSMs that are Ready.

4.2.4.1 Accessing the Autosampler Scheduling Menu screen

The Autosampler Scheduling menu can be accessed by following either step below:

- Select the **Autosampler Status Indicator** from the FLEX2 Status Bar to open the Autosampler Status Window. Select **Scheduling** from the bottom of the OLS Status Window.
- Navigate to the FLEX2 General Settings Menu, then select **Autosampler** from the options on the left of the screen.

Figure 4.12 Autosampler Scheduling Menu Screen

4.2.4.2 Scheduling Autosampler Analyses

1. Select the desired RSM(s) from the center of the Autosampler Scheduling screen. An RSM must be connected and configured to be available for selection.
   a. A selected RSM is highlighted in blue while an unselected RSM appears white.
   b. All RSMs can be selected or deselected at once by pressing **Select All/ Unselect All** in the Command Bar.
2. Select the **Analyze** icon.
3. Check the box next to **Active** to enable Scheduling.
4. Select a **Start Date** and **Start Time** for the schedule using the dropdown menu.

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

5. Select an **End Date** and **End Time** for the schedule using the dropdown menu.
6. Select the **Frequency** in hours at which sampling will occur by entering a value between 1-168.
   a. If retain collection is desired, check the box next to Follow with retain(s). Enter the desired volume of the retain sample and the number of retains to be collected per autosample.
7. Select **Save** in the Command Bar.
8. Repeat for other RSM(s) if different schedule is desired.

NOTE: *It is preferable to stagger the Start times for scheduled analyses at different RSMs. If multiple analyses are scheduled for the same time, sampling will proceed in this order: A1, B1, A2, B2…etc. or will begin with the first RSM that is available and Ready.*

The scheduled Autosampler analyses can now be viewed from the Scheduling menu, or by selecting the Next Scheduled Event icon in the FLEX2 Status Bar to open the New Schedule Event window.

Figure 4.13 FLEX2 Next Scheduled Event Window

4.2.4.3 Deactivating Scheduled Autosampler Analyses

At the conclusion of an experiment, it is important to deactivate the analysis schedule for the RSM(s) that will be disconnected from their vessels and/or shutdown.

To deactivate scheduled analysis for connected RSMs:

1. Select the desired RSM(s) from the center of the Autosampler Scheduling screen.
2. Select the **Analyze** icon.
3. Uncheck the box next to **Active** to disable Scheduling.
4. Select **Save** in the Command Bar.

If you must deactivate the analysis schedule for an RSM that has already been shut down/disconnected, follow the steps below.

To deactivate scheduled analysis for disconnected RSMs:

1. Navigate to the Autosampler Scheduling screen.
2. Select **Unschedule Disconnected RSMs**.

4.2.5 Manual OLS Analysis

Once an RSM has been connected and configured and both the STM and RSM are initialized and in Ready status, an Autosampler analysis can be manually initiated in one of two ways: (1) From the FLEX2 Autosampler Analysis screen or (2) From the local RSM User Interface Home Screen.

4.2.5.1 Accessing the Autosampler Analysis Menu

To access the FLEX2 Autosampler Analysis menu follow the steps below:

1. Select the **Analysis** icon from the Left Home Screen of the FLEX2 User Interface.

Figure 4.14 Analysis Icon

2. Select the **Autosampler** icon from the analysis mode options listed on the left of the screen.

Figure 4.15 Autosampler Icon

Figure 4.16 Autosampler Selected for Analysis

4.2.5.2 Manually Initiating Autosampler Analyses from FLEX2 UI

To initiate an analysis manually from the FLEX2 Autosampler Analysis Menu screen:

1. Select an available RSM icon so that it is highlighted in blue.
    a. An RSM must be in the Ready state to be available for selection.
    b. Only one RSM can be selected at a time.
2. Modify Sample Type and Sample Information as needed.

NOTE: *Any modifications made to the Sample Type, Sample Information (including vessel conditions), or modules/dilution ratio selections on the Analysis screen are not sticky; they only apply to the current analysis.*

    a. If a Sample Type and Sample Information have been configured for the RSM on the Autosampler Configuration screen, this information automatically populates upon RSM selection.
    b. Sample information (modules, dilution ratios, etc.) and the Sample Type can be modified from the Analysis screen as needed.
    c. Configuration inputs (i.e. Sample Volume) cannot be modified from the Analysis menu and must be modified through the RSM Configuration menu.
    d. The Total Required Sample Volume (µL) is displayed based on the RSM configuration.
3. Select **Analyze** in the Command Bar.
4. Sample acquisition and analysis begins.

NOTE: *Multiple analyses can be manually scheduled at once. If the resources are not available for the analysis to begin (i.e. the STM is not ready, or another analysis/cleanup on the same bank is in progress), the manually initiated analysis enters the scheduled sample queue upon selecting Analyze. Queued analyses occur in the order in which they were scheduled or will proceed at the first RSM that is Ready.*

5. Once the analysis completes, navigate to the FLEX2 Historical Results menu screen to review the sample results.

4.2.5.3 Manually Initiating Autosampler Analyses from the RSM UI

To initiate an analysis from the RSM User Interface:

1. Ensure the RSM has been programed with its correct STM address, configured on the FLEX2 User Interface, and has its status square shown Ready (white).
2. Select **Analyze** from the RSM Home Screen to open the Analysis Confirmation screen.
3. Select ✓ to begin sample acquisition. Select ✗ to cancel and return to the Home. If no selection is made within a few seconds, the RSM UI returns Home.

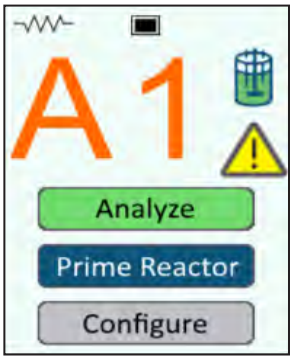


Figure 4.17 RSM Home Screen

Figure 4.18 Analysis Confirmation Screen

4. Sample acquisition and analysis proceeds using the configured settings, Sample Type, and sample information customized on the FLEX2 Configuration screen.

NOTE: *Note, if the resources are not available for the analysis to begin (i.e. the STM is not ready, or another analysis/cleanup on the same bank is in progress), the analysis enters the scheduled sample queue upon selecting Analyze. The analysis will begin once the RSM is Ready.*

5. Once the analysis completes, navigate to the FLEX2 Historical Results menu screen to review the sample results.

NOTE: *Retains cannot be collected when starting an analysis from the RSM.*

4.2.6 Sample Management

If a sample is initiated manually from the FLEX2 Autosampler Analysis Screen or the RSM User Interface and is not able to start immediately, it enters the FLEX2 scheduler and begins once the required resources are available. Select the **Next Scheduled Event** icon in the FLEX2 Status Bar to display manually queued samples alongside scheduled analyses and maintenance sequences. Manually queued autosampler analyses are managed in the Autosampler Management window, while scheduled autosampler analyses are managed in the Autosampler Scheduling menu.

Figure 4.19 FLEX2 Next Scheduled Event Window

4.2.6.1 Accessing the Autosampler Management Window

To access the Autosampler Management window, Select the **Autosampler Status Indicator** from the FLEX2 Status Bar to open the Autosampler Status Window, then select **Management** from the bottom of the OLS Status Window.

Figure 4.20 Autosampler Management Window

4.2.6.2 Managing Queued Samples

1. Open the Autosampler Management Window.
2. Select a sample from the list or press **Select All** to highlight the entire list.
3. Select **Suspend** to suspend all scheduled autosampler samples. Any analysis already in progress will proceed to completion.
4. Select **Delete** to remove the selected sample(s) from the Queued Samples list and the scheduler.
5. Press **Resume** to continue sampling once the queue is managed.
6. Select ✕ to close the Autosampler Management Window.

5 Maintenance

This section explains the maintenance procedures for the Autosampler and how to use them most effectively. Autosampler maintenance procedures are enabled on the FLEX2 User Interface and can be performed directly from the FLEX2 Autosampler Maintenance menu. Of these procedures, some are automated while others require that flowpath components be removed or disconnected from the system. Maintenance procedures pertaining to external tubing replacement are performed outside of the Maintenance menu.

System status data relevant to automated maintenance can be obtained through the FLEX2 OLS Status Window, and Maintenance menu sequences are tracked in the FLEX2 Maintenance Log.

Maintenance should be performed according to the appropriate recommended schedule or as needed to improve performance and troubleshoot any errors.

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.*

The various maintenance procedures are outlined in the following sections:

- Smart Maintenance
- Automated Maintenance Procedures
- Component Replacement

5.1 Smart Maintenance

Smart Cleanup is executed at the end of every sampling sequence, as soon as the sample has been delivered to the FLEX2. The automated Smart Cleanup sequence proceeds as follows:

1. The RSM syringe pump delivers Depro solution from the Fluid Pack through the Reactor port and into the Vent Line of the reactor line.
2. The Sample Line of the RSM that was just analyzed is flushed with System Fluid, Depro, and Shutdown solution, then purged with air.
3. The Depro is drawn back out of the Vent Line and dumped to waste, leaving a sterile air gap in the Reactor Line between the pinch valve and syringe pump.
4. After all RSM/STM sequences have been addressed, one available RSM is assigned to clean the SRCS.

The status of the FLEX2 Autosampler is constantly monitored on both the FLEX2 and RSM UI, and the system will automatically try to resolve any errors that might be present at a given time. After two failed attempts to resolve an issue on a given RSM, the system will stop trying to fix it and manual operator intervention may be required.

NOTE: *The RSM will not automatically attempt to re-prime a reactor after a failed prime.*

Like the other FLEX2 module reagent cartridges, the RSM Fluid Pack is Smart Maintenance-enabled, meaning reagent management information such as expiry and fluid remaining are tracked in the OLS Status Window on the FLEX2 UI.

5.2 Automated Maintenance

This section details the maintenance sequences available in the FLEX2 Autosampler Maintenance menu. From within the Autosampler Maintenance menu, an operator with appropriate privileges can perform the following procedures for any available RSM(s):

- Prime Reactor
- Prime Pack
- Initialize RSM
- Change Syringe
- Clean Sample Line
- Intensive Clean Sample Line
- Intensive Clean Reactor Line
- Long-Term Shutdown

The following system maintenance sequences can also be performed:

- Initialize STM
- Depro System

Of these procedures, some are fully automated using onboard reagents contained within the RSM Fluid Pack, while others require that flowpath components be connected to or disconnected from the system for the introduction of external cleaning solution to the system.

5.2.1 Accessing the FLEX2 Autosampler Maintenance Menu

To access the Autosampler Maintenance menu follow either step below:

- From the FLEX2 Left Home Screen, select **Maintenance** to view the system maintenance screen. Select **Autosampler** in the Command Bar to open the Autosampler Maintenance menu.
- Select the **OLS Status Indicator** from the FLEX2 Status Bar to open the Autosampler Status Window. Select **Maintenance** from the bottom of the OLS Status Window.

Figure 5.1 FLEX2 Autosampler Maintenance menu screen

5.2.2 Using Automated Maintenance Procedures

An RSM must be connected and configured for it to be selectable from the Maintenance menu. Only one RSM icon can be selected at a time. Selecting an available RSM will enable the Maintenance icons, and will populate that RSM's status information in the table at the top of the screen. Once a maintenance sequence is started for a given RSM, an operator can immediately proceed to the next RSM and begin maintenance (i.e. operator need not wait for first RSM's maintenance to finish to attempt maintenance at a second RSM). Note that if the resources are not available for a maintenance sequence to begin, the procedure will begin once the required resources are available.

5.2.2.1 Prime Reactor

The Prime Reactor function allows an operator with appropriate privileges to prime the Reactor Line of a selected RSM once it is connected to the bioreactor. The reactor must be primed prior to sample analysis. During a reactor prime the RSM syringe pump draws fresh culture from the reactor followed by Depro solution from the RSM Fluid Pack through the Reactor Line to flush and decontaminate the flow path. The RSM syringe pump deposits the culture and Depro solution into the self-contained Fluid Pack waste bag. Once the priming sequence is completed, the length of Reactor Line tubing between the pinch valve and syringe pump will be filled with sterile air. The green tubing of the Reactor Line will be filled with cell culture material. The Prime Reactor sequence can be scheduled to occur at regular intervals from the FLEX2 Autosampler Scheduling menu.

To Prime Reactor:

1. Verify the Reactor Line is securely connected to the cell culture vessel.
2. Select an available RSM from the Autosampler Maintenance screen.
3. Select the **Prime Reactor** icon so that it is highlighted in blue.
4. Select **Start** from the Command Bar to begin the Prime sequence.
   a. This sequence takes approximately 3.5 minutes to complete.

NOTE: *A Reactor Prime can also be initiated locally at the RSM User Interface by selecting* ***Prime Reactor*** *from the RSM Home Screen.*

To schedule Prime Reactor:

1. Navigate to the FLEX2 Autosampler Scheduling menu screen.
2. Select the desired RSM(s) from the left side of the Autosampler Scheduling screen. An RSM must be connected and configured to be available for selection.
   a. A selected RSM is highlighted in blue while an unselected RSM appears white.
   b. All RSMs can be selected/deselected at once by pressing **Select All/Unselect All** in the Command Bar.
3. Select the **Prime Reactor** icon.
4. Check the box next to **Active** to enable Scheduling.
5. Select a **Start Date** and **Start Time** for the analysis using the dropdown menu.

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

6. Select an **End Date** and **End Time** for the schedule using the dropdown menu.
7. Select the **Frequency** in hours at which priming will occur by entering a value between 1-168.
8. Select **Save** in the Command Bar.
9. Repeat for other RSM(s) if a different schedule is desired.

NOTE: *It is preferable to stagger the Start times for scheduled primes at different RSMs. If multiple primes are scheduled for the same time, priming will proceed in this order: A1, B1, A2, B2…etc. or will begin with the first RSM that is available and Ready.*

5.2.2.2 Prime Pack

Like the other FLEX2 module reagent cartridges, the RSM Fluid Pack is Smart Maintenance-enabled, meaning reagent management information such as expiry and fluid remaining are tracked in the OLS Status Window on the FLEX2 UI. When a new RSM Fluid Pack is installed, the pack prime will initiate automatically.

The Prime Pack function allows an operator with appropriate privileges to prime the internal RSM tubing from the Fluid Pack to the syringe pump. During a pack prime, the RSM syringe pump draws System Fluid to the Sample Line to calibrate the Sample Line Air Detector.

To Prime the RSM Pack:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Prime Pack** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the Prime sequence.
   a. This sequence takes under a minute to complete.

5.2.2.3 Initialize RSM

The Initialize RSM function allows an operator with appropriate privileges to initialize the RSM hardware and reset the RSM syringe pump valve.

To Initialize the RSM:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Initialize RSM** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the Initialize sequence.

5.2.2.4 Clean Sample Line

The Clean Sample Line function allows an operator with appropriate privileges to clean the Sample Line and Reactor Line using onboard reagents from the RSM Fluid Pack. During the Clean Sample Line sequence, the Reactor Line is purged of culture to Pack Waste and filled with Depro. The Sample Line is flushed with System Fluid, Depro, Shutdown Solution, then air (in order) through to the external waste container. The Depro is drawn out of the Reactor Line and pushed to Pack Waste.

To run a Clean Sample Line sequence:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Clean Sample Line** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the sequence.
   a. The sequence takes approximately 7 minutes to complete.

5.2.2.5 Change Syringe

The Change Syringe function allows an operator with appropriate privileges to replace the plunger and barrel of the RSM syringe pump assembly (PN 46915).

NOTE: *Nova Biomedical recommends the Syringe is replaced annually, or as needed, for optimal performance.*

To change the RSM syringe:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Change Syringe** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar.
4. The RSM will position the plunger at the bottom of its axis for replacement.
5. Remove the exisiting syringe:
   a. Once the plunger is down, remove the syringe by loosening the thumb screw at the bottom.
   b. Unthread the syringe from the valve assembly.
   c. Remove and discard of the syringe.

Figure 5.2 Bottom Thumb Screw

Figure 5.3 Unthread Syringe Barrel

Figure 5.4 Remove Syringe Assembly

6. Install the new syringe, making sure to secure the threaded connections.
7. The syringe pump will automatically initialize before the next sequence initiates.

5.2.2.6 Intensive Clean Sample Line

The Intensive Clean Sample Line function allows an operator with appropriate privileges to perform a deep clean of the Sample Line using external cleaning solution. This sequence requires the RSM External Cleaning Tubing Adapter (PN 62877) and an external cleaning solution such as Nova's External Depro Solution (PN 46850).

CAUTION: *Other than Nova's External Depro solution, a 3rd party enzymatic solution that is non-caustic and non-corrosive like TrypLE™ Select Enzyme (1x), no Phenal Red (Thermofisher™), or equivalent, can be used for these cleaning sequences.*

To run an Intensive Clean Sample Line sequence:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Intensive Clean Sample Line** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the sequence.
4. A window will appear with the prompt "Provide External Cleaning Solution at the RSM External Line."
5. Remove the black fitting from the *External* (12 o'clock) port on the RSM syringe pump valve and fasten the External Cleaning Tubing Adapter in its place.
6. Insert the free end of the External Cleaning Tubing into the cleaning solution and press **Continue**, or press **Cancel** to dismiss the window and not run the sequence.
7. The RSM syringe pump draws the external cleaning solution through the External Cleaning Tubing Adapter, pushes it into the Sample Line and holds it for 10 minutes to soak the line.
   a. The sequence takes approximately 16 minutes to complete.
8. Shutdown solution is flushed through the Sample Line to STM Bottle Waste (or the remote waste receptacle).
9. The Sample Line is purged with air.
10. Remove the External Cleaning Tubing Adapter and cleaning solution from the syringe pump, reinstall the black fitting.

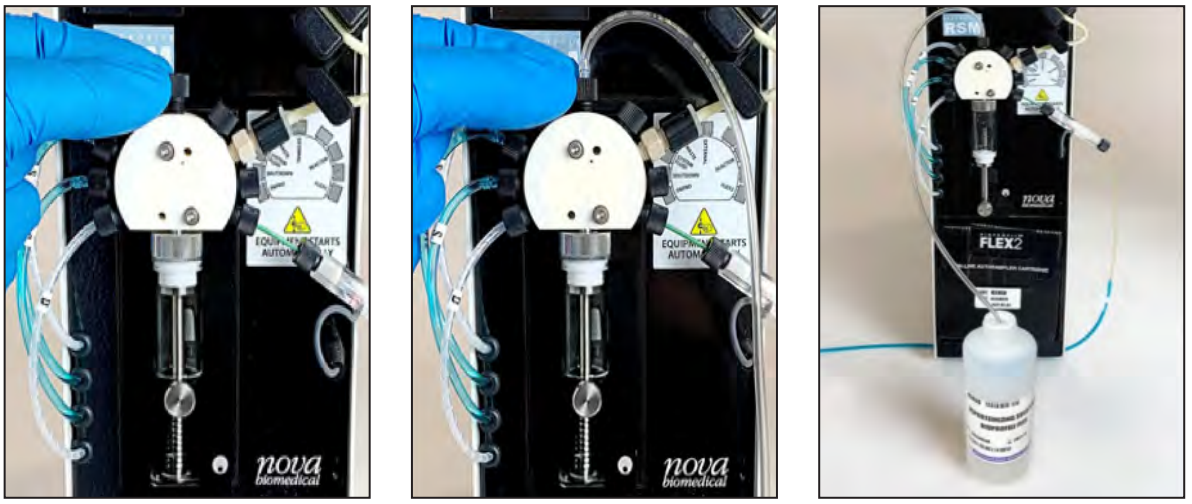


Figure 5.5 Installing the External Cleaning Line

5.2.2.7 Intensive Clean Reactor Line

The Intensive Clean Reactor Line function allows an operator with appropriate privileges to clean the Reactor line using external cleaning solution such as Nova's External Depro Solution (PN 46850).

CAUTION: *If using a third party cleaner other than Nova's External Depro solution, ensure the material is non-caustic and non-corrosive.*

NOTE: *Nova Biomedical recommends cleaning the Reactor Line once a bioreactor run is complete and the reactor line has been disconnected from the vessel.*

To run an Intensive Clean Reactor Line sequence:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Intensive Clean Reactor Line** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the sequence.
   a. A window appears with the prompt "Provide External Cleaning Solution at the Reactor Line."
4. Disconnect the Reactor Line from the reactor and insert the tubing into a cleaning solution such as Nova's Depro Solution.
   a. If using the Reactor Line Adapter tubing, disconnect the Adapter from the reactor and insert the Vessel end of the adapter tubing into the cleaning solution.
5. Select **Continue** to proceed or **Cancel** to dismiss the window and not run the sequence.
   a. The sequence takes approximately 2 minutes to complete, after which the Reactor Line/Adapter can be reinstalled on the vessel.

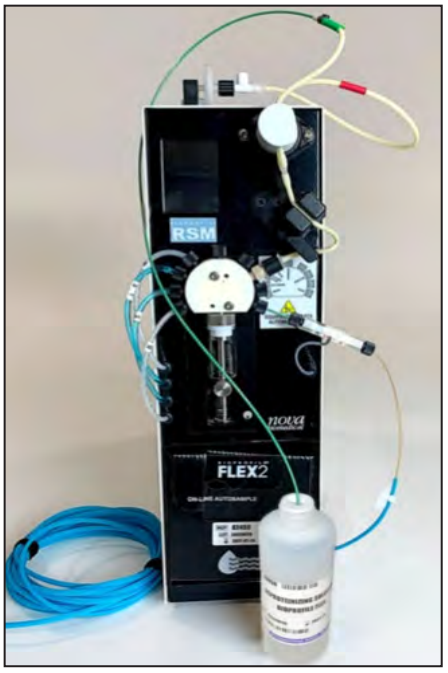


Figure 5.6 Reactor Line in External Depro Solution

5.2.2.8 Long-Term Shutdown

The Long-Term Shutdown sequence allows an operator with the appropriate privileges to effectively shut down the RSM in preparation for an extended period of non-use. During a Long-Term Shutdown, the RSM internal lines are backflushed with Shutdown Solution from the RSM Fluid Pack and then purged with air. Once the Long-Term Shutdown sequence has completed, the RSM Fluid Pack will be invalidated.

NOTE: *If the RSM is going to be idle for longer than a month, please follow the procedure outlined below to prevent salt buildup/microbial growth in the flow path.*

To run put an RSM into Long-Term Shutdown:

1. Select an available RSM from the Autosampler Maintenance screen.
2. Select the **Long-Term Shutdown** icon so that it is highlighted in blue.
3. Select **Start** from the Command Bar to begin the sequence. A window appears with the prompt “This will invalidate the RSM pack.”
4. Select **Continue** to proceed or **Cancel** to dismiss the window and not run the sequence.
   a. This sequence takes approximately 5 minutes to complete.
5. Once the sequence is completed and the pack status reads “Expired” on the FLEX2 UI, power off the RSM by flipping the rear power switch.

5.2.2.9 Initialize STM

The Initialize STM function allows an operator with appropriate privileges to initialize the STM hardware.

NOTE: *The STM should be initialized whenever an RSM address is assigned, or in the event that the system is rebooted or loses power for any reason.*

To Initialize the STM:

1. Select **Initialize STM** from the Command Bar.
2. Select **Start** to begin the sequence.
   a. This sequence takes approximately 10 seconds to complete.

5.2.2.10 Depro System

The Depro System function allows an operator with appropriate privileges to Depro all available RSMs. When the sequence is initiated, all available RSMs prime their respective Sample Lines with Depro solution from the Fluid Packs. Depro is drawn and pushed through to the STM Waste Bottle and held for 20 minutes. Then, the lines are flushed with Shutdown Solution and purged with air in preparation for the next sample. This sequence also calibrates the STM Air Detectors.

To run a System Depro:

1. Select **Depro System** from the Command Bar.
2. Select **Start**. A window appears with the prompt “This will Depro all available RSM sample lines.”
3. Select **Continue** to proceed or **Cancel** to dismiss the window and not run the sequence.

5.3 Component Replacement / Manual Maintenance

The OLS flow path must be maintained by the End User through onboard and external cleaning sequences, and replacement of the external tubing sets at regular intervals. The recommended replacement frequency for each component is listed in Table 5.1, but the optimal maintenance schedule will ultimately depend on sample throughput and sample composition and should therefore be performed as needed.

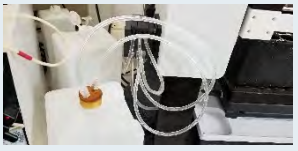
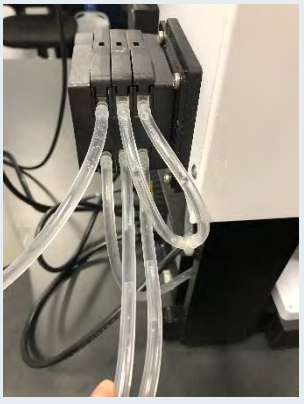
NOTE: *Replacement of the external tubing sets and Sample Line Air Detector is not monitored by FLEX2 Smart Maintenance.*

Table 5.1 Required Flow Component Details

Component		P/N	Replace After
STM Tubing Harness		61372	6 months
Waste Tubing Harness		61371	12 months
External Waste Line Kit (Optional)		62878	12 months
Sample Line		61376	12 months
Sample Line Air Detector		62849	12 months
RSM Syringe		46915	12 months
Reactor Line		61370	5 autoclave cycles (Max)*
Reactor Line Adapter (Optional)		63274	5 autoclave cycles (Max)*
Sample Port Septa		Included in 62450	30 days

*Based on an autoclave cycle at 250°F (20-minute sterilization time, 15-minute dry time)

Table 5.1 Required Flow Component Details

Component	P/N	Replace After
STM + Tubing Sets: Rotary Valve to 3-Way Valve and 3-Way Valve to RC AD 	63470 63471	6 months
SRCS Wash/Waste Tubing  	63534	12 months
SRCS Sample Line 	63472	6 months

5.3.1 Tubing Replacement Procedures

The following sections detail how to replace the various lines of tubing that comprise the external OLS flow path.

5.3.1.1 Replacing the STM Tubing Harness

1. **Remove the existing STM Tubing Harness**
   a. Use the wrench to loosen all connectors on the FLEX2 cover and STM a ¼-turn counterclockwise.
   b. Remove lines **SA**, **WA**, **SB**, and **WB** from the FLEX2 interface ports by turning each connector counterclockwise until released.
   c. Remove lines VA and VB from the STM 3-Way valves by loosening until released.
   d. Remove **Rotary Valve to 3-Way Valve** lines from the both A and B valves
   e. Remove Lines **A** and **B** from the STM Air Detectors.
   f. Lift pinch tubing segments **A** and **B** (and **RC** if installed) out of their respective pinch valves.
   g. Discard the old STM tubing harness.
2. **Install the new STM Tubing Harness**

1. Install the STM harness on the FLEX2. Secure Sample Inlet A (SA), Waste Outlet A (WA), Sample Inlet B (SB), and Waste Outlet B (WB) to the first (front-most), second, .

Figure 5.7 Sample Inlets and Outlets

2. Ensure the STM is positioned such that the STM Harness will not be under strain, then secure the other ends of Sample Inlets A and B (VA and VB) to the right-side ports on STM 3-Way Valves A and B, respectively.

Figure 5.8 Sample Inlet A (VA)

3. Secure the other ends of Waste Outlets A and B (A and B) to the ports left of Air Detectors A and B, respectively.

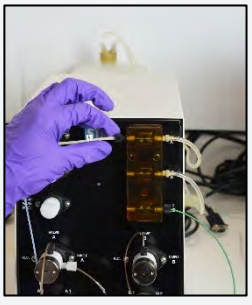


Figure 5.9 Waste Outlet A

4. Seat the outlet pinch tubing segments fully inside Pinch Valves A and B.

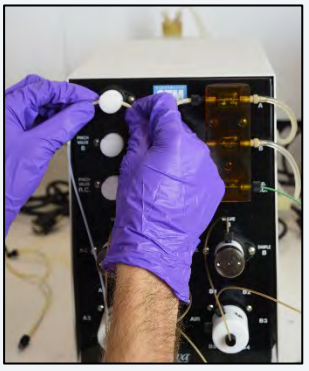


Figure 5.10 Pinch Segment A

5. If a Retain Collector is installed, follow steps 5-7. Connect the Rotary Valve to 3-Way Valve tubing to the center of the rotary valve to the top port of the 3-way valve for both A and B banks.

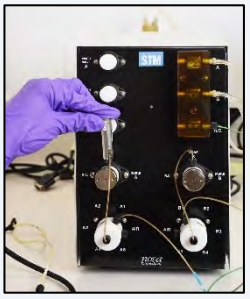


Figure 5.11 3-Way Valve Sample A

6. Secure the 3-Way Valve to RC AD tubing to both of the right-side ports for the 3-way valves to both A and B banks.

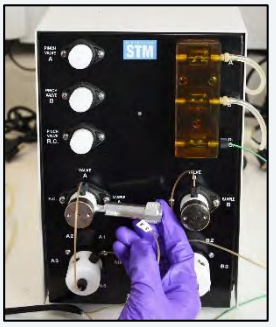


Figure 5.12 3-Way Valve A Bank to RC AD

7. Seat the 3-Way Valve to RC AD tubing in the pinch valve in the same manner as steps 3 and 4.

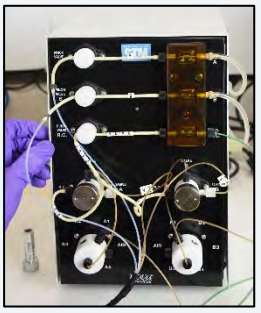


Figure 5.13 RC AD tubing in Pinch Valve

8. Use the wrench to tighten up all connections an additional ¼-turn.

Figure 5.14 Tighten the Connections

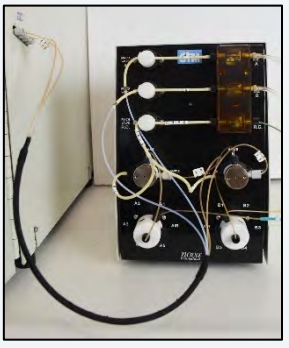


Figure 5.15 STM Tubing Harness Installed

5.3.1.2 Replacing the Waste Tubing

To replace the waste tubing lines:

1. **Remove the old Waste Tubing Lines**
   a. Disconnect each Waste Line Luer fitting from the Waste Bottle cap.
   b. Disconnect the other end of each Waste Line from the STM Air Detectors by turning the connectors counterclockwise until released.
   c. Discard the old Waste Tubing Lines.
2. **Install the new Waste Tubing Lines**
   a. Fasten the ¼”-28 fitting at the end of one waste line to the right side of STM Air Detector A, and the other line to Air Detector B.

Figure 5.16 Luer Connection on Cap

   b. Secure the Luer connection at the other end of each waste line to a port on the Waste Bottle cap.

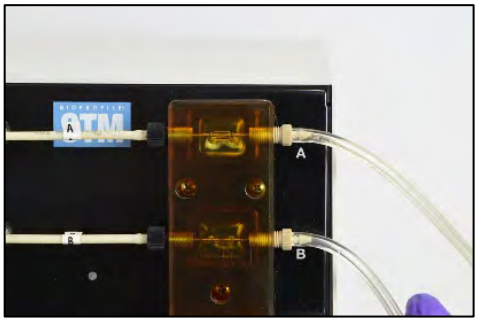


Figure 5.17 Waste Line A

To replace the External Waste Line Kit:

1. **Remove the old External Waste Lines**
   a. Disconnect each External Waste Line from the external waste receptacle.
   b. Disconnect the other end of each Waste Line from the STM Air Detectors.
2. **Install the new External Waste Lines**
   a. Fasten the fitting at the end of one waste line to the right side port of STM Air Detector A, and the other line to the right side port of STM Air Detector B.
   b. Secure the free ends of both tubing lines to the remote waste receptacle.
   c. Use the provided rip-ties and/or clamps to neatly dress the waste lines.

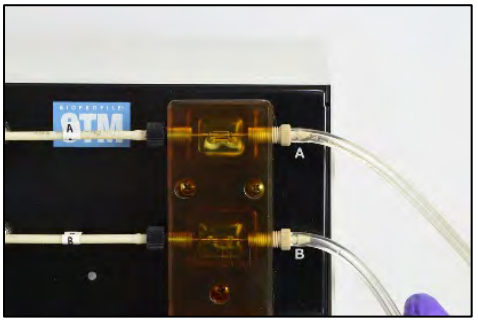


Figure 5.20 Waste Line A

Figure 5.21 External Waste Line Kit Installed

5.3.1.3 Replacing the SRCS Wash/Waste Tubing

1. **Remove the old SRCS Wash/Waste Tubing**
   a. Disconnect both waste lines from the waste bottle cap and from the top positions of the peristaltic pump.
   b. Pull out the straw from the wash bottle and disconnect the other end of the line from the bottom position of the peristaltic pump.
   c. Disconnect the waste line Y-tubing from the bottom positions of the peristaltic pump and disconnect from the bottom of the wash station waste.
   d. Disconnect the wash line from the top position of the peristaltic pump and disconnect from the wash cup on the wash station.
2. **Install the new SRCS Wash/Waste Tubing**
   a. Connect the Wash/Waste lines to the peristaltic pump as shown.

Connect the Wash/Waste tubing to the peristaltic pump as shown, (1) From Wash bottle Straw Cap, (2) To Wash Station Cup, (3) 2 lines Y-tubing from bottom of Wash Station Waste, (4) 2 lines to the Waste Bottle Cap.

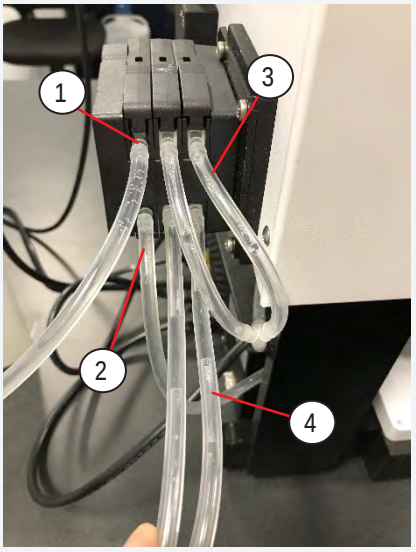


Figure 5.22 SRCS Peristaltic Pump

Connect the Wash/Waste tubing to the Wash Station and to their designated bottles as shown, (1) Wash Bottle Cap, (2) Wash Station Cup, (3) Wash Station Waste, (4) Waste Bottle Cap.

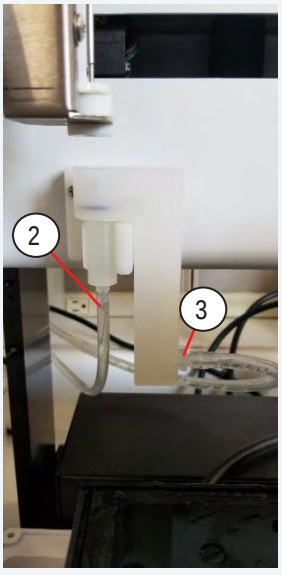


Figure 5.23 Wash Station and Wash/Waste Bottles

5.3.1.4 Replacing the Sample Line

1. To avoid confusion when multiple RSMs are installed, label the tabs on both ends of each new Sample Line with its intended, unique address. Do this for all Sample Lines, then replace the Sample Line for one RSM at a time.
2. Remove the old Sample Line:
   a. Use the wrench to loosen the Sample Line connector installed on the STM Rotary valve. Then continue loosening with finger until released.
   b. Loosen the Sample Line connector installed on the Sample Line Air Detector and continue loosening until the tubing is released from the air detector.
3. Install the new Sample Line Assembly:
   a. Secure the new Sample Line to an available port on the perimeter of either STM rotary valve.
   b. Use the wrench to tighten the Sample Line an additional ¼-turn on the STM rotary valve.
   c. Run the Sample Line tubing from the STM rotary valve to the RSM and secure it to the air detector body.

NOTE: *The fittings at either end of the Sample Line tubing are identical, therefore its orientation is arbitrary.*

Figure 5.26 Sample Line on Rotary Valve A

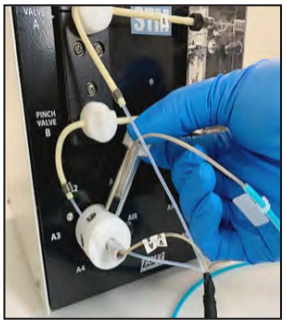


Figure 5.24 Rotary Valve A

Figure 5.25 Sample Line and Sample Line Air Detector

4. If the Sample Line is installed on a different STM Rotary valve port than the old Sample Line, make sure to update the RSM address accordingly on the RSM UI.
5. Use the provided rip-ties to neatly dress the Sample Line.

CAUTION: *The Sample Line air detector is required for proper function of the system. Do not install the Sample Line directly to the RSM syringe pump.*

5.3.1.5 Replacing the Sample Line Air Detector

1. **Remove the old Sample Line Assembly**
   a. Disconnect the Sample Line Tubing from the Air Detector body, using the wrench if needed.
   b. Unplug the air detector cable from the jack on the front of the RSM.
   c. Use the wrench to loosen the connector on the short length of green tubing installed on the *FLEX2* port of the RSM syringe pump valve.
   d. Continue loosening until the air detector is released from the syringe pump.

2. **Install the new Sample Line Assembly**

NOTE: *Refer to the sticker label on the front of the RSM when installing the Sample Line Air Detector on the syringe pump valve.*

a. Fasten the connector on the short length of green tubing to the *FLEX2* port on the RSM syringe pump valve and use the wrench to tighten.
b. Plug in the air detector cable to the jack on the front of the RSM.
c. Secure the Sample Line tubing to the Air Detector Body.

Figure 5.27 Tighten Air Detector

Figure 5.28 Air Detector Cable Jack

Figure 5.29 Sample Line and Sample Line Air Detector

5.3.1.6 Replacing the Reactor Line

1. **Remove the old Reactor Line Assembly**
   a. Run an Intensive Clean Reactor Line maintenance sequence. This involves disconnecting the Reactor Line from the vessel and inserting the tubing into cleaning solution.
   b. Once the Reactor Line is disconnected from the vessel and cleaned, remove it from the RSM:
      i. Remove the Reactor Line from the RSM syringe pump by turning the Luer connector counterclockwise until it releases.
      ii. Remove the Reactor Line from the RSM Air Detector by pulling the tubing forward from both sides of the air detector.
      iii. Remove the Reactor Line tubing segments from the RSM pinch valve by lifting the Vent Line and Reactor Line out of the valves.
2. **Follow the instructions outlined in Section 3.4.6 for making the vessel connection and autoclaving the Reactor Line and vessel. Once the new reactor line (and adapter, if applicable) have been autoclaved and attached to the reactor, follow the steps below to install the reactor line on the RSM.**
3. Remove the new Reactor Line from its autoclavable pouch and seat the tubing segments in the RSM pinch valve according to the colored labels on the valve and each line of tubing.

   a. Seat the vent line in the rear pinch valve position (red) by pulling on the tubing to either side of the valve until it is seated fully inside.

   b. Seat the sterile air vent filter in the designated mounting bracket atop the RSM.

   c. Seat the beige segment of the reactor line fully in the front valve position (green) by pulling on the tubing to either side of the valve until it is seated fully inside.

Figure 5.30 Vent Line in Pinch Valve

Figure 5.31 Filter Mount

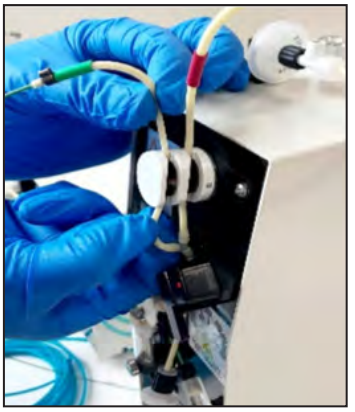


Figure 5.32 Reactor Line in Pinch Valve

CAUTION: *The Reactor Line pinch valve remains closed to isolate the bioreactor from the rest of the OLS flow path. It is important to seat the Reactor line pinch tubing in the valve before removing the sterile filter from the bottom of the tubing line for sterility.*

4. Remove the sterile air filter from the bottom (*RSM* end) of the reactor line tubing and secure the connector to the beige Luer fitting installed on the *Reactor* port of the syringe pump valve.

Figure 5.33 Air Filter Removed

Figure 5.34 Reactor Port Luer Fitting

5. Seat the Reactor Line inside the RSM air detector by sliding it into place. Avoid stretching the tubing while performing this step.

Figure 5.35 RSM Air Detector

6. Adjust the tubing so the Y-fitting is as close to the pinch valve as possible, and ensure the tubing is not twisted or crimped between the pinch valve and air detector, or between the air detector and syringe pump.

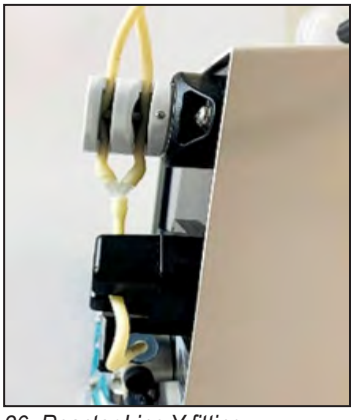


Figure 5.36 Reactor Line Y-fitting

5.3.2 Emptying the Waste Bottle

Note there is no Reagent Management System for the OLS External Waste Bottle or for the SRCS External Waste Bottle. The Waste Bottles have a 4L capacity, and it is the responsibility of the End User to make sure that the waste bottles are emptied regularly as to prevent overflow.

Figure 5.37 Nova OLS Waste Bottle with Waste Tubing Harness Installed

5.3.3 Cleaning FLEX2 + OLS Surfaces

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

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

6 Troubleshooting

This section describes the errors and explains the troubleshooting procedures for the BioProfile FLEX2 On-line Autosampler.

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.*

The recommended troubleshooting procedures use the most logical and direct steps to resolve the error code. These steps are also organized to prevent unnecessary consumable replacement. If the recommendations given here do not resolve the problem, contact Nova Technical Services for troubleshooting assistance. It is helpful to have printed or written down the error codes, flow times, and slope performance numbers.

FOR TECHNICAL ASSISTANCE CALL:

USA: 1-800-545-NOVA

CANADA: 1-800-263-5999

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

6.1 Error Codes

NOTE: *The error codes below use "{0}" to denote the STM identifier (A or B) and/or RSM identifier (A1-5 or B1-5).*

Autosampler No Sample Detected at RSM {0} Reactor AD

During sample acquisition from the reactor, the RSM air detector failed to see fluid when expected.

Recommended Solution

1. Verify the reactor line tubing is properly installed in the RSM air detector and pinch valve.
2. Check for leaks or blockages between the reactor and the RSM syringe pump.
3. Run a Prime Reactor.
4. Rerun sample.

Autosampler No Sample Detected at STM AD {0}

During sample delivery to the FLEX2, the STM air detector failed to see fluid when expected.

Recommended Solution:

1. Verify the RSM address matches the STM valve position where the Sample Line is connected for the RSM that was just analyzed.
2. Check for leaks or blockages between the RSM and STM waste.
3. Run Clean Sample Line.
4. Rerun sample analysis.

Autosampler No Air Detected at STM AD {0}

Prior to sample acquisition from the reactor, the STM air detector failed to see air when expected. This would indicate that the prior Sample Line cleanup sequence did not complete properly. Verify the following for RSMs on the respective STM Valve Bank:

Recommended Solution:

1. Verify all RSM addresses match the STM valve positions of their respective sample lines.
2. Check for leaks or blockages between RSM and STM waste (start with the last RSM that was analyzed).
3. Run Clean Sample Line.
4. Rerun sample analysis.

Autosampler No Air Detected at RSM {0} Reactor AD

Prior to sample acquisition from the reactor or following a cleanup sequence, the RSM air detector failed to see air when expected.

Recommended Solution:

1. Verify the reactor line tubing is properly installed in the RSM air detector and pinch valve.
2. Check for leaks or blockages between the reactor and the RSM syringe pump.
3. Run Prime Reactor.
4. Rerun sample analysis.

Autosampler No Air Detected at RSM {0} Sample Line AD

Prior to sample acquisition from the reactor or following a cleanup sequence, the Sample Line air detector failed to see air when expected.

Recommended Solution:

1. Check for leaks or blockages between RSM and STM waste.
2. Run Prime Pack.

Autosampler No System Fluid Detected {0}

During a Fluid Pack prime or a Sample Line cleanup sequence, the Sample Line air detector failed to see System Fluid when expected.

Recommended Solution:

1. Verify the Fluid Pack is properly installed, with more than 10% fluid remaining.
2. Verify the RSM address matches its Sample Line STM valve position.
3. Check for leaks and blockages between the RSM and STM Waste.
4. Prime the Fluid Pack.
5. Run Clean Sample Line.

Autosampler No Depro Detected {0}

During a Sample Line cleanup, the Sample Line air detector failed to see Depro solution when expected; or during a Sample Line cleanup or reactor prime, the RSM air detector failed to see Depro solution when expected.

Recommended Solution:

1. Verify the Fluid Pack is properly installed, with more than 10% fluid remaining.
2. Verify the RSM address matches its Sample Line STM valve position.
3. Verify the reactor line tubing is properly installed in the air detector and pinch valve.
4. Check for leaks or blockages between RSM and STM waste, or between the reactor and syringe pump.
5. Prime the Fluid Pack.
6. Run Clean Sample Line.

Autosampler No Air Detection Time Exceeded {0}

During sample delivery, the STM air detector failed to see fluid within the allotted time.

Recommended Solution:

1. Verify the RSM address matches its Sample Line STM valve position.
2. Check for leaks or blockages between the RSM and STM waste.
3. Run Clean Sample Line.
4. Rerun sample analysis.

Autosampler Air Detector Calibration Failed on STM AD {0}

During the Depro System sequence, the STM air detector failed to calibrate.

Recommended Solution:

Verify the following for each RSM on the applicable STM bank:

1. Verify the Fluid Pack is properly installed, with more than 10% fluid remaining.
2. Verify the RSM address matches its Sample Line STM valve position.
3. Check for leaks or blockages between RSM and STM waste.
4. Run Depro System.

Autosampler Air Detector Calibration Failed on RSM AD {0}

During the pack prime sequence, the Sample Line air detector failed to calibrate.

Recommended Solution:

1. Verify the Fluid Pack is properly installed, with more than 10% fluid remaining.
2. Check for leaks or blockages between RSM and STM waste.
3. Run Prime Pack.

Autosampler Reactor Line Failed to Prime on RSM {0}

This is posted when a Prime Reactor sequence fails.

Recommended Solution:

1. Verify the reactor line tubing is properly installed in the RSM air detector and pinch valve.
2. Check for leaks or blockages between the reactor and the RSM syringe pump.
3. Run Prime Reactor.

Autosampler RSM Invalid Address

This indicates a software issue where the RSM/STM identifier cannot be parsed.

Recommended Solution:

1. Contact Technical Support.

Autosampler Reactor Line Clean Failed RSM {0}

This is posted when the reactor line cleaning fails during reactor prime or Sample Line cleaning. This is usually posted with another more specific error, i.e. no Depro or no air detected.

Recommended Solution:

1. Verify the reactor line tubing is properly installed in the air detector and pinch valve.
2. Check for leaks or blockages between the reactor and the RSM syringe pump.
3. Run Clean Sample Line.
4. Run Prime Reactor.

Autosampler Reactor Line Purge Failed RSM {0}

No air was found at RSM reactor air detector while attempting to purge the Depro with air.

Recommended Solution:

1. Verify the reactor line tubing is properly installed in the air detector and pinch valve.
2. Check for leaks or blockages between the reactor and the RSM syringe pump.
3. Run Prime Reactor.

Autosampler Sample Line Cleaning Failed RSM {0}

The Sample Line Cleanup sequence did not complete properly.

Recommended Solution:

1. Verify the Fluid Pack is properly installed, with more than 10% fluid remaining.
2. Verify the RSM address matches its Sample Line STM valve position.
3. Check for leaks and blockages between the RSM and STM Waste (start with the last RSM that was analyzed).
4. Prime the Fluid Pack.
5. Run Clean Sample Line.

Autosampler RSM {0} Failed to Initialize

The RSM syringe pump failed to reset or there is a communication issue with the RSM.

Recommended Solution:

1. Run Initialize RSM.
2. Contact Technical Support.

Autosampler STM {0} Failed to Initialize

The STM rotary valve failed to reset.

Recommended Solution:

1. Run Initialize STM.
2. Contact Technical Support.

Autosampler Multiple RSMs With Same Address

This indicates that during a periodic check or STM initialization sequence, two RSMs were found to be configured with the same alphanumeric address.

Recommended Solution:

1. Verify that all RSM addresses are unique.
2. If there is a redundant address, re-configure one RSM address from the RSM UI.
3. Verify the RSM address matches its Sample Line STM valve position.
4. Run Initialize STM.

ASX Unresponsive

This indicates that the Retain Collector is either powered off or has lost connection with the STM.

Recommended Solution:

1. Check the connection of the RC COM cable between the RC and the STM.
2. Run Initialize STM.
3. Contact Technical Support

Movement Error/Internal Error

This indicates that either the Retain Collector arm or probe hit an object while moving.

Recommended Solution:

1. Verify that there is nothing that could be impeding the RC arm from moving.
2. Contact Technical Support.

A Appendix

A.1 Spare Parts and Supplies List

You can order the following parts and supplies from Nova Biomedical Order Services 1-800-822-0911 or novaorders@novabio.com or your local Nova Biomedical Distributor.

Description *Part Number (PN)*

Fluid Packs

FLEX2 Online Autosampler Fluid Cartridge (2/pack)..... 62450
FLEX2 STM Waste Bottle Pack (4/pack) 46400

Tubing

FLEX2 20 ft Autosampler Sample Line 61376
FLEX2 Autosampler RSM Sample Line Air Detector Assembly 62849
FLEX2 Autosampler STM Tubing Harness..... 61372
FLEX2 Autosampler STM Waste Tubing Harness 61371
FLEX2 Autosampler STM External Waste Line Kit..... 62878
FLEX2 Reactor Line Assembly (for use with 1/4"-28 Dip Tubes)..... 61370
FLEX2 OLS Reactor Line Adapter (for use with Barbed Dip Tubes)..... 63274
SRCS Wash/Waste Tubing Set..... 63534
STM to Rotary Valve to 3-Way Valve Tubing Set 63470
STM 3-Way Valve to RC Air Detector Tubing Set..... 63471
STM RC Air Detector to FLEX2 Tubing Set..... 63472
External RSM Cleaning Tubing Adapter..... 62877

Table A.1 Bioreactor Dip Tube Options

Part Number:	Dip Tube Description:	Reactor Line Adapter Required (Yes/No)
47456	10 mm Headplate Port, 7 in (17.78 cm) Threaded Dip Tube Assembly	No, connects directly to Reactor Line Assembly
50251	10 mm Headplate Port, 9 in (22.86 cm) Threaded Dip Tube Assembly	No, connects directly to Reactor Line Assembly
47457	10 mm Headplate Port, 15 in (38.10 cm) Threaded Dip Tube Assembly	No, connects directly to Reactor Line Assembly
63003	10 mm Headplate Port, 5.63 in (14.30 cm) Threaded Dip Tube Assembly	No, connects directly to Reactor Line Assembly
55505	10 mm Headplate Port, 7 in (17.78 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter
55506	10 mm Headplate Port, 9 in (22.86 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter
55885	10 mm Headplate Port, 5.63 in (14.30 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter

Table A.1 Bioreactor Dip Tube Options		
Part Number:	Dip Tube Description:	Reactor Line Adapter Required (Yes/No)
55881	10 mm Headplate Port, 15 in. (38.10 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter
55887	12 mm Headplate Port, 8.88 in. (22.56 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter
55882	12 mm Headplate Port, 7.75 in. (19.69 cm) Barbed Dip Tube Assembly	Yes, requires Reactor Line Adapter

Description *Part Number (PN)*

Communication Cables

FLEX2 Autosampler Communications Cable - 10 ft 62563

Accessories

FLEX2 RSM Replacement Syringe 46915

Deproteinizing Solution (4 oz bottle) 46850

Pre-Pierced Septum (for ESM, Gas QC, OLS and EOLS) 3/pack 59228

SRCS Consumables

Retain Collector 2 mL Sample Tubes (package of 100) 63674

Retain Collector 15 mL Sample Tubes (package of 25) 63765

Retain Collector 50 mL Sample Tubes (package of 25) 63766

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:

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, 02453, USA.