Symphony® X

User Manual

GYROS PROTEIN Technologies

®

Symphony X

Peptide Synthesizer

USER MANUAL

GYROS PROTEIN Technologies

4675 S. Coach Dr.
Tucson, AZ 85714 USA

Document # D0028120 / E

WARNING ALL REACTION VESSELS MUST BE IN PLACE AT ALL TIMES.

WARNING ONLY GLASS REACTION VESSELS SHOULD BE USED ON INSTRUMENTS WITH INFRARED HEATERS TO AVOID RISK OF FIRE AND DAMAGE TO INSTRUMENT AND LABORATORY. DO NOT USE PLASTIC.

WARNING SAFETY SHIELD DOORS MUST BE CLOSED WHILE A SYNTHESIS OR CLEAVAGE IS RUNNING.

WARNING SYNTHESIS WILL HALT IF WASTE CONTAINER IS FULL.

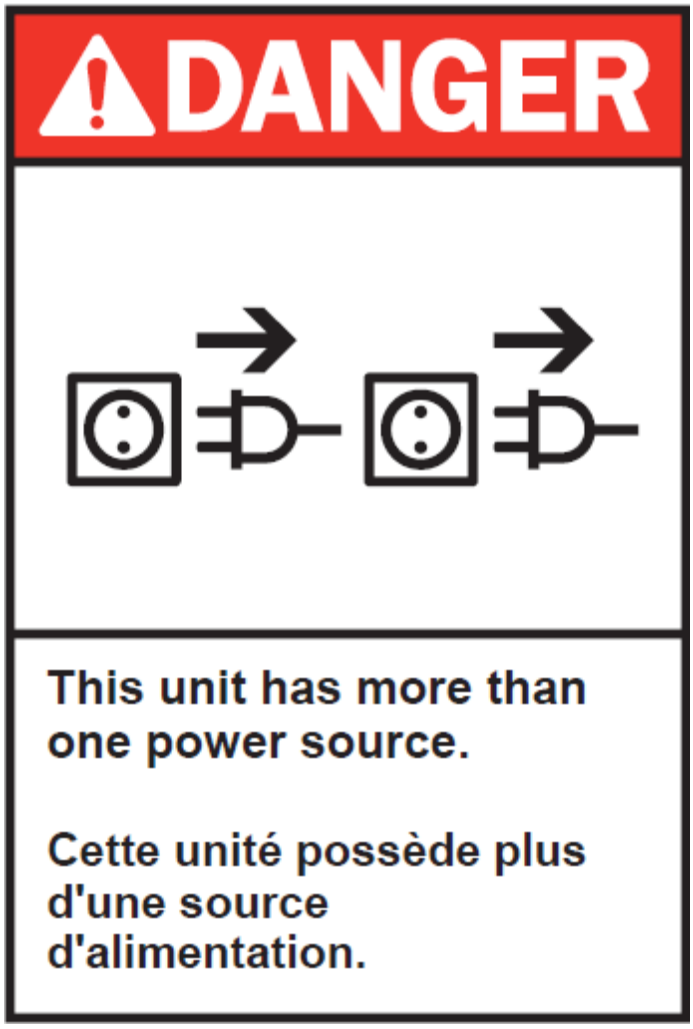
WARNING DO NOT ATTEMPT TO MOVE THE INSTRUMENT WHILE ANY OF THE SOLVENT OR WASTE CONTAINERS CONTAIN LIQUIDS.

WARNING THIS INSTRUMENT CONTAINS SOLVENTS AND CHEMICALS THAT SHOULD BE HANDLED CAREFULLY. MANY ARE EASILY ABSORBED THROUGH THE SKIN AND CAN CAUSE ADVERSE HEALTH EFFECTS. WEAR SAFETY GLASSES, PROTECTIVE CLOTHING AND RUBBER GLOVES AT ALL TIMES. FOLLOW MSDS HANDLING GUIDELINES PROVIDED WITH THE INDIVIDUAL REAGENTS. RESPIRATORS AND ABSORBENT SHOULD BE AVAILABLE IN THE EVENT OF A SPILL.

<u>WARNING:</u> BURN HAZARD

TOUCHING THIS SURFACE OR SURFACES INSIDE THE RV ENCLOSURE COULD RESULT IN BODILY INJURY.

TO REDUCE RISK OF INJURY,
ALLOW TO COOL BEFORE TOUCHING.


DANGER
This unit has more than one power source.
Cette unité possède plus d'une source d'alimentation.

AVERTISSEMENT : TOUS LES RÉCIPIENTS DE REACTION DOIVENT ÊTRE EN PLACE À TOUT MOMENT.

AVERTISSEMENT: SEULEMENT LES BATEAUX DE RÉACTION EN VERRE DEVRAIENT ÊTRE UTILISÉS SUR DES INSTRUMENTS AVEC DES CHAUFFEURS INFRAROUX POUR ÉVITER LE RISQUE D'INCENDIE ET LES DOMMAGES AUX INSTRUMENTS ET AU LABORATOIRE. NE PAS UTILISER DE PLASTIQUE.

AVERTISSEMENT : LE COUVERCLE DE SÉCURITÉ DOIT ÊTRE TOUJOURS FERMÉ PENDANT LA SYNTHÈSE OU LE CLIVAGE.

AVERTISSEMENT: LA SYNTHÈSE S'ARRETERA SI LE CONTENEUR DE DECHETS EST PLEIN.

AVERTISSEMENT: NE PAS TENTER DE DÉPLACER L'INSTRUMENT PENDANT QU'UN DES SOLVANTS OU DES CONTENEURS DE DÉCHETS CONTIENNENT DES LIQUIDES.

ATTENTION : CET INSTRUMENT CONTIENT DES SOLVANTS ET DES PRODUITS CHIMIQUES QUI DOIVENT ETRE MANIPULÉS ATTENTIVEMENT. NOMBREUX SONT FACILEMENT ABSORBÉS PAR LA PEAU ET PEUVENT PROVOQUER DES EFFETS NÉFASTES SUR LA SANTÉ. PORTER DES LUNETTES DE SÉCURITÉ, VÊTEMENTS DE PROTECTION ET DES GANTS DURANT LEUR UTILISATION.
SUIVRE LES DIRECTIVES DE MANIPULATION FOURNIS AVEC LES REACTIFS INDIVIDUELS. DES RESPIRATEURS ET ABSORBANT DOIVENT ÊTRE DISPONIBLE EN CAS DE FUITE.

<u>WARNING:</u> BURN HAZARD

TOUCHER CETTE SURFACE OU SURFACES À L'INTÉRIEUR DE L'ENCEINTE DE VACANCES POURRAIENT RESULTANT DE BLESSURES CORPORELLES.

POUR RÉDUIRE LE RISQUE DE BLESSURES, PERMET DE REFRIGER AVANT DE TOCER.

Contents

INTRODUCTION

Thank you for purchasing your new Symphony® X peptide synthesizer from Protein Technologies, Inc. The Symphony® X is a fully automated peptide synthesizer featuring 12 independent reaction vessel fluid paths capable of carrying up to 24 reaction vessels, 8 solvent positions and 40 amino acid positions. It was designed for users ranging from novice to expert peptide chemists and features an easy setup and simple software while providing the ultimate in flexibility and power. The Symphony® X can generate peptides in an easy to use, high-throughput format, while providing the user the ultimate in flexibility and efficiency. It can perform automated cleavage on the same platform without user intervention, and it also features user logging, E-mail notification and a Safe-Response for interrupted syntheses. UV-monitoring and infrared (IR) heating are additional optional features available as field-upgrades.

I.1 About The Manual

In this manual:

- Chapter 1, **General Information**, describes the instrument layout, basic installation procedures and Symphony® X accessories available for purchase from Protein Technologies, Inc.
- Chapter 2, **Introduction to Software**, explains the function of each software screen
- Chapter 3, **Running A Synthesis**, explains the basic steps for setting up and running a synthesis
- Chapter 4, **Cleaning and Maintenance**, explains post-synthesis cleaning, shutdown and maintenance procedures.
- Chapter 5, **Errors and Recovery**, describes Symphony® X error messages and recovery steps.

I.2 About The Company

Protein Technologies, Inc. (PTI) is a private company based in Tucson, Arizona. Founded in 1985 by researchers affiliated with the University of Arizona, PTI has a long history of developing and manufacturing high quality peptide synthesizers.

Our instruments are used in major universities, pharmaceutical companies and biotechnology companies worldwide. We support our products with a dedicated field service team and are proud of our reputation for reliability. We value the trust our customers and partners have placed in PTI. Today, we continue to grow and innovate to serve the needs of the solid-phase synthesis market.

I.3 Common Abbreviations

AA	-	Amino acid
ACT	-	Activator
CAP	-	Capping
Cat	-	Catalog
DEP	-	Deprotection Solution
DCM	-	Dichloromethane (Methylene Chloride)
DMF	-	Dimethylformamide
E-Stop	-	Emergency Stop
Fmoc	-	9-Fluorenylmethyloxycarbonyl
FNPT	-	Female National Pipe Thread
HBTU	-	2-(1H-Benzotriazol-1-yl-)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU	-	O-(6-Chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
In Hg	-	Inches of Mercury
Inc	-	Increment
L	-	Liter
lbs	-	Pounds
mL	-	Milliliter(s)
uL	-	Microliter(s)
mmol	-	Millimoles
N2	-	Nitrogen
NMM	-	N-Methylmorpholine
Ops	-	Operations
Pkg	-	Package
PP	-	Polypropylene
Psi	-	Pounds Per Square Inch
REAG	-	Reagent
REP	-	Repetitions
RV	-	Reaction Vessel
SOLV	-	Solvent
Syn	-	Synthesis
TFA	-	Trifluoroacetic Acid
USB	-	Universal Serial Bus
Vac	-	Vacuum
Vol	-	Volume

CHAPTER 1: GENERAL INFORMATION

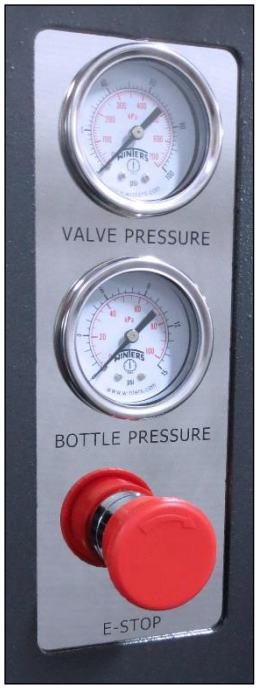
1.1 General System Description

1.1.1 Symphony® X Front

1.1.2 Symphony® X Back

On the top left is the nitrogen inlet. On the top right is the Back Panel (See Section 1.1.5). In the back of the solvent cabinet is the 4” vent.

1.1.3 Symphony® X Left Front Panel



The Symphony® X Left Front Panel contains:

1. Valve Pressure – Displays the valve pressure (in psi). The valve pressure should be at 50 psi.

2. Bottle Pressure – Displays the solvent bottle pressure (in psi). The bottle pressure should be at 10 psi.

3. E-Stop button – Press down to stop the Symphony X in the event of an emergency. All actions will cease and all bottles will be vented. Simply twist clockwise to release.

1.1.4 Symphony® X Right Front Panel

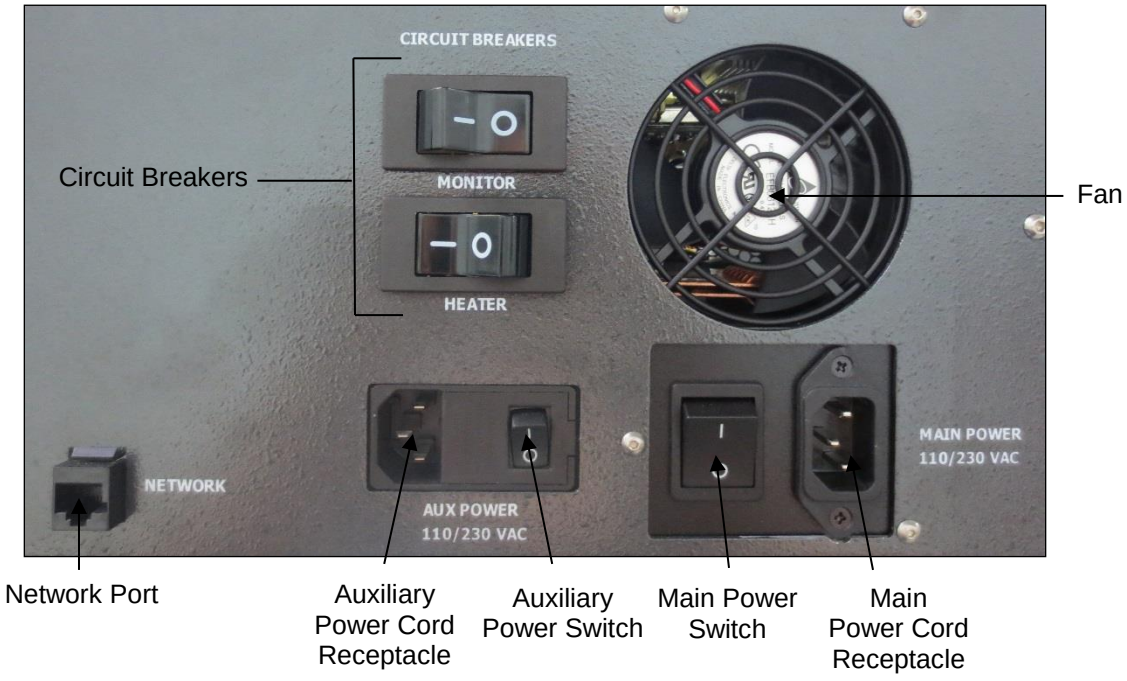
The Symphony® X Right Front Panel contains:

1. N2 Mix – Flow knob controls the nitrogen flow during a mix. Counter clockwise to increase, clockwise to decrease.

2. USB Ports – Used to transfer files between the Symphony X and an external computer. Alternately, ports may also be used for a keyboard, mouse, or printer.

3. Vacuum – Displays the vacuum pressure (in mm Hg). The <u>minimum</u> vacuum should be -17 in Hg.

1.1.5 Symphony® X Back Panel



The Symphony® X Back Panel contains:

1. Circuit Breakers – Circuit breakers for monitor and heating element
2. Fan – Fan for power supply
3. Network Port – Attach network cable to run E-mail notification option and connect to Network
4. Auxiliary Power Cord Receptacle – Attach power cord and plug other end into a wall outlet to power monitor and infrared heater
5. Auxiliary Power Switch – Turns the Symphony X monitor and infrared heater on and off.
6. Main Power Switch – Turns the Symphony X on and off
7. Main Power Cord Receptacle – Power for Symphony X. Attach power cord and plug other end into a wall outlet.

1.1.6 Reaction Vessel System

The Symphony® X reaction vessel system is designed around a simple and reliable quick release mechanism. Cam levers allow the operator to remove and install the 24 reaction vessels quickly and easily. An 'In-Place' detection sensor verifies that an RV is present prior to solution transfer operations. The top row of reaction vessels (PV1 – PV12) may be used as preactivation vessels for the bottom row (RV1 – RV12) of reaction vessels, or as separate reaction vessels for the synthesis of up to 24 separate peptides. PTI, a subsidiary of Gyros Protein Technologies, strongly recommends using resin that is 74 microns or larger (mesh size 200 or smaller), including standard 100-200 mesh resins. Using resin of a smaller size (larger mesh) will produce poor results and may damage the synthesizer. Using resins that are not recommended may void PTI warranty and service contract coverage.

NOTE All active RV's must be in place at all times for the instrument to function.

1.1.7 Cleavage Collection System

The collection system for the Symphony® X accepts 50 mL polypropylene conical vials. The system is made of materials resistant to the acidic reagents associated with cleavage solutions. A positive seal ensures cleavage solution vapors are vented. An 'In-Place' detection sensor verifies that a vial is present at all times.

NOTE All twelve collection vials must be in place at all times for the instrument to function.

CAUTION Since the collection tubing is not rinsed automatically it is important to perform a **Collect Clean** after each collection operation. To prevent contamination use different vials designated for this cleaning procedure. See Section 2.8.4 for detailed information on the **Collect Clean**.

1.1.8 Amino Acid Bottle System

28 amino acid bottle positions are located on the front of the Symphony® X. Amino acid bottles are available in 10, 120 and 400 mL capacities. The instrument uses nitrogen to pressurize the bottles and transfer fluid. The **Bottle Prep** screen (Section 2.5) controls this operation. The fluid is measured by sensors in 1000 or 1500 μL aliquots. Each amino acid bottle has a bottle filter to prevent particulates from entering the fluid system. These filters should be changed on a regular basis depending on the quality and concentration of reagent utilized. See Section 4.6, **Bottle Filter Replacement** for instructions.

The natural 20 amino acids usually go into bottles 1-20 in alphabetical order according to the single letter code. Any non-natural or proprietary residues can be put into bottles 21-28. The default concentration in DMF or NMP is 0.1 mM. These assignments may be changed in the **Amino Acid Editor** (Section 2.7.1). PTI, a subsidiary of Gyros Protein Technologies, strongly recommends using resin that is 74 microns or larger (mesh size 200 or smaller), including standard 100-200 mesh resins. Using resin of a smaller size (larger mesh) will produce poor results and may damage the synthesizer. Using resins that are not recommended may void PTI warranty and service contract coverage.

CAUTION DO NOT install or remove amino acid bottles when they are pressurized.

IMPORTANT Amino acid bottles 1-14, 15-21 and 22-28 are each connected to a separate pressurization manifold. All amino acid bottles in each group are vented or pressurized together. All amino acid positions in a group must have bottles in place for that group to pressurize. Empty bottles should be placed in any unused positions.

IMPORTANT The amino acid manifold seals are not affected by DMF or NMP. THF and DCM can also be utilized without destruction of the seals if extra caution is used to prevent the liquid from contacting the seals. Contact PTI's Technical Service Department if alternative solvents are desired. Under no circumstances should TFA be used in the amino acid manifold system—destruction of the seals will occur! See Section 4.7 **Amino Acid Bottle Seal Replacement** for replacement procedures.

1.1.9 Solvent Bottle System

The eight solvent bottles are located in the solvent cabinet. These glass bottles are pressurized with nitrogen to accomplish solution transfer. Safety-coated glass bottles should always be used. The solvent and reagent bottles are controlled using the **Bottle Prep** screen (Section 2.5).

CAUTION Safety-coated glass bottles are supplied by PTI with each instrument and should always be used with this instrument. Using regular glass bottles may result in serious bodily injury.

Bottle positions 1-6 are located on the same valve block, and solvent 1 is used to rinse that block after solvent deliveries. These positions can precisely measure volumes in 1000 or 1500 μL aliquots using a metering loop. Bottles 7 and 8 are located on a separate valve block, and solvent 7 is used to rinse that block after deliveries. Solvents 7 and 8 are delivered in 2500 μL aliquots using a metering loop. Bottle position 8 is specifically intended for the delivery of cleavage solution and is therefore pressurized and primed on demand for safety reasons. Throughout the Symphony® X software program, each bottle position is referred to by an abbreviated title. These assignments may be changed in the **Solvent Editor** (Section 2.7.2). The titles, standard abbreviations, bottle volumes, and typical solution composition of each solvent bottle position for Fmoc chemistry are as follows:

SOLV 1 or DMF: 20 L pressure vessel for the primary wash solvent, typically synthesis grade (low amine content) DMF. DMA or NMP may also be used. Because this solvent is quite stable, the 20 L pressure vessel may be installed and left in place throughout several sets of syntheses. This solvent is utilized in the automated cleaning operations for the valve fluid system. Therefore, SOLV 1 must be in place for normal operation of the instrument. This solvent position is also utilized during the **Flush Solv** (Section 2.5), and **System Solvent Flush** (Section 2.8.3) cleaning operations.

SOLV 2 or DEP: 4 L safety-coated glass bottle for deprotectant to remove the N-terminal Fmoc protecting group. The standard composition is 20% (v/v) piperidine in DMF. This solution is also quite stable and may be installed and used for several sets of syntheses. Other reagents may be loaded for alternate chemistries.

SOLV 3 or ACT1: 4 L safety-coated glass bottle for activator solution to form the activated Fmoc amino acid for the coupling reaction. The standard composition is 0.1 M HCTU in 0.4 M NMM in DMF. Other reagents may be loaded for alternate chemistries.

NOTE The activator solution must be equimolar concentration with the amino acid solutions if both are using the same volume deliveries. The activator solution should be prepared fresh for each synthesis.

SOLV 4 or ACT2: 1 L safety-coated glass bottle for additional reagent or activator solution.

SOLV 5 or BASE: 1 L safety-coated glass bottle for base, if separation of base and coupling reagent is desired. The standard base composition is 0.4 M NMM in DMF. Bottle 3 or 4 should then contain a coupling reagent such as 0.1 M HCTU in DMF.

SOLV 6 or DCM: 1 L safety-coated glass bottle for a secondary wash solvent, such as DCM for safe response or to wash the peptide-resin in preparation for automated cleavage. DCM may be installed and left in place for several sets of syntheses.

SOLV 7 or DCM: 1 L safety-coated glass bottle for a secondary solvent for automated cleavage. This bottle position is utilized during the **System Solvent Flush** (Section 2.8.3), **Cleave Bottle Solvent Back Flush** (Section 2.5), cleaning operation. DCM may be installed and left in place for several sets of syntheses.

SOLV 8 or TFA: 1 L safety-coated glass bottle of cleavage reagent for cleavage of the peptide from the resin after synthesis. This position is specifically designed to handle the aggressive TFA cleavage solution. It can be accessed through the **Cleave & Collect** operation or the **Bottom Delivery** operation.

NOTE The cleavage solution should be prepared fresh for each synthesis.

Each solvent position has a bottle filter to prevent particulates from entering the fluid system. For replacement procedures, see Section 4.6. A teflon encapsulated o-ring in the bottle cap insert establishes the bottle seals and is inert to the reagents. Damage to the insert or o-rings will result in nitrogen leakage and potential loss of reagent (volatiles like TFA, DCM). For bottle seal replacement procedures see Section 4.8.

Custom bottle configurations and assemblies can be arranged through technical service.

1.1.10 Waste System

The only exit for the closed fluid flow paths of the instrument is through the waste system. Waste exits the Symphony® X to the waste containers. The waste

containers are vented through a tube attached to a fitting on the 4” vent duct. The waste container is a 5 gallon carboy fitted with a waste level sensor to prevent overfilling. The solvent waste will be directed to waste container number two if waste container number one is full. If both containers are full, all operations in the instrument will stop automatically. No operations will be allowed until the container is emptied and reconnected.

IMPORTANT When both waste containers are full the Symphony® X will automatically pause all operations to prevent overfilling of the waste containers. To resume operations, first empty and reconnect the waste containers. Go to the **RV Automated Operations** or **RV Manual Operations** screens (Section 2.6.1 or 2.6.3) and press the **Resume** button to continue the operations on the paused reaction vessels.

The waste level sensor is wired in a normally closed (NC) configuration so if the switch is disconnected, it is the same as if the container is full. This logic prevents waste from being delivered when the container is not connected. The connectors are resistant to the aggressive waste solutions and fumes. Do not attempt to disassemble the switch connector assembly.

1.1.11 Ventilation System

The Symphony® X has a 4-inch vent hole at the back of the solvent cabinet. It comes equipped with an adjustable angle adaptor. The adaptor has a tube fitting for attaching the waste container vent line. The Symphony® X should be connected to lab ventilation with a 4-inch (10 cm) duct hose supplied by the user. The ducting should be made of a chemically resistant material such as PVC, urethane, or aluminum (no rubber). A minimum flow of 100 cubic feet/min (CFM) must be maintained at the instrument.

1.1.12 Nitrogen System

The nitrogen inlet is located on the back of the instrument. A minimum of 70 psi must be supplied for the instrument to operate. The lack of nitrogen is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. No operations will be allowed until the supply is restored.

The high pressure nitrogen from the inlet is diverted into three regulators:

1. **Valve Pressure** – Used to seal the valve membranes. Should be set to 50 psi. User should not adjust.

2. **Nitrogen Pressure** – Used for mixing and delivering fluid. Should be set to 5 psi. User should not adjust.

3. **Bottle Pressure** – Used to pressurize the bottles. Should be set to 10 psi. User should not adjust.

The Valve and Bottle pressures are displayed on pressure gauges on the left front panel of the instrument.

The intensity of the RV mixing and is controlled using the following **Mix Flow Controller.** This control is a black knob located on the right front panel of the instrument. Turn the knob counter clockwise to increase the mixing in the RV's and clockwise to decrease the mixing. If there is no mixing in the RV's, the mix flow controller may be turned all the way down.

IMPORTANT Adjusting the mixing or delivery flows too high can cause resin to stick to the top of the reaction vessels and possible reagent loss. This can lead to incomplete reactions. It is important to adjust the mixing to a reasonable level.

1.1.13 Vacuum System

Vacuum is supplied by a vacuum pump located inside the instrument. The vacuum is displayed on the vacuum gauge on the front right panel of the instrument. The normal operating range is -10 to -26 in Hg. When the vacuum drops below -10 in Hg, the vacuum pump will turn on. The vacuum is diverted directly to the valve blocks and is used to lift the valve membranes to allow fluid flow from different locations. The lack of vacuum is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. This occurs when the vacuum pump fails to bring the vacuum >10 in Hg. No operations are allowed until the vacuum is restored.

1.1.14 Computer System

The Symphony® X has an internal computer that operates the Symphony® X software. A touchscreen monitor is used to program the Symphony® X. Data may be transferred from the computer using the USB ports on the right front panel of the instrument or via a network connection.

1.1.15 Safety Shield

Safety doors are installed for the protection of the user. The doors of the safety shield MUST be CLOSED when the Symphony® X is running. Before opening the doors, all of the reaction vessels must be in a non-operational state (i.e. paused or completed) and drained of all fluids.

IMPORTANT Minimum safety equipment to be used at all times are: NIOSH/MSHA-approved respirator, face shield, chemically resistant gloves, lab coat, protective clothing, and closed toe shoes.

1.2 Instrument Setup

1.2.1 Instrument Installation Procedure

IMPORTANT Installation of the Symphony® X should be performed by trained personnel only. Improper installation may result in damage to the instrument or operators.

To install the Symphony® X, you will need:

1. OUTLET: A grounded primary power source. Two 6-foot power cords are supplied with the Symphony® X. Plug the instrument into the power outlet.

2. NITROGEN: A relatively pure (>99.9%) and dry source of pressurized nitrogen. A 10-foot tubing set with a ¼ inch FNPT fitting is supplied with the Symphony® X. Attach this fitting to the pressure regulator on the nitrogen tank. Connect the other end of the tube directly to the "quick-disconnect" bulkhead fitting labeled "N2 70/80 PSI" on the back of the Symphony X. Turn on the nitrogen tank and adjust the regulator to 80 psi. Check for pressure leaks and tighten the fittings if needed.

IMPORTANT Securely fasten the cylinder with a safety strap to prevent it from falling, and do not move a cylinder or undo safety straps unless the metal cap is in place.

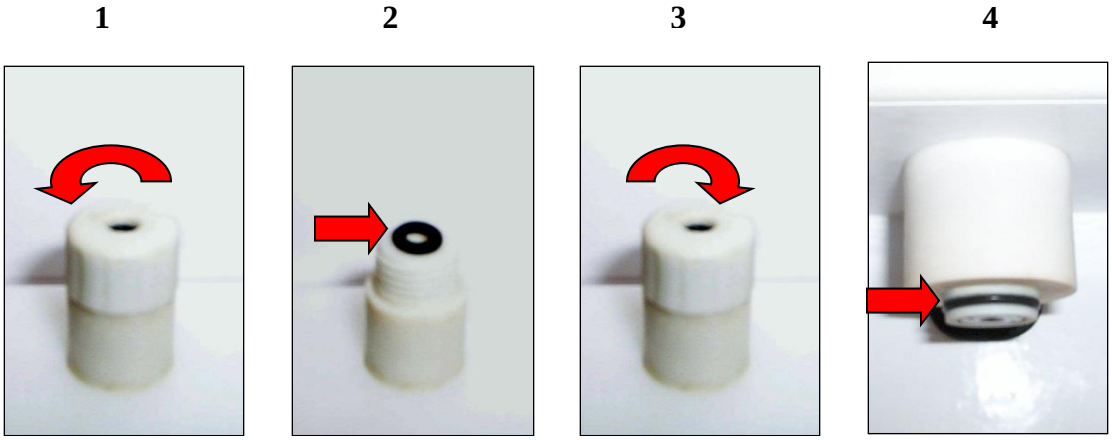
3. VENTILATION: A four inch (10 cm) exhaust line pulling at least 100 CFM located within 15 feet of the instrument (or an "elephant-trunk" exhaust line). This will allow for safe disposal of waste products and their vapors.

To install the Symphony® X:

- Verify ambient temperature should be 10-37.8°C(50-100°F) with relative humidity below 90%.
- Do not lift the machine without proper protection against injury. Unit weights more than 400LBs(181.4Kg).
- Determine location for instrument which should be in a laboratory setting capable of 110/230 VAC 50/60Hz (Main Power: 1.0A (Internally Fused), Heater Power:10.0A (Fuse Value = 10.0A), / Main Power: 0.5A (Internally Fused), Heater Power: 5.0A (Fuse Value = 5.0A).
- For European customers, Fuses are available from PTI with PTI P/N:4700015 for the 5.0A.
- To uncrate the Symphony X, remove any straps on the outside of crate and bring down the ramp. Open crate up to expose the Symphony X. By using the ramp, roll the unit onto the floor. Once the Symphony X is on the floor, remove items in solvent cabinet. Move the Symphony X into desired place (use caution >400 lbs(181.4Kg).).
- Recycle any material from crate.
- Install adjustable vent adaptor in 4” hole in rear of instrument and bend flaps out to fasten.
- Attach waste level sensor cable connectors (4 pin) to receptacles located at the top back of the inside of the solvent cabinet.
- Place waste tank(s) in the solvent cabinet and dress cleanly. Attach the other end of the connectors to the waste level sensor(s) on the waste container(s) by lining up the red dots, then pushing down.
- Attach the long 1/4" vent line from the waste tanks to vent duct adaptor fittings.
- Install RV’s on instrument (Section 1.3.1).
- Install collect tubes on instrument. (Section 1.3.2)
- Install AA bottles on instrument (Section 1.3.3).
- Install Solvent 1 pressure vessel and Solvent 2 through Solvent 8 bottles (Section 1.2.5) in solvent cabinet.
- Attach power cords to power cord receptacles (Section 1.1.5) and plug in.
- Attach nitrogen supply line.
- Turn on main power switch, auxiliary power switch.
- Sign in and verify all systems alarms are OK (appropriate icons at lower right of screen are green)
- Perform Nitrogen Leak Check (see Section 4.5).

1.2.2 RV & O-Ring Installation

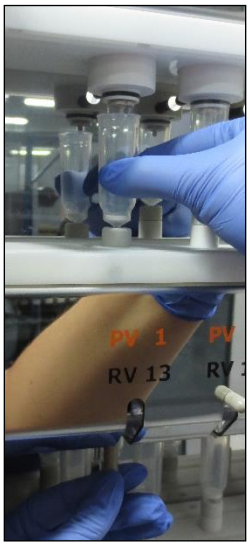
To install reaction vessel o-rings:



1. Unscrew the cap from the lower RV seat on the instrument.
2. Place a reaction vessel bottom o-ring in the center of the lower RV seat.
3. Screw the cap on over the o-ring until tight.
4. Slide a reaction vessel top o-ring into the groove on the upper RV seat.
5. Test for leaks by installing an empty reaction vessel (below) and performing a **Top Delivery** using the **RV Manual Operations** screen (Section 2.6.3).

To install reaction vessels:

1 **2**



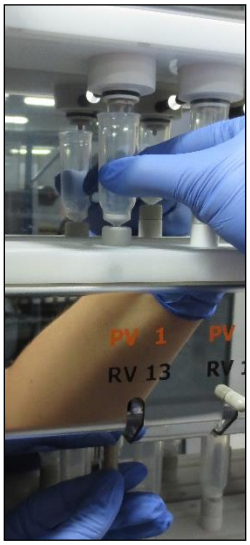
1. Lower the cam lever to the vertical position. Push the RV firmly into the lower seat first.

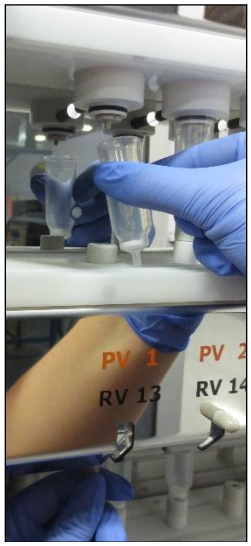
CAUTION Make sure the RV bottom is pressed through the bottom o-ring in the lower RV seat, or the RV may leak. A slight twisting may help.

2. Line up RV with the upper seat and raise the cam lever up into a horizontal position to lock the RV in place.

To remove reaction vessel:

1 **2**





1. Hold the RV with one hand while lowering the cam lever into the vertical position.

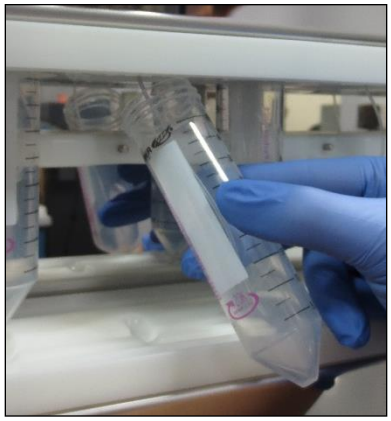
2. Remove RV from the instrument.

1.2.3 Collect Vial Installation

IMPORTANT It is not recommended to have cold ether in the collect vial when the cleavage solution is collected. The vial may overfill during the collection of the product causing both loss of the product and potential damage to the instrument from the TFA solution. Instead, allow the instrument do deliver cleavage solution to the collect vials, then remove the collect vial from instrument and precipitate peptide with cold ether (< 0°C).

To install collect vial:

1 **2**

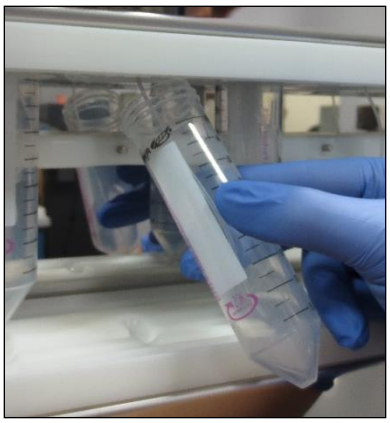


1. Tilt the vial to insert into the upper receptacle.

2. Screw the vial into place.

To remove collection vial:

1 **2**



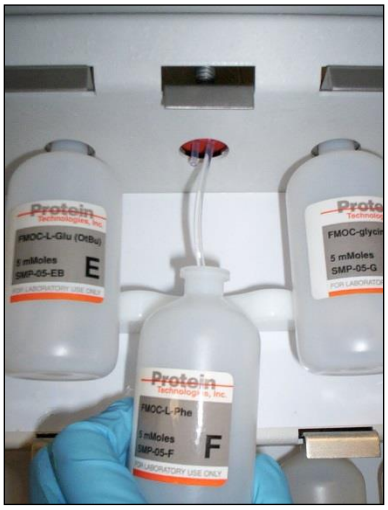
1. Unscrew the collect vial from the receptacle.

2. Tilt the vial from the bottom and remove from the instrument.

1.2.4 Amino Acid Bottle Installation

To install an amino acid bottle, first make sure the bottle position is vented (See **Bottle Prep** screen, Section 2.5) then:

1 **2**



1. Make sure the metal slide is pushed all the way in. Insert the bottle filter and tube into the bottle, and push the amino acid bottle upward.

2. The metal slide is spring-loaded and will pop out when the bottle is in place.

NOTE Check that the bottle filter is resting against the lower rear of the bottle. This will ensure that most of the reagent in the bottle will be used.

To release the bottle, make sure the bottle position is vented. Hold the amino acid bottle with one hand while pushing in the metal slide with the other. Carefully slide the bottle off the tubing and filter.

CAUTION Failure to hold the bottle while releasing will cause the bottle to fall and spill, which may result in personal injury, loss of reagent and/or damage to the instrument.

1.2.5 Solvent/Reagent Bottle Installation

To install a solvent/reagent bottle:

1. Make sure the solvent/reagent bottle position is vented (See **Bottle Prep** screen, Section 2.5).

2. Verify the o-ring is properly installed on the cap insert and that the insert is in the cap. Also, verify that the fluid line has a bottle filter with frit attached.

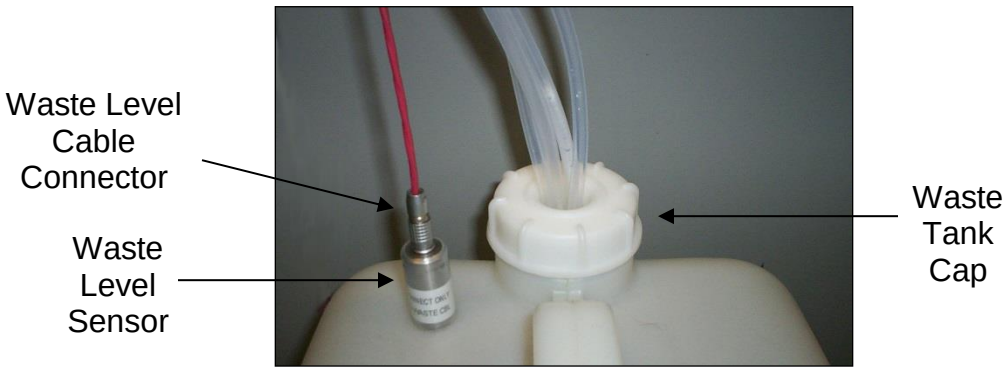
3. Place the bottle in the bottle container. Insert the line so that it is straight and at the bottom of the bottle (the tubing can be 'molded' by gentle bending—Do not 'kink' or the tubing integrity will be compromised).

4. Attach the cap and tighten to a firm hand tight.

To remove the bottle, make sure the bottle position is vented and unscrew the cap while carefully guiding the tubing and filter out of the bottle.

1.2.6 Waste Container Installation

To install a waste container:



1. Place the waste container into the solvent cabinet chemical spill tray.

2. Align both red dots to properly insert the waste level cable connector into the waste level sensor located at the top of the waste container. Plug the other end of the waste level cable connector into the appropriate waste level sensor port located at the top back inside of the solvent cabinet.

3. Screw the cap onto the waste tank.

To empty a full container:

1. Carefully disconnect the waste level sensor connector by grasping the knurled area of the fitting firmly and pulling directly up.

CAUTION If waste is being emptied while the instrument is running, wait until the status icon for the waste container turns red at the lower right of the screen before removing cap from container. If the icon has not turned red, fluid may still be draining into the container as part of the currently running operation.

2. Unscrew the cap. Empty the waste container and place it back into the solvent cabinet.

3. Screw the cap back on and reconnect the waste level sensor, being careful to line up the red dots before applying pressure. Do not force. Ensure the o-ring is in place to seal the cap on the waste container.

1.3 Accessories

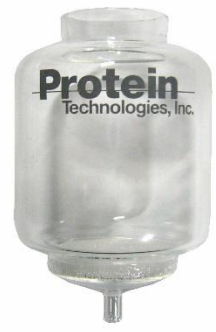
1.3.1 Reaction Vessels

10 mL, Disposable
Cat#: PPS-R10-030, 30
Cat#: PPS-R10-090, 90
Cat#: PPS-R10-180, 180

10 mL, Glass
Cat#: TPS-GRV10-1, 1 ea.
Cat#: TPS-GRV10-10, 10

10 mL, Frosted Glass
Cat#: TPS-FGRV10-1, 1 ea.
Cat#: TPS-FGRV10-10, 10

45 mL, Disposable
Cat#: PPS-R45-030, 30
Cat#: PPS-R45-090, 90
Cat#: PPS-R45-180, 180



40 mL, Glass
Cat#: TPS-GRV40-1, 1 ea.
Cat#: TPS-GRV40-10, 10

40 mL, Frosted Glass
Cat#: TPS-FGRV40-1, 1 ea.
Cat#: TPS-FGRV40-10, 10

1.3.2 Cleavage Vials

50 mL
Cat#: CLV-050-030, 30
Cat#: CLV-050-090, 90
Cat#: CLV-050-180, 180

1.3.3 Amino Acid Bottles

10 mL Single-Shot™
Cat#: AAR-SSI, 1 ea
Cat#: AAR-SSX, 10

120 mL
Cat#: SMP-VX-20, 20
Cat#: SMP-VX-100, 100

400 mL
Cat#: AAR-400-I, 1 ea.
Cat#: AAR-400-X, 10

1.3.4 Amino Acids & Reagents for Peptide Synthesis

Protein Technologies, Inc. supplies high quality, pre-tested N-Fmoc-protected amino acids preweighed in 5 mmol, 10 mmol and 20 mmol quantities in synthesizer-ready bottles (see Appendix A.1 for listings), as well as bulk N-Fmoc-protected amino acids preweighed in 25 g and 100 g quantities (See Appendix A.2 for listings). We recommend using our amino acids for all of your synthesis needs. Protein Technologies, Inc. also supplies reagents and kits for peptide synthesis on the Symphony X™ (See Appendix A.3 for listings).

1.3.5 Replacement Parts/Accessories

Protein Technologies, Inc. supplies replacement parts for the Symphony X as well as various accessories, including bottles and waste containers. A partial listing of replacement parts and accessories is located in Appendix B. For additional part and accessory information, please call our support desk at 1-800-477-6834.

Chapter 2: Introduction to Software

This chapter covers the function of each software screen.

2.1 Input Screens

There are four types of screens for inputting data on the Symphony® X:

1. Alpha Keyboard
2. Numeric Keyboard
3. Time Keyboard
4. Unit Selection

Operation of these four screens will be covered in the following sections.

2.1.1 Alpha Keyboard

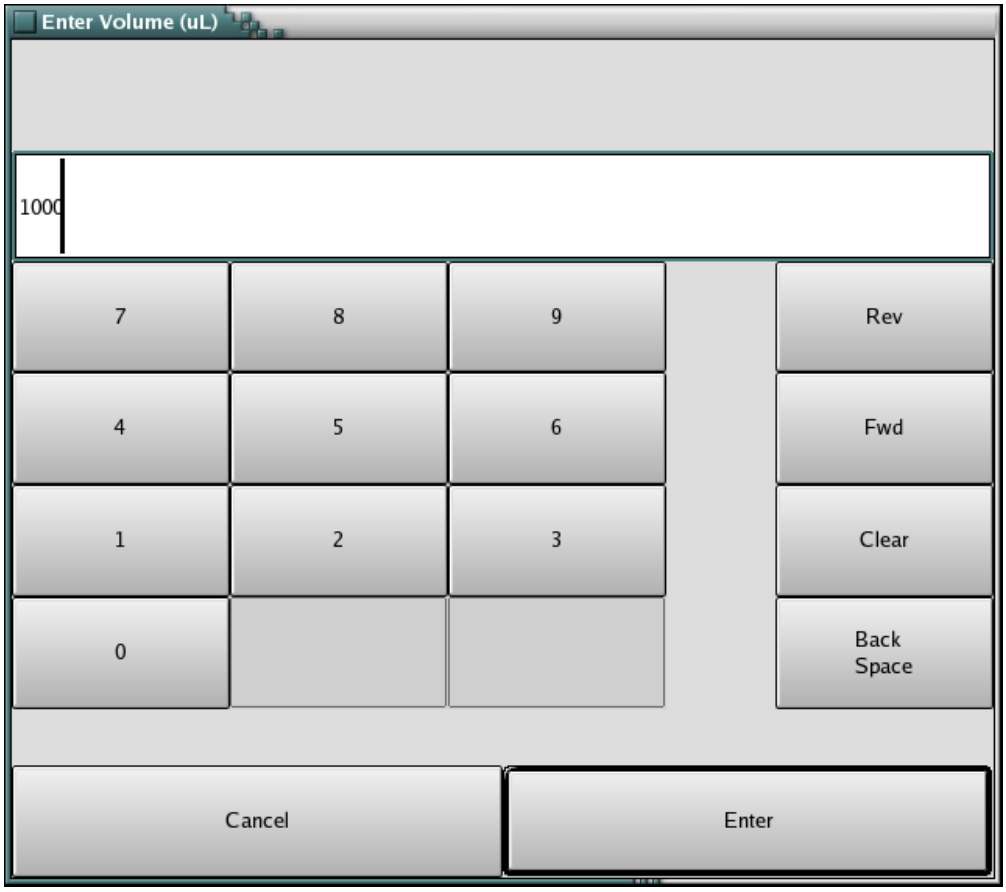
The **Alpha Keyboard** screen is for the input of anything involving letters and numbers. It includes all 26 letters of the alphabet as well as a numeric keypad and some symbols.

The functions of the remaining buttons are as follows:

1. **Back Space** – Erases the last character of the entry
2. **Caps Lock** – Switches between upper and lowercase letters. Also reveals different symbols.
3. **Clear** – Erases the entire entry
4. **Left** – Moves the cursor one character to the left
5. **Right** – Moves the cursor one character to the right
6. **Cancel** – Cancels input
7. **Enter** – Confirms entry and returns to previous screen

2.1.2 Numeric Keyboard

The **Numeric Keyboard** is for the input of anything involving numbers.



The function of the four buttons on the right are:

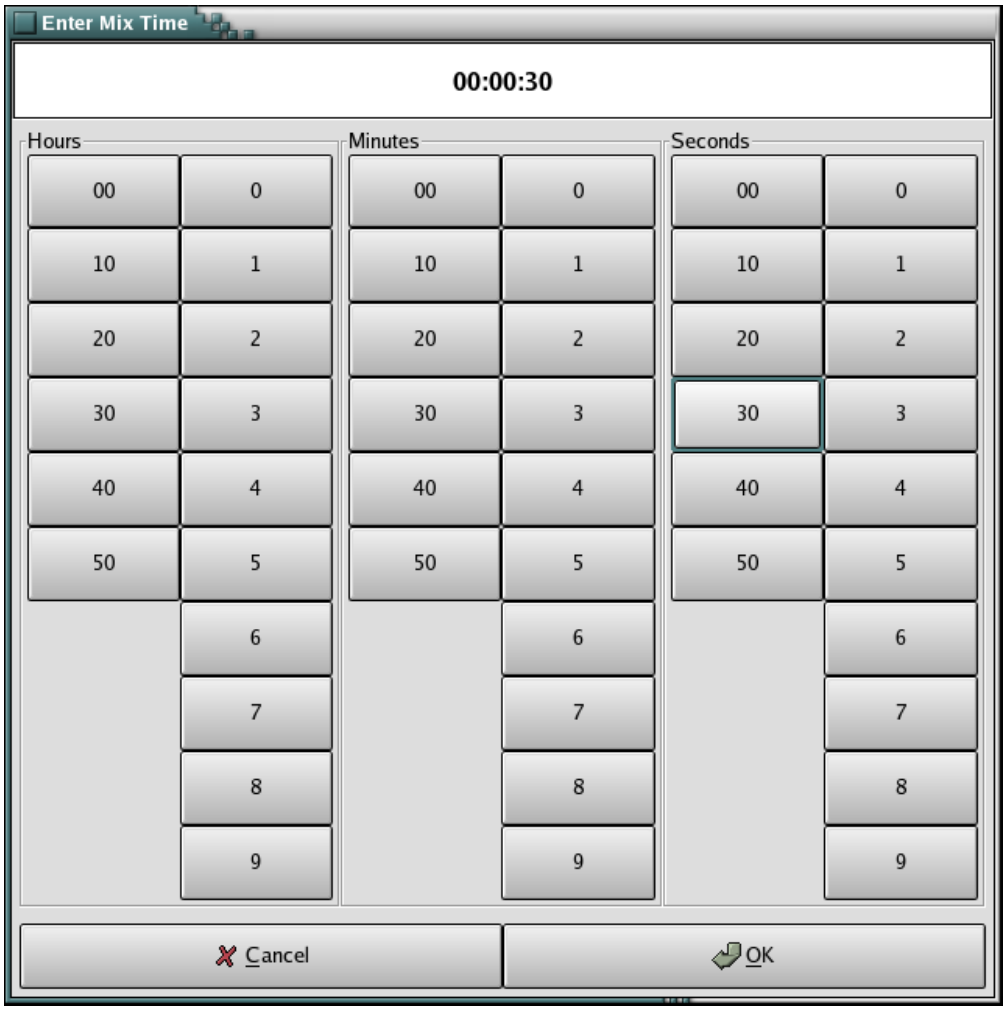
1. **Rev** – Moves the cursor one character to the left
2. **Fwd** – Moves the cursor one character to the right
3. **Clear** – Erases the entire entry
4. **Back Space** – Erases last character of entry

The remaining buttons are:

1. **Cancel** – Cancels entry
2. **Enter** – Confirms entry and returns to previous screen

2.1.3 Time Keyboard

The **Time Keyboard** is for the input of times.



It is separated into three sections:

1. **Hours** – Input number of hours
    a. Left Column – Tens value (00 – 50)
    b. Right Column – Ones value (0 – 9)
2. **Minutes** – Input the number of minutes
    a. Left Column – Tens value (00 – 50)
    b. Right Column – Ones value (0 – 9)
3. **Seconds** – Input the number of seconds

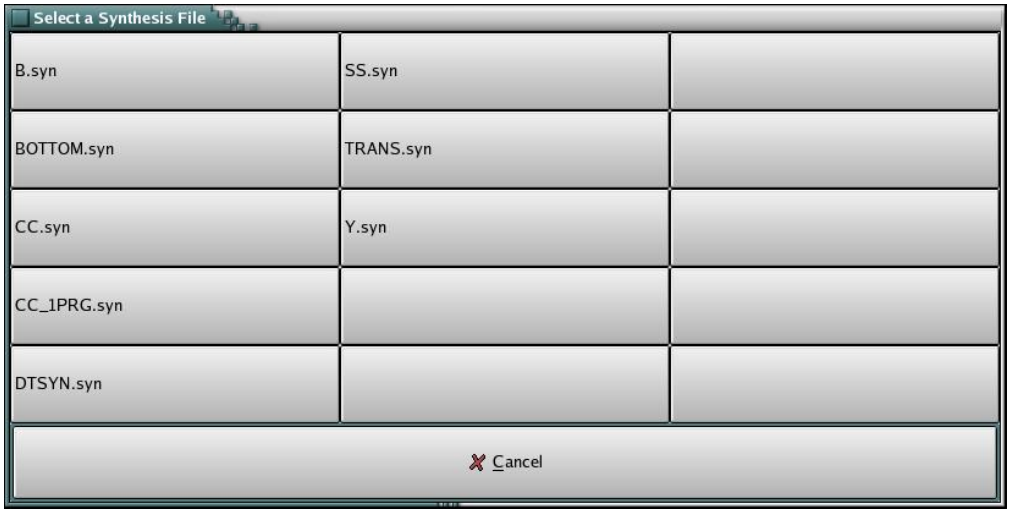
a. Left Column – Tens value (00 – 50)
b. Right Column – Ones value (0 – 9)

The remaining buttons are:

1. **Cancel** – Cancels entry
2. **OK** – Confirms entry and returns to previous screen

2.1.4 Selection Screen

The **Selection Screen** appears when a selection must be made (program file, program operation, synthesis file etc.) and lists all available selections.

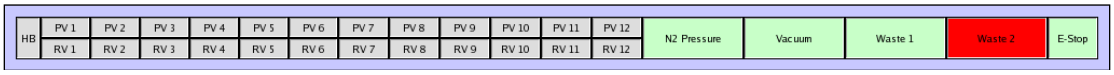


The buttons on the bottom of the screen are as follows:

1. **Prev Page** – Scrolls the list to the left (when number of entries exceeds the space on the screen)
2. **Next Page** – Scrolls the list to the right (when number of entries exceeds the space on the screen)
3. **Cancel** – Returns to previous screen

2.1.5 Instrument Status

The **Instrument Status** section is located at the bottom of all screens except the **Main Menu**.



At the far left is the heartbeat (HB) and PV and RV status displays.

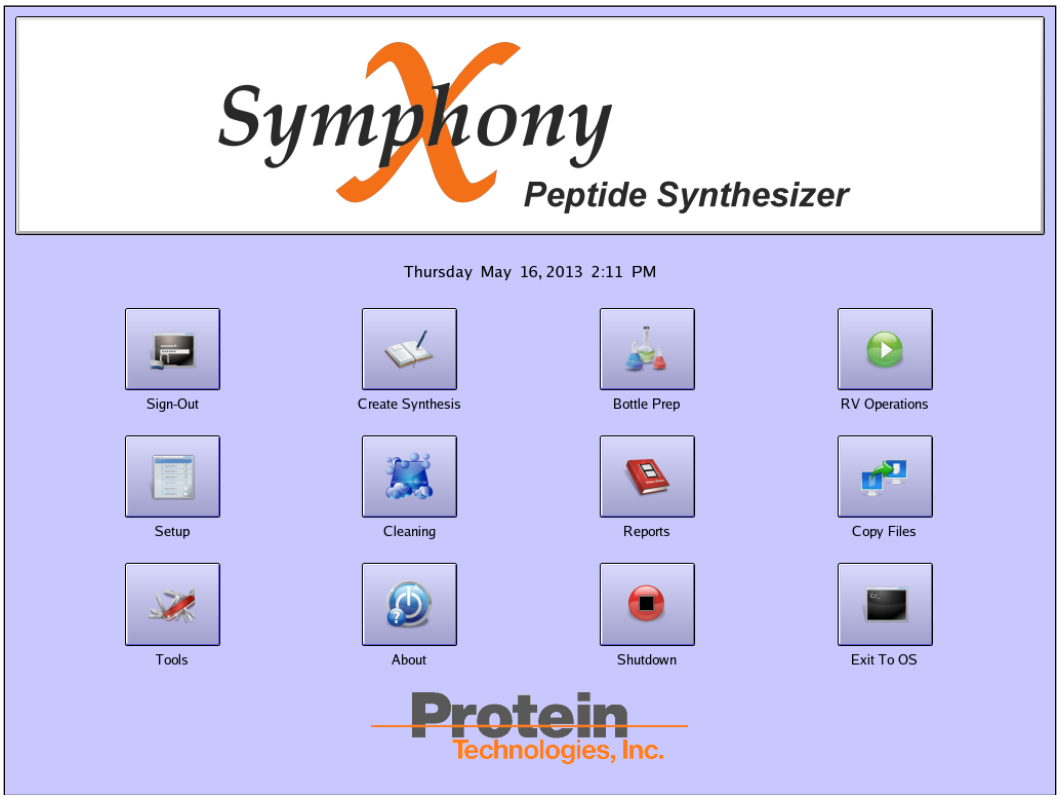
The HB status flashes green when the computer is communicating effectively with the instrument. The PV and RV statuses turn green when the instrument is running or paused, they flash red during an error and they turn orange when a synthesis is canceled.
The five instrument status displays on the lower right of the screen turn green to indicate the following:

1. **N2 Pressure**– Indicates adequate pressure (70 to 90 psi) supplied by the external nitrogen source
2. **Vacuum** – Indicates adequate vacuum (-10 to -26 in Hg) supplied by the internal vacuum pump
3. **Waste 1** – Waste 1 tank not full
4. **Waste 2** – Waste 2 tank not full
5. **E-Stop** – Emergency stop button not activated

These displays will flash red if any of the above conditions are not met.

2.2 Main Menu

When the Symphony® X is initialized, the **Main Menu** screen will open. The **Main Menu** allows the user to access all other screens.



There are 12 selections:

1. **Sign In/Sign Out** – Allows you to sign in or sign out as a user.

2. **Create Synthesis** – Allows you to create, edit or delete syntheses, and assign programs using the **Synthesis Editor** screen

3. **Bottle Prep**– Allows you to pressurize, prime, vent and backflush solvent and amino acid bottles, and set the current amino acid and solvent files

4. **RV Operations** – Allows you to access the RV Automated Operations, RV Cycle Progress and RV Manual Operations screens.

5. **Setup** – Opens the **Setup** menu which leads to the **Amino Acid Editor**, **Solvent Editor**, **Program Editor**, **Sequence Editor**, **Good Laboratory Practice**, **User Editor**, **Operations Times**, and **Settings** screens

6. **Cleaning** – Allows you to perform automated cleaning operations

7. **Reports** – Allows you to access the **View Reports** screen and **Log Files** screen.

8. **Copy Files** – Allows you to select a current drive and copy synthesis, program, sequence, amino acid, solvent or job files between drives

9. **Tools** – Opens the **Tools** menu which leads to the **Calculations**, **Machine Status**, and **Diagnostics** screens.

10. **About** – Displays information about the Symphony® X

11. **Shutdown** – Shuts down the software

12. **Exit to OS** – Exits software to Linux operating system

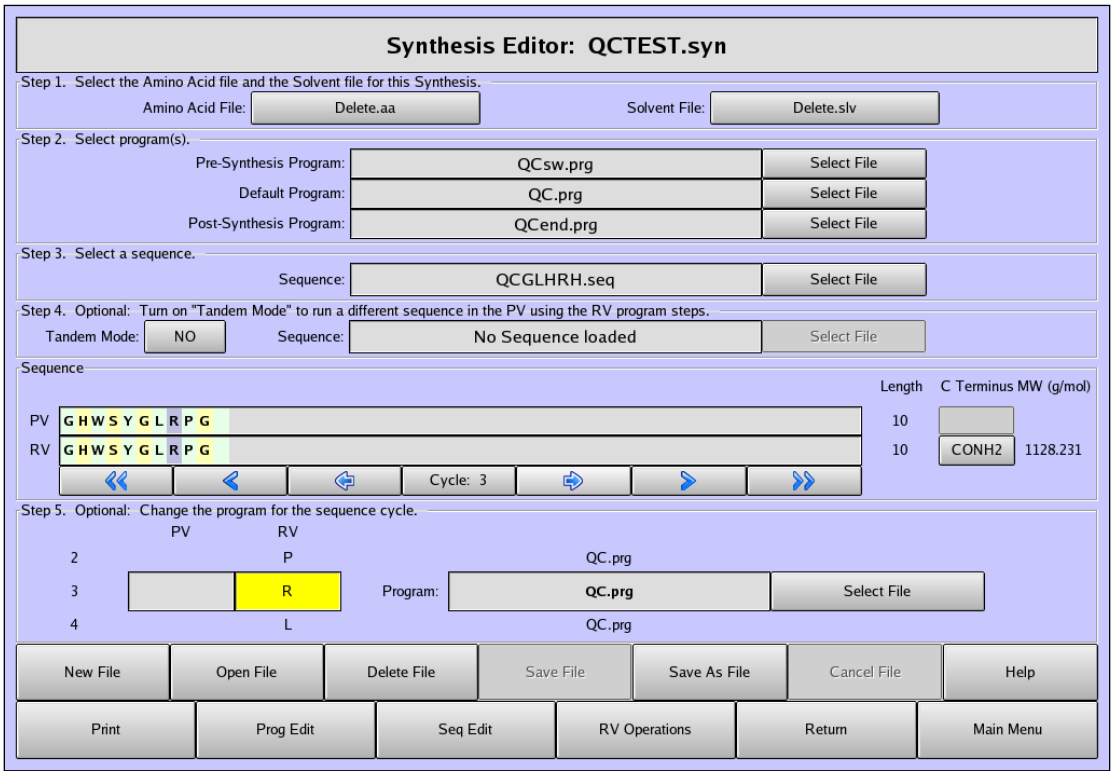
2.3 Sign In/Sign Out

To sign in a user, select the **Sign In** button from the **Main Menu** screen. Select a username from the pop-up list, and enter the password to sign in.

To sign out the current user, select the **Sign Out** button from the **Main Menu** screen.

2.4 Create Synthesis

The **Synthesis Editor** screen allows the user to create, edit or delete synthesis files. To open the **Synthesis Editor** screen, select **Create Synthesis** from the **Main Menu**.



The name of the synthesis file is displayed at the top of the screen.

In Step 1, select the amino acid and solvent files using the following buttons:

1. **Amino Acid File** – Select amino acid file for the synthesis. Only program and sequence files that use this amino acid file may be selected in the synthesis.
2. **Solvent File** – Select solvent file for the synthesis. Only program files that use this solvent file may be selected in the synthesis.

In Step 2, use the **Select File** button to select the following programs for the synthesis:

1. **Pre-Synthesis Program** – This program will be performed at the beginning of the synthesis and is not associated with an amino acid in the sequence.

2. **Default Program** – This program will automatically be assigned to all amino acids in the selected sequence(s).
3. **Post-Synthesis Program** – This program will be performed at the end of the synthesis and is not associated with an amino acid in the sequence.

In Step 3, use the **Select File** button to select a sequence to run in the RV position.

In Step 4, select the **Tandem Mode** setting. **NO** means only one sequence will run in the RV position, while the PV position may be used as a pre-activation chamber for the RV. **YES** means a separate sequence may be run in the PV position using the same programs assigned to the RV position. Use the **Select File** button to select a sequence to run in the PV position.

NOTE There must be no PV operation steps in the programs selected in Step 2, or **Tandem Mode** may not be used.

NOTE The RV sequence must be the same length or longer than the PV sequence.

The RV and optional PV sequence(s) are displayed in the **Sequence** section. To the right of the sequence(s) are the following:

1. **Length** – Displays sequence length
2. **C-Terminus** – Select COOH or CONH2 for C-terminus. CONH2 starts the synthesis from the rightmost amino acid position, while COOH assumes a pre-loaded resin will be used and starts the synthesis from the second amino acid position from the right.
3. **Molecular Weight** – Displays molecular weight for the sequence and selected C-terminal group.

In Step 5, assign custom programs to selected cycles. Use the arrow keys in the section above to navigate to different cycles:

1. **<<** – Moves to leftmost position in sequence
2. **<** – Moves five cycles to the left
3. **Left Arrow** – Moves one cycle to the left
4. **Cycle** – Displays current cycle
5. **Right Arrow** – Moves one cycle to the right
6. **>** – Moves five cycles to the right
7. **>>** – Moves to rightmost position in sequence

The remaining buttons are as follows:

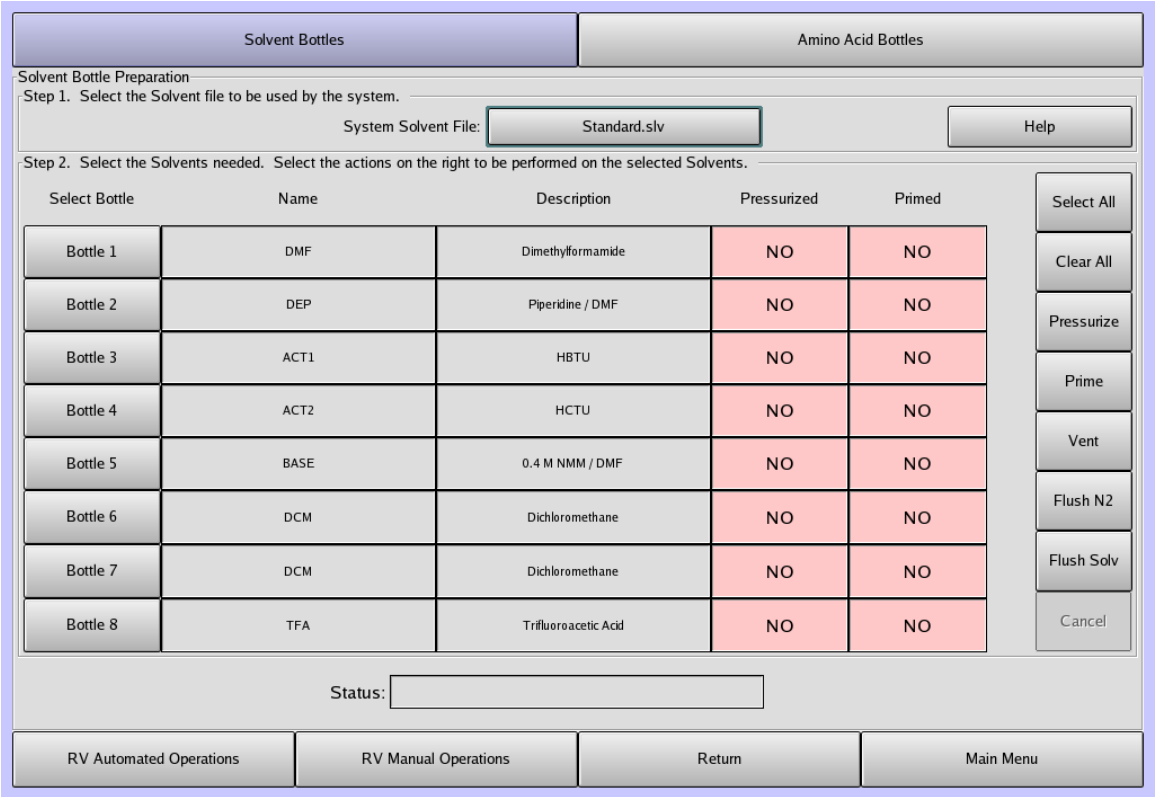
1. **New File** – Use to create new synthesis file
2. **Open File** – Use to edit existing synthesis file

3. **Delete File** – Use to delete existing synthesis file
4. **Save File** – Saves changes to synthesis
5. **Save As File** – Saves synthesis under different name
6. **Cancel File** – Cancels all changes
7. **Help** – Displays **Synthesis Editor Help** screen
8. **Print** – Prints current synthesis file
9. **Prog Edit** – Shortcut to **Program Editor** screen
10. **Seq Edit** – Shortcut to **Sequence Editor** screen
11. **RV Operations** – Shortcut to the **RV Operations** screen
12. **Return** – Returns to previous screen
13. **Main Menu** – Returns to **Main Menu**

2.5 Bottle Prep

The **Bottle Prep** screen allows the user to set the current bottle configuration for the instrument and pressurize, prime, vent, and back flush the amino acid and solvent bottles with nitrogen or solvent. To open the **Bottle Prep** screen, select **Bottle Prep** from the **Main Menu**.

There are two screens. The first is the **Solvent Bottles** screen:



In Step 1 are the following 2 buttons:

1. **System Solvent File** – Select a solvent file to define the current bottle configuration for the instrument. Only syntheses which use the currently loaded solvent file may be loaded on the **RV Automated Operations** screen. Currently loaded solvent files may not be edited on the **Solvent Editor** screen.
2. **Help** – Displays the **Bottle Prep Help** screen

In Step 2 are the following 5 columns:

1. **Select Bottle** – Select solvent bottle(s).
2. **Name** – Displays abbreviated solvent name
3. **Description** – Displays more detailed description of solvent
4. **Pressurized** – Indicates whether bottle is pressurized or vented
5. **Primed** – Indicates whether bottle is primed or not

The buttons to the right of the screen are as follows:

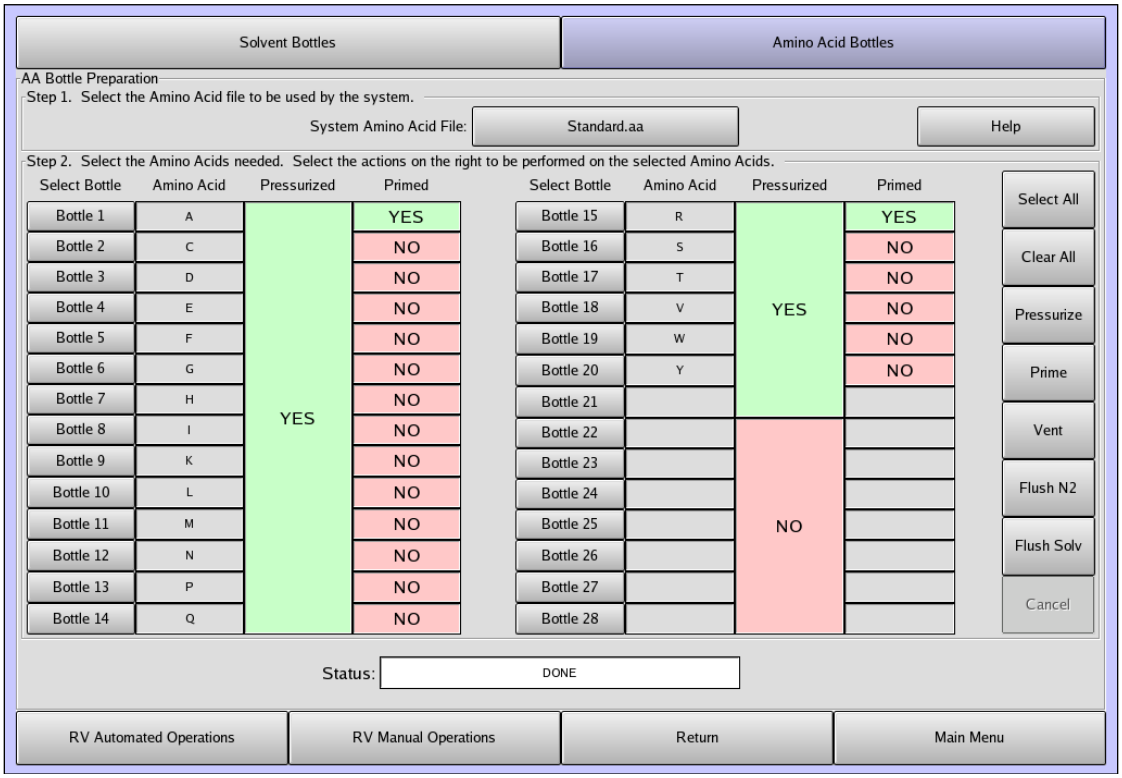
1. **Select All** – Selects all bottle positions
2. **Clear All** – Deselects all bottle positions
3. **Pressurize** – Pressurizes selected bottles with nitrogen
4. **Prime** – Primes selected bottles
5. **Vent** – Vents selected bottles
6. **Flush N2** – Back flushes selected bottles with nitrogen
7. **Flush Solv** – Back flushes selected bottles with Solvent 1 or Solvent 7. Bottles 2-6 are back flushed with Solvent 1 and Bottle 8 is back flushed with Solvent 7. Flush Solv cannot be performed on Bottles 1 or 7.
8. **Cancel** – Cancels current operation

The remaining buttons are as follows:

1. **RV Automated Operations** – Shortcut to **RV Automated Operations** screen
2. **RV Manual Operations** – Shortcut to **RV Manual Operations** screen
3. **Return** – Return to previous screen
4. **Main Menu** – Return to the **Main Menu** screen

CAUTION Bottles are under pressure. Use with caution. Protein Technologies, Inc. recommends using safety-coated bottles on the Symphony® X to prevent bodily damage if a bottle should break under pressure.

The second screen is the **Amino Acid Bottles** screen:



In Step 1 are the following 2 buttons:

1. **System Amino Acid File** – Select an amino acid file to define the current bottle configuration for the instrument. Only syntheses which use the currently loaded amino acid file may be loaded on the **RV Automated Operations** screen. Currently loaded amino acid files may not be edited on the **Amino Acid Editor** screen.
2. **Help** – Displays the **Bottle Prep Help** screen

In Step 2 are the following 4 columns:

1. **Select Bottle** – Select amino acid bottle(s).
2. **Amino Acid** – Displays 1-letter code for amino acid
3. **Pressurized** – Indicates whether bottle is pressurized or vented
4. **Primed** – Indicates whether bottle is primed or not

The buttons to the right of the screen are as follows:

1. **Select All** – Selects all bottle positions
2. **Clear All** – Deselects all bottle positions
3. **Pressurize** – Pressurizes selected bottles with nitrogen

4. **Prime** – Primes selected bottles
5. **Vent** – Vents selected bottles
6. **Flush N2** – Back flushes selected bottles with nitrogen
7. **Flush Solv** – Back flushes selected bottles with Solvent 1
8. **Cancel** – Cancels current operation

The remaining buttons are as follows:

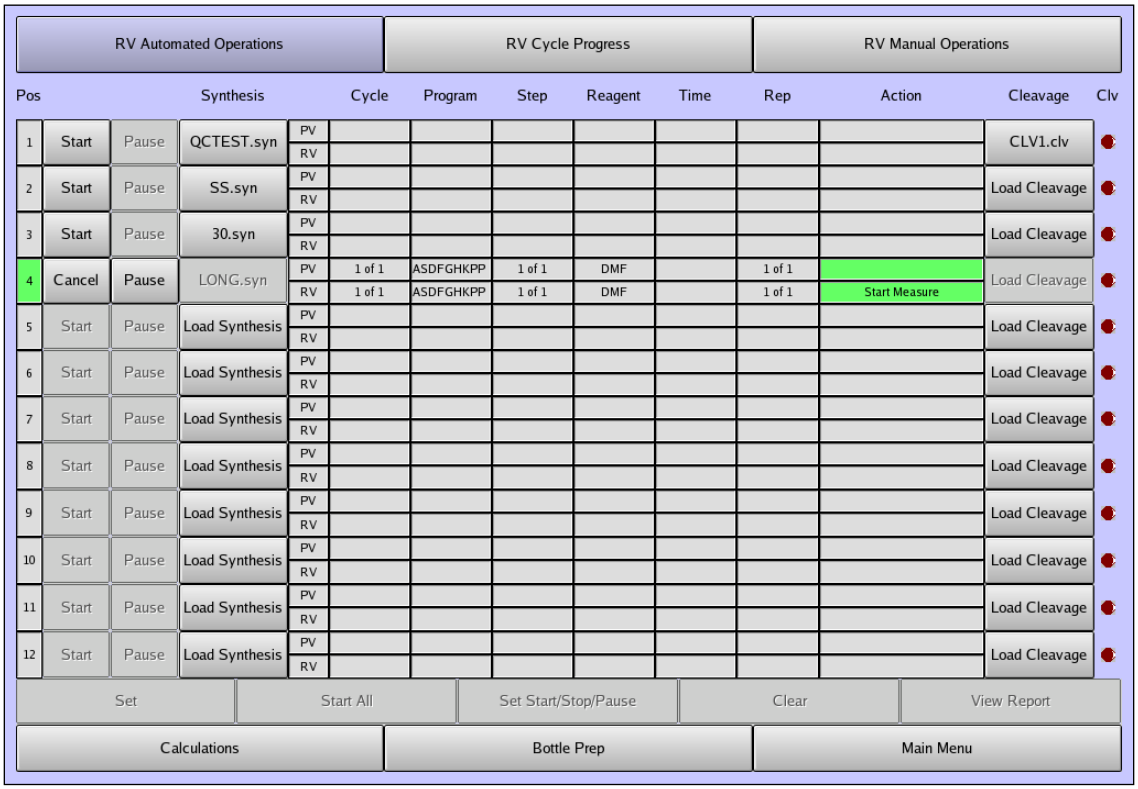
1. **RV Automated Operations** – Shortcut to **RV Automated Operations** screen
2. **RV Manual Operations** – Shortcut to **RV Manual Operations** screen
3. **Return** – Return to previous screen
4. **Main Menu** – Return to the **Main Menu** screen

2.6 RV Operations

Select **RV Operations** from the **Main Menu** to access the **RV Automated Operations**, **RV Cycle Progress** and **RV Manual Operations** screens. The function of each screen is detailed in the sections below.

2.6.1 RV Automated Operations

The **RV Automated Operations** screen allows you to run syntheses or cleavage programs on RV and PV positions. You can also set Start/Stop/Pause steps. To open the **RV Automated Operations** screen, select **RV Operations** from the **Main Menu**, then select the **RV Automated Operations** tab.



There are 14 columns:

1. **Pos** – RV/PV position
2. **Start/Restore/Cancel** – Press **Start** to start a loaded synthesis. Press **Cancel** to cancel a synthesis. Press **Restore** to select the cycle, step and rep to restart the synthesis following an error.
3. **Pause/Resume** – Press **Pause** to pause the synthesis. Press **Resume** to restart the synthesis after a pause or error.
4. **Synthesis** – Select a **Load Synthesis** button to load a synthesis file for a specific position. Only syntheses which contain the current solvent and

amino acid files may be loaded. The current solvent and amino acid files are selected on the **Bottle Prep** screen.

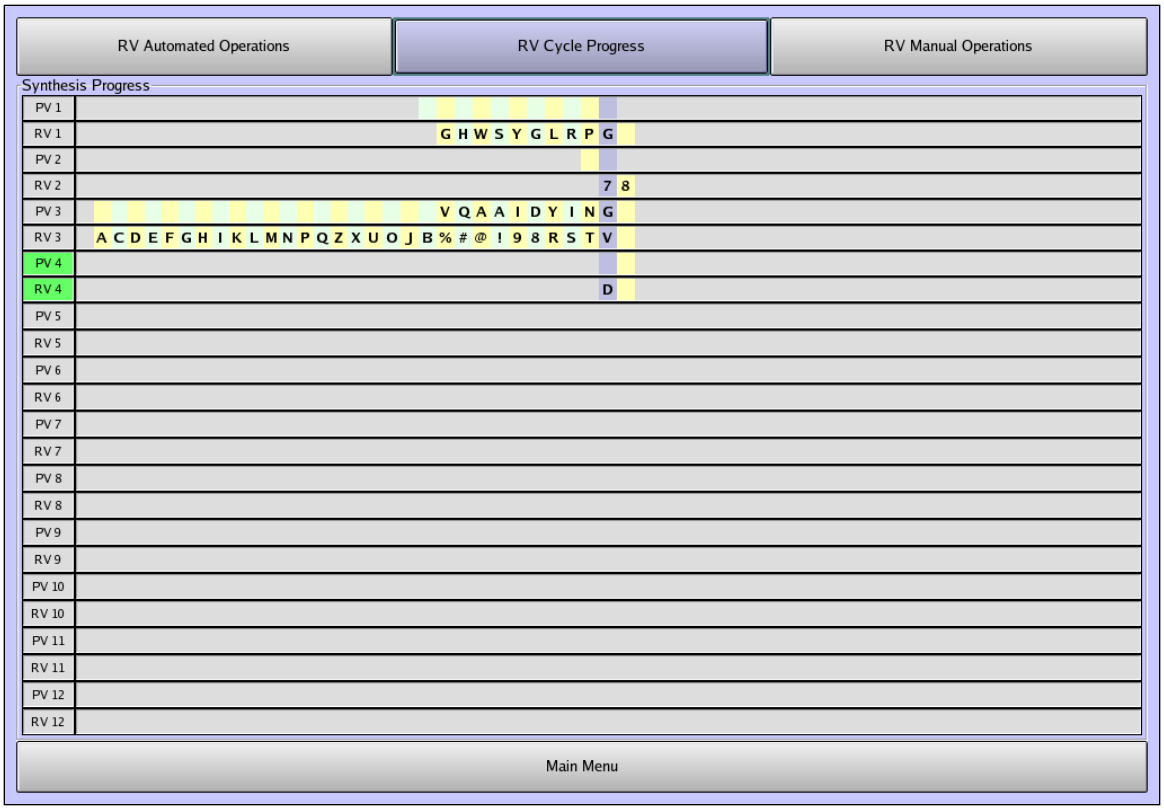
5. **PV/RV** – Labels where PV or RV data displayed
6. **Cycle** – Displays the current cycle while the synthesis is running. "Pre-synth" means the pre-synthesis program is running, and "post-synth" means the post-synthesis program is running.
7. **Program** – Displays the current program while the synthesis is running.
8. **Step** – Displays the current program step while the synthesis is running.
9. **Reagent** – Displays the reagent being delivered in the current program step.
10. **Time** – Displays the remaining mix time for the current program step.
11. **Rep** – Displays the current rep number for the program step.
12. **Action** – Displays the current action for the program step.
13. **Cleavage** – Select a **Load Cleavage** button to load a cleavage program for a specific position. Only programs which contain the current solvent and amino acid files may be loaded. The current solvent and amino acid files are selected on the **Bottle Prep** screen.
14. **Clv –** Visual indicator that cleavage program is running.

At the bottom of the screen are 8 buttons:

1. **Set** – Use to load synthesis files on multiple positions at the same time
2. **Start All** – Press to start all loaded syntheses
3. **Set Start/Stop/Pause** – Opens the **Start/Stop/Pause** screen which allows you to program where the synthesis starts, stops and pauses for each position.
4. **Clear** – Unloads a synthesis file(s) from selected positions.
5. **View Report** – Shortcut to **View Report** screen
6. **Calculations** – Shortcut to **Calculations** screen
7. **Bottle Prep** – Shortcut to **Bottle Prep** screen
8. **Main Menu** – Return to **Main Menu**

2.6.2 RV Cycle Progress

The **RV Cycle Progress** screen allows you to view the cycle progress during a synthesis. To open the **RV Cycle Progress** screen, select **RV Operations** from the **Main Menu**, then select the **RV Cycle Progress** tab.



The PV and RV positions are displayed in the column at the left, and the sequences are displayed with the current cycle highlighted in purple. At the bottom of the screen, select the **Main Menu** button to return to the **Main Menu**.

2.6.3 RV Manual Operations

The **RV Manual Operations** screen allows the user to perform manual operations on the reaction vessels. To open the **RV Manual Operations** screen, select **RV Operations** from the **Main Menu**, then select the **RV Manual Operations** tab.

The **RV Manual Operations** screen is divided into twelve rows, one for each RV/PV position. There are twelve columns:

1. **Pos** – RV/PV position.
2. **Start/Cancel** – Press **Start** to start an operation. Press **Cancel** to cancel an operation.
3. **Operation** – Operation.
4. **RV/PV** – Select between RV or PV position.
5. **Bottle** – Bottle position from which fluid will be delivered.
6. **Volume (uL)** – Volume of fluid delivered (in microliters).
7. **Mix Type** (RV 1 for IR only) – Select between N2 and/or Vortex mixing. All other positions use N2 mixing.
8. **Time** – Mix time (HH:MM:SS).
9. **Temp (°C)** (IR only) – Input temperature for mix step in degrees Celsius.
10. **Drain** – Y indicates the fluid will be drained from the reaction vessel at the end of the step. N indicates the fluid will not be drained.
11. **Reps** – Number of times the step will be performed.
12. **Status** – Displays status of running operation.

Available Actions are:

1. **AA Delivery** – Delivers the selected AA to RV or PV then optionally Mix and Drain. AA's can be delivered in 1000 or 1500 µL aliquots, or by Single-Shot™ deliveries.

2. **Bottom Delivery** – Delivers fluid to RV or PV from the bottom, then optionally Mix and Drain. Valid for Solvents 7 and 8, and CV 1-12 when **Collect Mode** set to **Single Shot Amino Acid** on the **Settings** screen. Solvents 7 and 8 are delivered in 2500 µL aliquots. CV's 1-12 are delivered as Single-Shots™.
3. **Cleave & Collect** – Delivers solvent 8 to RV or PV from the bottom, then optionally Mix and Collect. Only available when **Collect Mode** set to **Cleavage** on the **Settings** screen.
4. **Cleave Mix** – Nitrogen burst for one second with a 2 minute delay between bursts (default) to prevent evaporation of TFA over the course of a cleavage step
5. **Collect** – Empties the RV or PV to collect vial
6. **Collect Clean** – Rinses the RV or PV lines and the collect lines.
7. **Drain Dry** – Empties the RV or PV to waste, Time to Dry
8. **Mix**– Mix then empty RV or PV to waste. Nitrogen burst for one second with a 10 second delay between bursts (default).
9. **PV to RV** – Transfers contents of PV to RV
10. **Top Delivery** – Delivers fluid to RV or PV from the top, then optionally Mix and Drain. Select from Solvents 1-6 or AA 1-28. Deliveries may be made in 1000 or 1500 uL aliquots, or by Single-Shot™ deliveries for AA 1-28.
11. **Vent Wash** – Top delivery of Solvent 1 to RV or PV through the vent line out to waste.

The bottom section contains 5 buttons:

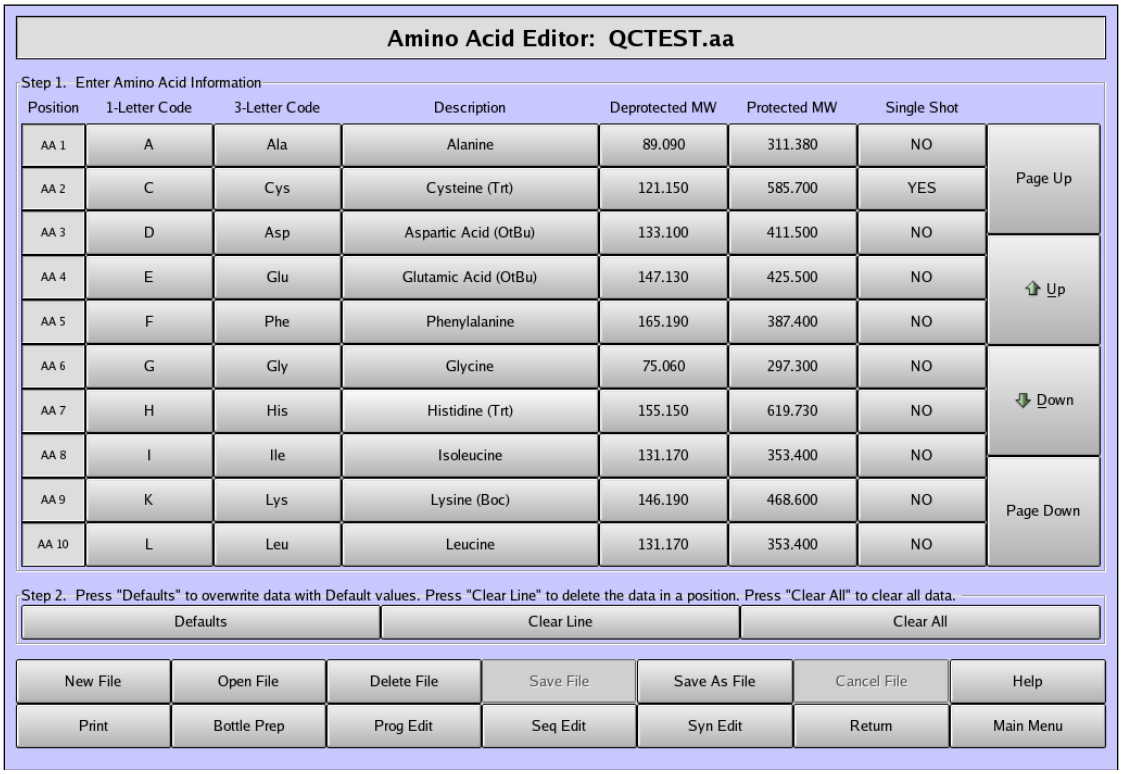
1. **Set** – Use to load synthesis files on multiple positions at the same time
2. **Start All** – Press to start all loaded syntheses
3. **Clear** – Unloads a synthesis file(s) from selected positions.
4. **Bottle Prep** – Shortcut to **Bottle Prep** screen
5. **Main Menu** – Return to **Main Menu** screen

2.7 Setup

The **Setup** menu allows the user to access the **Amino Acid Editor**, **Solvent Editor**, **Program Editor**, **Sequence Editor**, **Good Laboratory Practice Editor**, **User Editor**, **Operation Times** and **Settings** screens. To open the **Setup** menu, select **Setup** from the **Main Menu**.

2.7.1 Amino Acid Editor

The **Amino Acid Editor** screen allows the user to define the amino acids and special reagents available for a synthesis. To open the **Amino Acid Editor** screen, select **Setup** from the **Main Menu**, then select **Amino Acid Editor**.



The name of the amino acid file is displayed at the top of the screen.

In Step 1, enter the following for each amino acid position:

1. **Position** – Amino acid bottle position. AA refers to the 28 amino acid bottle positions, while CV refers to the 12 collect vial positions. Collect vial positions may be used as additional Single-Shot™ amino acid positions when **Collect Mode** is set to **Single Shot Amino Acid** in the **Settings** screen.
2. **1-Letter Code** – Enter 1-letter abbreviation for amino acid
3. **3-Letter Code** – Enter 3-letter abbreviation for amino acid
4. **Description** – Enter detailed description of amino acid
5. **Deprotected MW** – Enter the deprotected molecular weight of the amino acid (in g/mol)
6. **Protected MW** – Enter the protected molecular weight of the amino acid (in g/mol)
7. **Single Shot** – YES turns the Single-Shot™ feature on for that bottle position, so the entire contents of the bottle will be delivered to the selected reactor in an AA Delivery or Top Delivery step regardless of the programmed volume. Selecting “NO” turns the Single-Shot™ feature off for that bottle position, so only the programmed volume will be delivered.

Use the four buttons to the right to maneuver between positions:

1. **Page Up** – Displays positions one page above current display
2. **Up** – Displays one position above current display
3. **Down** – Displays one position below current display
4. **Page Down** – Displays positions one page below current display

In Step 2, use the following three buttons to edit entries:

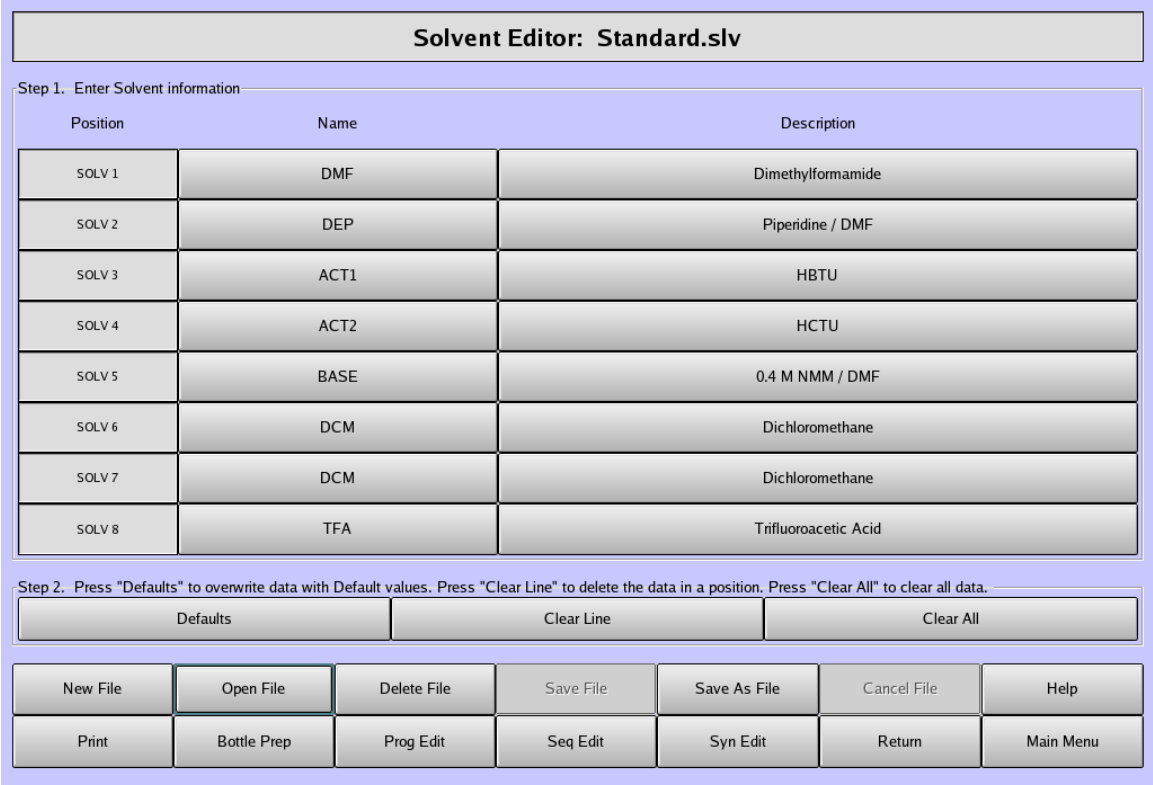
1. **Defaults** – Replaces current entries with default values
2. **Clear Line** – Select a line number to clear contents
3. **Clear All** – Clears entries from all amino acid positions

The remaining buttons at the bottom of the screen are:

1. **New File** – Use to create new amino acid file
2. **Open File** – Use to open existing amino acid file
3. **Delete File** – Use to delete existing amino acid file
4. **Save File** – Saves changes to amino acid file
5. **Save As File** – Saves amino acid file under different name
6. **Cancel File** – Cancels all changes
7. **Help** – Displays the **Amino Acid Editor Help** screen
8. **Print** – Prints current amino acid file
9. **Bottle Prep** – Shortcut to **Bottle Prep** screen
10. **Prog Edit** – Shortcut to **Program Editor** screen
11. **Seq Edit** – Shortcut to **Sequence Editor** screen
12. **Syn Edit** – Shortcut to **Synthesis Editor** screen
13. **Return** – Returns to previous screen
14. **Main Menu** – Returns to **Main Menu**

2.7.2 Solvent Editor

The **Solvent Editor** screen allows the user to define the solvents available for a synthesis. To open the **Solvent Editor** screen, select **Setup** from the **Main Menu**, then select **Solvent Editor**.



The name of the solvent file is displayed at the top of the screen.

In Step 1, enter the following for each solvent position:

1. **Position** – Solvent bottle position.
2. **Name** – Input abbreviated solvent name
3. **Description** – Input detailed description of solvent

NOTE The abbreviations in the **Name** column will determine the bottle choices in the **Program Editor** and other screens.

In Step 2, use the following buttons to edit entries:

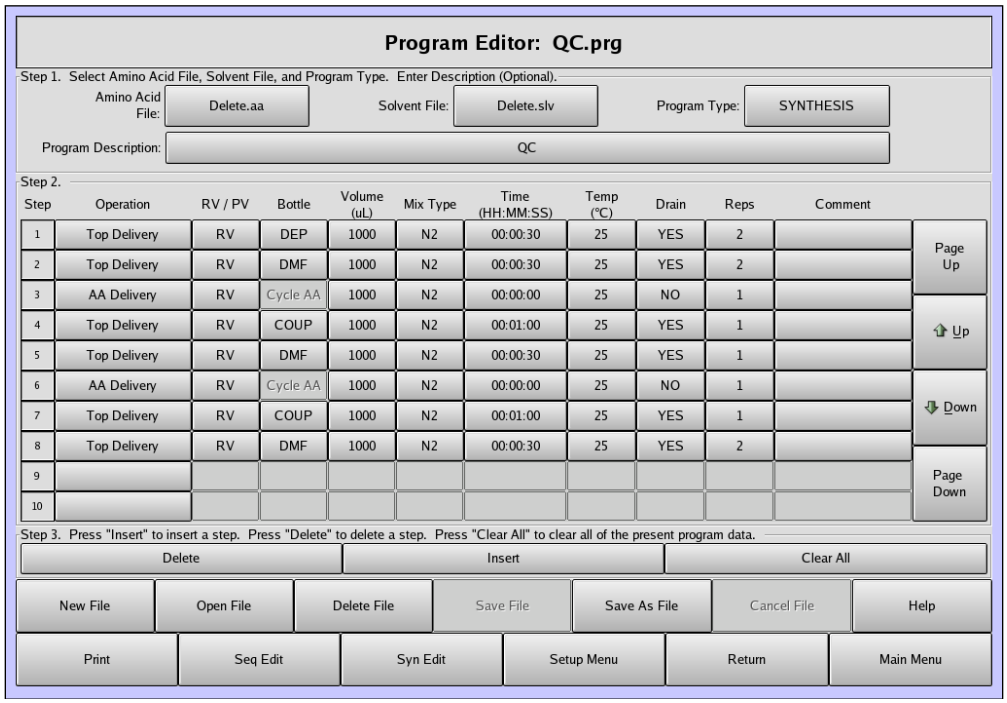
1. **Defaults** – Replaces current entries with default values
2. **Clear Line** – Select a line number to clear contents
3. **Clear All** – Clears entries from all solvent positions

The remaining buttons at the bottom of the screen are:

1. **New File** – Use to create new solvent file
2. **Open File** – Use to open existing solvent file
3. **Delete File** – Use to delete existing solvent file
4. **Save File** – Saves changes to solvent file
5. **Save As File** – Saves solvent file under different name
6. **Cancel File** – Cancels all changes
7. **Help** – Displays the **Solvent Editor Help** screen
8. **Print** – Prints current solvent file
9. **Bottle Prep** – Shortcut to **Bottle Prep** screen
10. **Prog Edit** – Shortcut to **Program Editor** screen
11. **Syn Edit** – Shortcut to **Synthesis Editor** screen
12. **Return** – Returns to previous screen
13. **Main Menu** – Returns to **Main Menu**

2.7.3 Program Editor

The **Program Editor** screen allows the user to create, edit or delete programs. To open the **Program Editor** screen, select **Setup** from the **Main Menu**, then select **Program Editor**.



The name of the program file is displayed at the top of the screen.

In Step 1 are the following 4 buttons:

1. **Amino Acid File** – Select amino acid file for program. Amino acid file will define amino acid bottle and collect vial selections.
2. **Solvent File** – Select solvent file for program. Solvent file will define solvent bottle selections.
3. **Program Type** – Select between Synthesis or Cleavage program type. Synthesis programs may be assigned to amino acid cycles in the **Synthesis Editor** screen. Cleavage programs may be loaded on the **RV Automated Operations** screen.
4. **Program Description** – Enter comment for the program file.

In Step 2, enter the following for each program step:

1. **Step** – Denotes program step.
2. **Operation** – Program operation.
3. **RV/PV** – Reactor on which operation will be performed.
4. **Bottle** – Bottle position from which fluid will be delivered.
5. **Volume (uL)** – Volume of fluid delivered (in microliters).
6. **Mix Type** – N2 or vortex mixing. Vortex mixing will only be performed on RV 1 on instruments with the infrared heating option. All other positions use nitrogen bubbling only.
7. **Time (HH:MM:SS)** – Mix time.
8. **Temp (°C)** – Reaction vessel temperature for step. This selection is only valid for RV 1 on instruments with the infrared heating option.
9. **Drain** – YES indicates the fluid will be drained from the reaction vessel at the end of the step. NO indicates the fluid will not be drained.
10. **Reps** – Number of times the step will be performed.
11. **Comment** – User may enter a comment for the step.

Available operations are:

1. **AA Delivery** – Delivers AA to RV or PV according to sequence, then optionally Mix and Drain. AA's can be delivered in 1000 or 1500 µL aliquots, or by Single-Shot™ deliveries. Valid for **Synthesis** programs only.
2. **Bottom Delivery** – Delivers fluid to RV or PV from the bottom, then optionally Mix and Drain. Valid for Solvents 7 and 8, and CV 1-12 when **Collect Mode** set to **Single Shot Amino Acid** on the **Settings** screen. Solvents 7 and 8 are delivered in 2500 µL aliquots. CV's 1-12 are delivered as Single-Shots™.
3. **Cleave & Collect** – Delivers solvent 8 to RV or PV from the bottom, then Mix and Collect. Valid for **Cleavage** programs only.
4. **Cleave Mix** – Nitrogen burst for one second with a 2 minute delay between bursts (default) to prevent evaporation of TFA over the course of a cleavage step.
5. **Collect** – Empties the RV or PV to collect vial.

6. **Drain Dry** – Empties the RV or PV to waste, Time to Dry.
7. **Email Notification** – Send an email notification with cell, cycle and step information.
8. **Mix**– Mix then empty RV or PV to waste. Nitrogen burst for one second with a 10 second delay between bursts (default).
9. **Pause** – Programmed Pause - Operator must press Resume to continue synthesis.
10. **PV to RV** – Transfers contents of PV to RV.
11. **Top Delivery** – Delivers fluid to RV or PV from the top, then optionally Mix and Drain. Select from Solvents 1-6 or AA 1-28. Deliveries may be made in 1000 or 1500 uL aliquots, or by Single-Shot™ deliveries for AA 1-28.
12. **Vent Wash** – Top delivery of Solvent 1 to RV or PV through the vent line.

Use the buttons to the right of the screen to maneuver between steps:

1. **Page Up** – Displays steps one page above current display
2. **Up** – Displays one step above current display
3. **Down** – Displays one step below current display
4. **Page Down** – Displays steps one page below current display

In Step 3, use the following buttons to delete, insert or clear program steps:

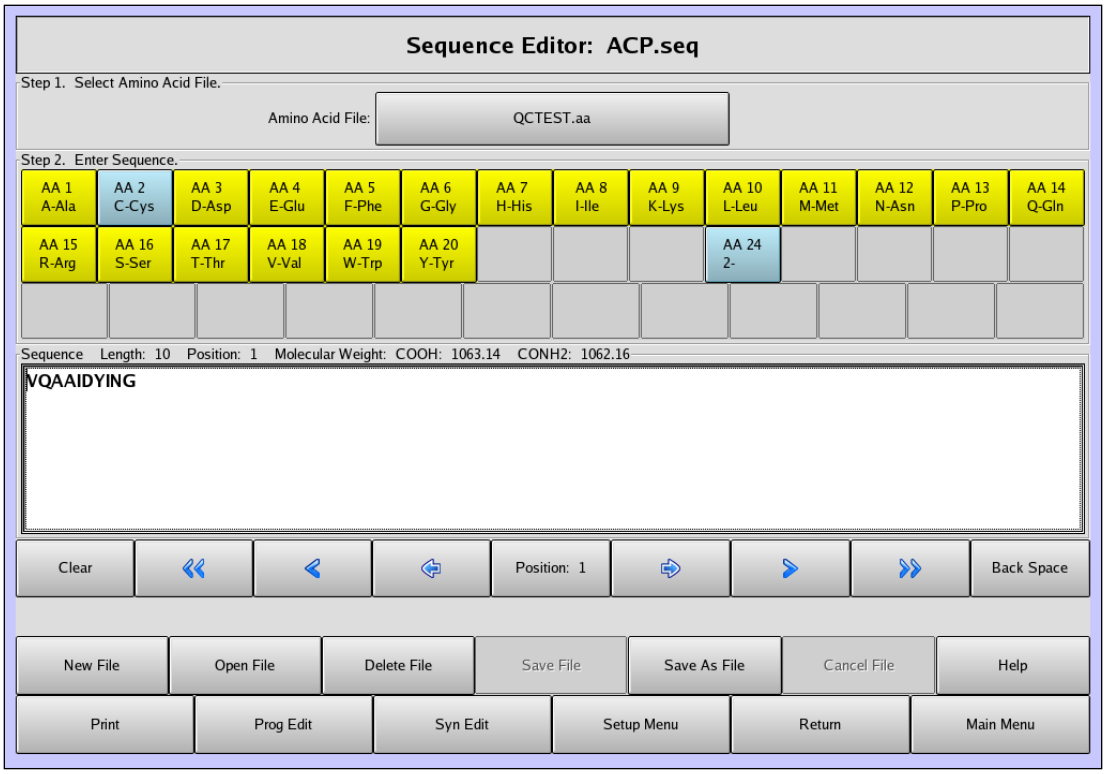
1. **Delete** – Input step number to delete
2. **Insert** – Input step number to insert new step
3. **Clear All** – Clears all steps in program

The remaining buttons are as follows:

1. **New File** – Use to create new program file
2. **Open File** – Use to open existing program file
3. **Delete File** – Use to delete existing program file
4. **Save File** – Saves changes to program
5. **Save As File** – Saves program under different name
6. **Cancel File** – Cancels all changes
7. **Help** – Displays the **Program Editor Help** screen
8. **Print** – Prints current program file
9. **Seq Edit** – Shortcut to **Sequence Editor** screen
10. **Syn Edit** – Shortcut to **Synthesis Editor** screen
11. **Setup Menu** – Shortcut to **Setup** menu
12. **Return** – Returns to previous screen
13. **Main Menu** – Returns to **Main Menu**

2.7.4 Sequence Editor

The **Sequence Editor** screen allows the user to create, edit or delete sequence files. To open the **Sequence Editor** screen, select **Setup** from the **Main Menu**, then select **Sequence Editor**.



The name of the sequence file is displayed at the top of the screen.

In Step 1, use the **Amino Acid File** button to select an amino acid file to define what amino acids may be used in the sequence.

In Step 2, the active amino acid positions defined by the selected amino acid file are displayed. Regular amino acid positions are shown in yellow, while Single-Shot™ amino acid positions are shown in teal. Use these buttons to enter a sequence which will be displayed in the white box in the center of the screen.

Above the white box are displayed the sequence length, cursor position, and molecular weight for both COOH and CONH2 C-terminal sequences.

Below the white box are the following buttons:

1. **Clear** – Clears all text from the sequence box
2. **<<** – Moves cursor to leftmost position of sequence
3. **<** – Moves cursor five characters to the left
4. **Left Arrow** – Moves cursor one character to the left

5. **Position** – Displays current position
6. **Right Arrow** – Moves cursor one character to the right
7. **>** – Moves cursor five characters to the right
8. **>>** – Moves cursor to rightmost position of sequence
9. **Back Space** – Deletes one character to the left

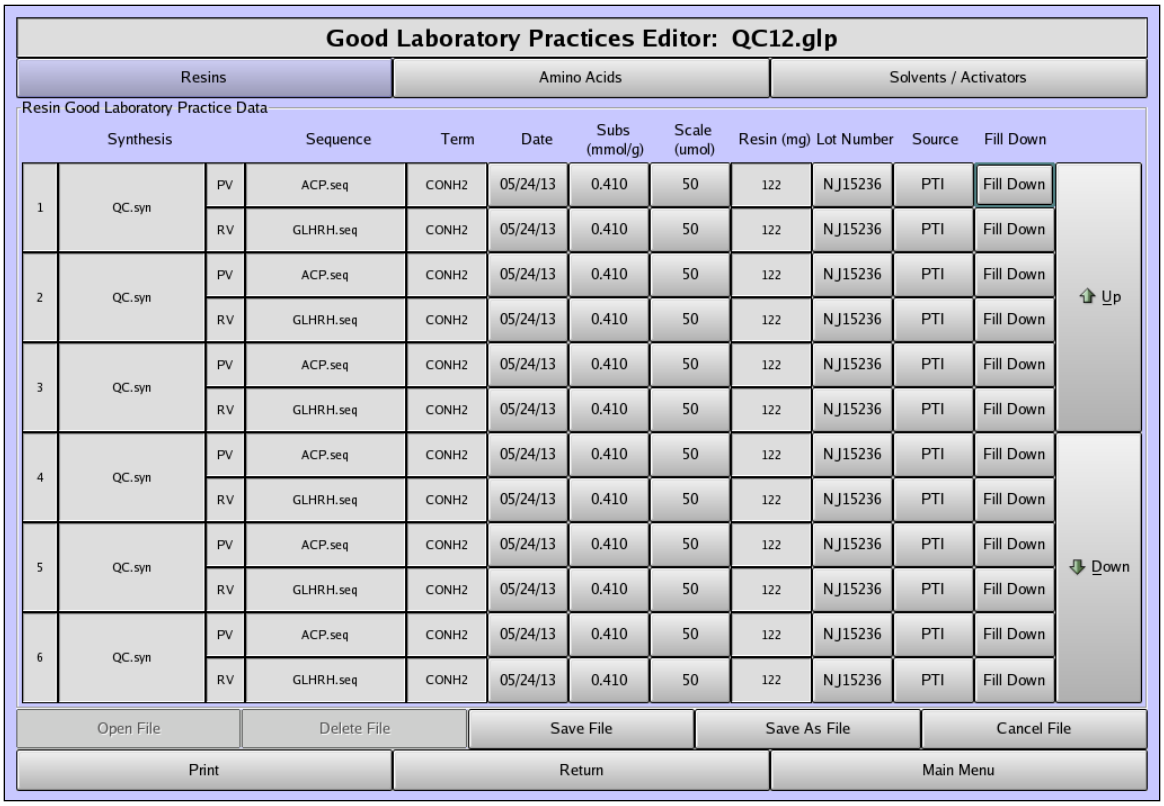
The remaining buttons are as follows:

1. **New File** – Use to create new sequence file
2. **Open File** – Use to open existing sequence file
3. **Delete File** – Use to delete existing sequence file
4. **Save File** – Saves changes to sequence file
5. **Save As File** – Saves sequence file under different name
6. **Cancel File** – Cancels all changes
7. **Help** – Displays the **Sequence Editor Help** screen
8. **Print** – Prints current sequence file
9. **Prog Edit** – Shortcut to **Program Editor** screen
10. **Syn Edit** – Shortcut to **Synthesis Editor** screen
11. **Setup Menu** – Shortcut to **Setup** menu
12. **Return** – Returns to previous screen
13. **Main Menu** – Returns to **Main Menu**

2.7.5 Good Laboratory Practice

The **Good Laboratory Practice Editor** screen allows the user to edit a good laboratory practice (GLP) file created on the **Calculations** screen. To open the **Good Laboratory Practice Editor** screen, select **Setup** from the **Main Menu**, then select **Good Laboratory Practice Editor**.

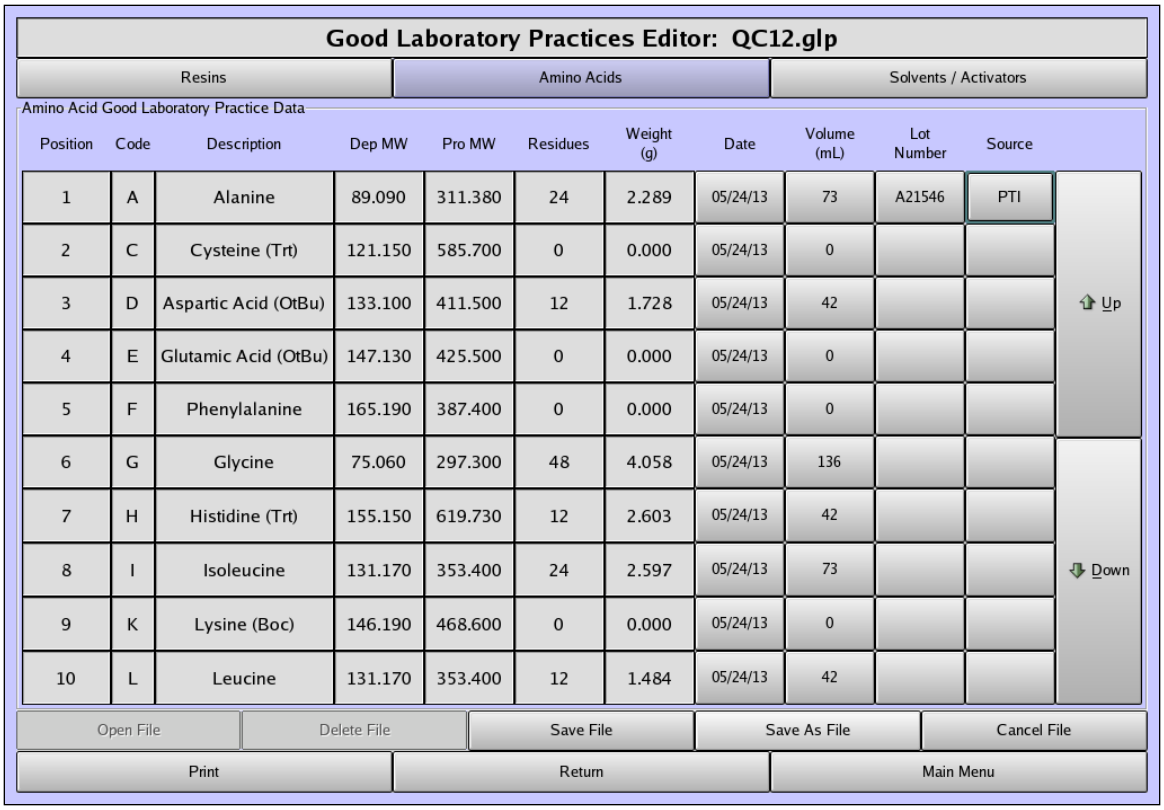
To create a Good Laboratory Practice file, load synthesis files on the **RV Automated Operations** screen, then go to the **Loaded Syntheses** tab on the **Calculations** screen, and select **CreateGLP**.



In the **Resins** section are the following columns:

1. **Position** – RV/PV pair position
2. **Synthesis** – Select a synthesis file
3. **PV/RV** – Indicates PV or RV position
4. **Sequence** – Displays loaded peptide sequence(s)
5. **Term** – Displays the C-terminal functional group (COOH or CONH2)
6. **Date** – Displays date in MM/DD/YY
7. **Subst (mmol/g)** – Input the resin substitution in (mmol/g)
8. **Scale (umol)** – Input the synthesis scale (in micromoles)
9. **Resin (mg)** – The software will calculate the amount of resin (in mg) needed
10. **Lot Number** – Enter lot number
11. **Source** – Enter source name
12. **Fill Down** – Copies the data in the **Date**, **Subst (mmol/g)**, **Scale (umol)**, **Resin (mg)**, **Lot Number** and **Source** columns into all rows below the selected row.

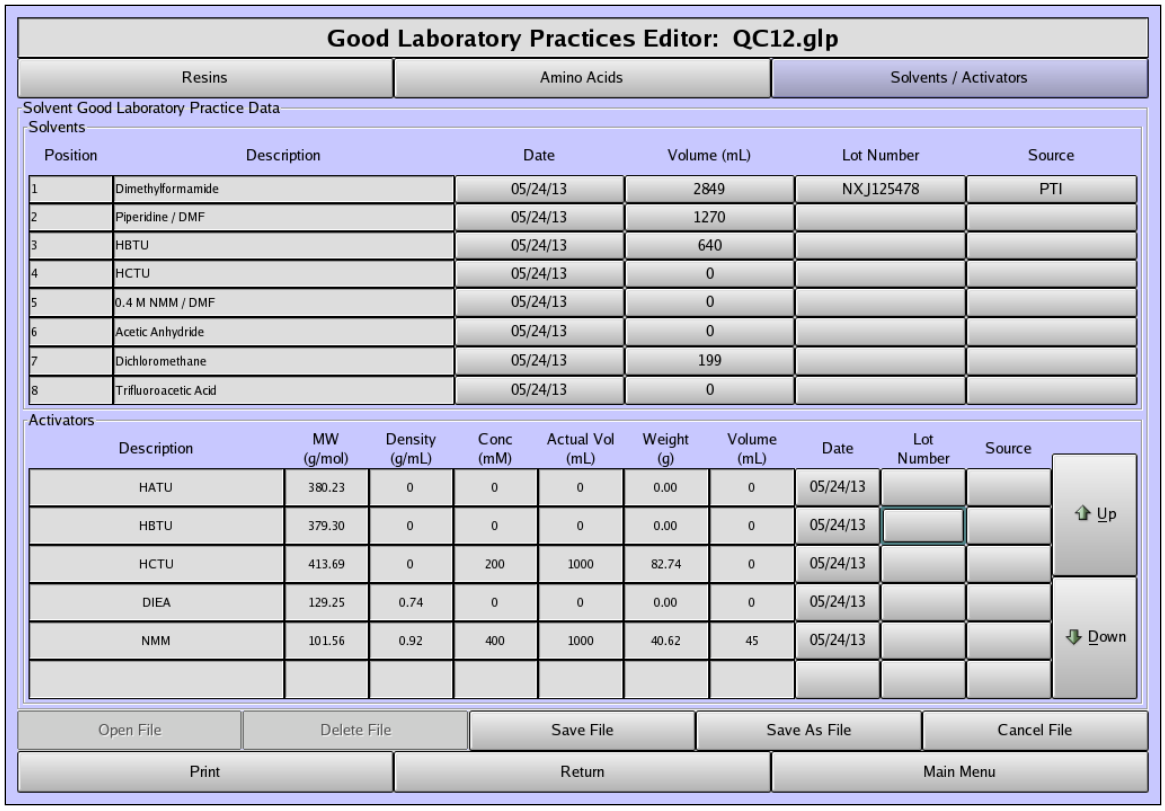
Use the up and down arrows to the right to scroll through the list.



In the **Amino Acids** section are the following columns:

1. **Position** – AA bottle position
2. **Code** – 1-letter code
3. **Description** – Description of amino acid or reagent
4. **Dep MW** – Deprotected molecular weight (g/mol)
5. **Pro MW** – Protected molecular weight (g/mol)
6. **Residues** – Number of times the amino acid is delivered
7. **Weight (g)** – Amount (in g) of dry amino acid needed for the amino acid
8. **Date** – Enter date MM/DD/YY
9. **Volume (mL)** – Volume suggested for the synthesis. This volume includes calculated and prime volumes and may be modified by the user.
10. **Lot Number** – Enter lot number
11. **Source** – Enter source name

Use the up and down arrows to the right to scroll through the list.



In the **Solvents / Activators** section are two sections: **Solvents** and **Activators**.

In the **Solvents** section are the following columns:

1. **Position** – Solvent bottle position
2. **Description** – Description of solvent bottle contents
3. **Date** – Enter date MM/DD/YY
4. **Volume (mL)** – Volume suggested for the synthesis. This volume includes calculated, priming and wash volumes and may be modified by the user.
5. **Lot Number** – Enter lot number
6. **Source** – Enter source name

Use the up and down arrows to the right to scroll through the list.

In the **Activators** section are the following columns:

1. **Description** – Input name of reagent.
2. **MW (g/mol)** – Input molecular weight (in g/mol) of the reagent
3. **Density (g/mL)** – Input density (in g/mL) of reagent if liquid
4. **Conc (mM)** – Input desired concentration (in mM) of reagent in solution
5. **Actual Vol (mL)** – Input desired solution volume (in mL)
6. **Weight (g)** – The software will calculate the weight (in g) of reagent needed to prepare the solution if the reagent is a solid

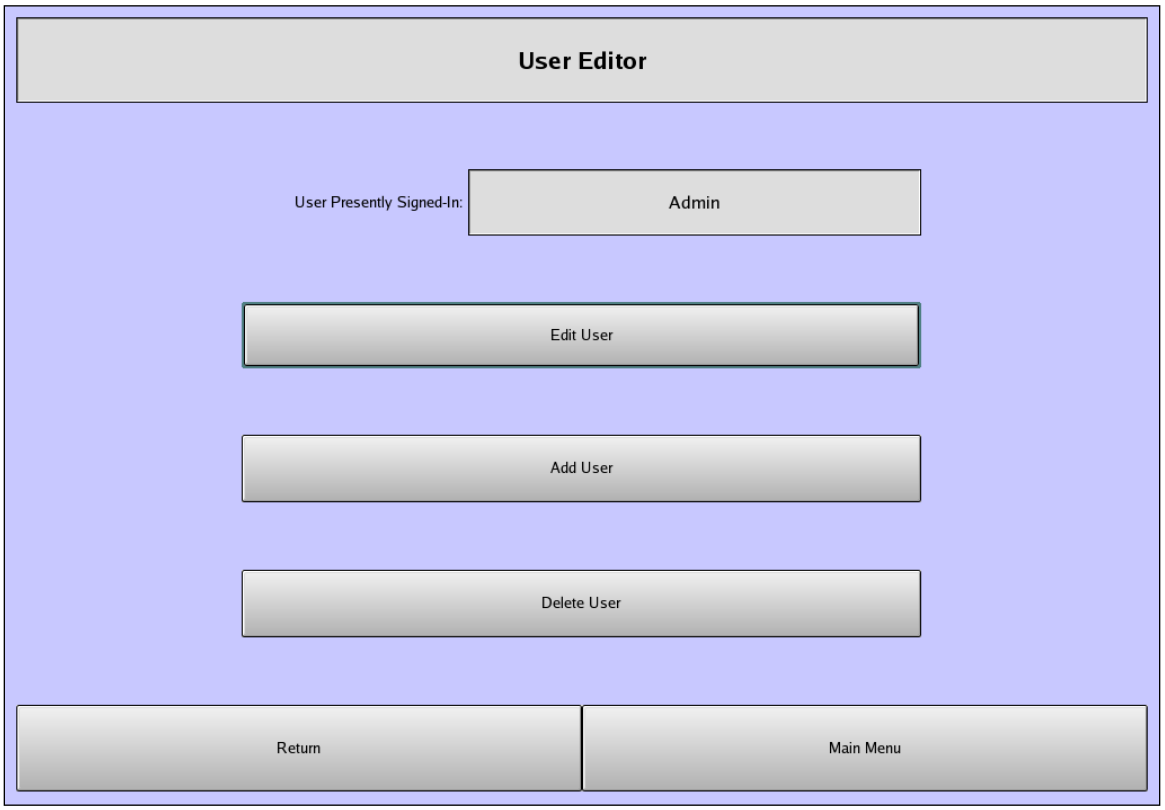
7. **Volume (mL)** – The software will calculate the volume (in mL) of reagent needed to prepare the solution if the reagent is a liquid
8. **Date** – Enter date MM/DD/YY
9. **Lot Number** – Enter lot number
10. **Source** – Enter source name

At the bottom of the screen are the following buttons:

1. **Open File** – Open a GLP file
2. **Delete File** – Delete a GLP file
3. **Save File** – Saves GLP file
4. **Save As File** – Save As GLP file
5. **Cancel File** – Cancel changes to GLP file
6. **Print** – Print GLP data
7. **Return** – Return to previous screen
8. **Main Menu** – Returns to the **Main Menu**

2.7.6 User Editor

The **User Editor** screen allows admin-level users to create, edit and delete users. To open the **User Editor** screen, select **Setup** from the **Main Menu**, then select **User Editor**.



The different user types are as follows:

1. **Administrator** – User may create or edit users (excluding **Service** or **Factory** users), delete report files, view log files, and access the **Diagnostics**, **Machine Status**, and **Settings** screens.
2. **Designer** – User may perform all functions related to designing and running a synthesis, excluding **Administrator** functions.
3. **Runner** – User may perform all **Designer** functions except edit or copy files.
4. **Service** – User may perform all functions except create or edit users other than **Service** users, edit programs or load synthesis conditions
5. **Factory** – User may perform all functions.

At the top of the screen is displayed the “User Presently Signed-In.”

Beneath that are 3 buttons:

1. **Edit User** – Use to edit user attributes including name, password and user type.
2. **Add User** – Use to create a new user
3. **Delete User** – Use to delete a user

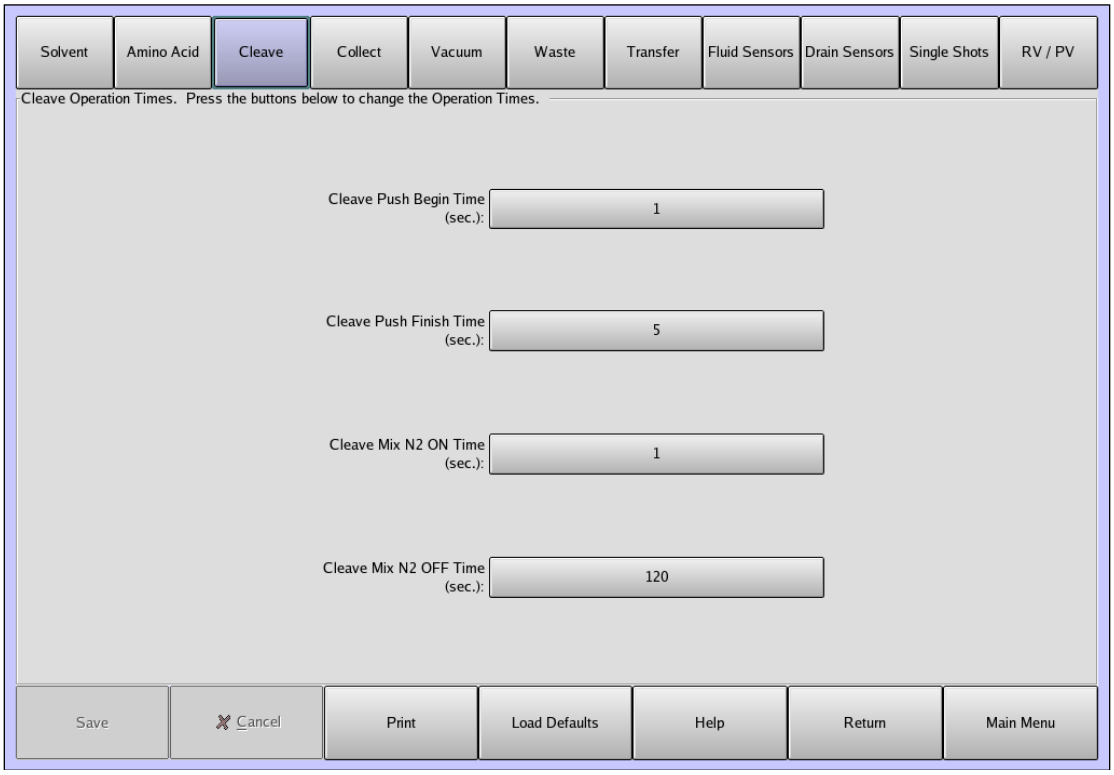
At the bottom of the screen are the following 2 buttons:

1. **Return** – Returns to previous screen
2. **Main Menu** – Returns to **Main Menu**

2.7.7 Operation Times

The **Operation Times** screen displays the operation times for the instrument and allows the user to edit the values. To open the **Operation Times** screen, select **Setup** from the **Main Menu**, then select **Operation Times**. There are 10 tabs at the top of the screen which can be used to select different screens. Admin-level users have access to the **Cleave** and **RV/PV** tabs. Operation times on the other tabs may be accessed using a daily password obtained from the factory. Operation times on other tabs should only be altered with supervision from your PTI service representative.

The **Cleave Operation Times** screen is shown below:



Admin-level users may change the following operation times on the **Cleave Operation Times** screen:

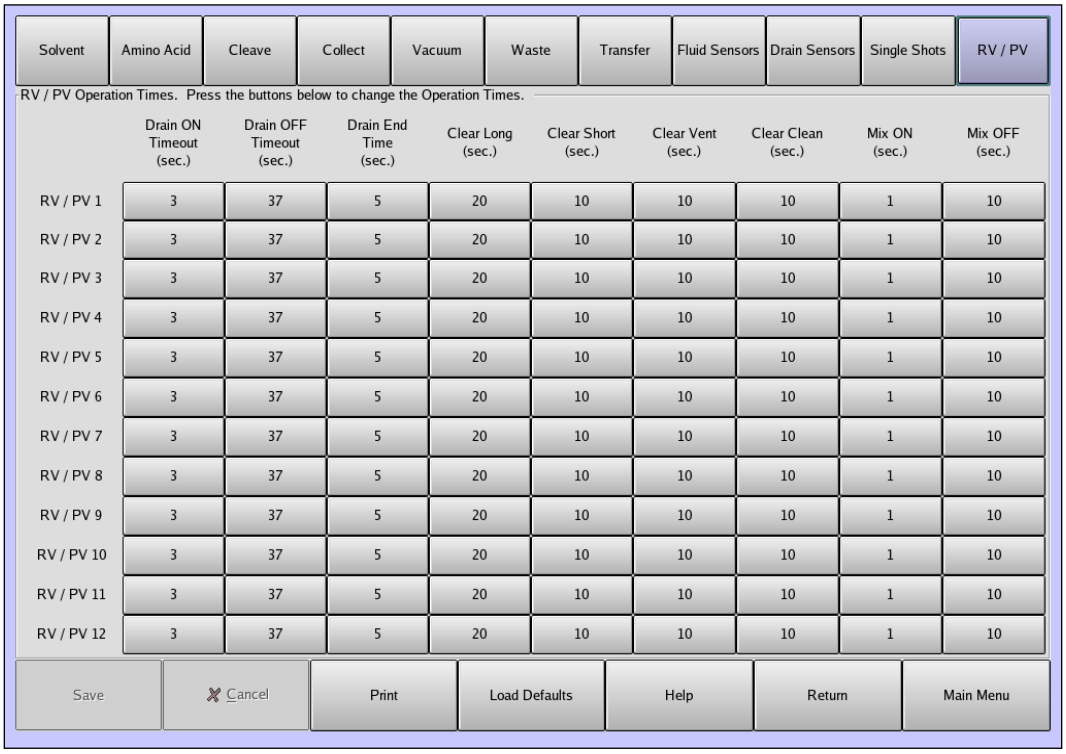
1. **Cleave Mix N2 ON Time (sec.)** – Number of seconds nitrogen turned on for nitrogen burst during Cleave Mix operation (Default: 1 sec.)
2. **Cleave Mix N2 OFF Time (sec.)** – Number of seconds between nitrogen bursts for Cleave Mix operation (Default: 120 sec.)

The **Single Shots Operation Times** screen is shown below:

Admin-level users may change the following operation times on the **Single Shots Operation Times** screen:

1. **Single Shot Delivery from Bottle Time (sec.)** – Number of seconds to transfer fluid from amino acid bottle to reaction vessel during a Single-Shot delivery (Default: 30 sec.)
2. **Single Shot Delivery from Collect Vial Time (sec.)** – Number of seconds to transfer fluid from collect vial to reaction vessel during a Single-Shot delivery (Default: 60 sec.)

The **RV/PV Operation Times** screen is shown below:



Admin-level users may change the following operation times on the **RV/PV Operation Times** screen:

1. **Drain ON Timeout (sec.)** – Maximum number of seconds for drain sensor to see fluid or it will go into error (Default: 3 sec.)
2. **Drain OFF Timeout (sec.)** – Maximum number of seconds for drain sensor to stop seeing fluid after seeing fluid or it will go into error (Default: 37 sec.)
3. **Drain End Time (sec.)** – Number of seconds to continue draining after sensor sees fluid, then sees no fluid (Default: 5 sec.)
4. **Mix ON (sec.)** – Number of seconds nitrogen turned on for nitrogen burst during Mix operation (Default: 1 sec.)
5. **Mix OFF (sec.)** – Number of seconds between nitrogen bursts for Mix operation (Default: 10 sec.)

The remaining buttons are as follows:

1. **Save** – Saves changes to operation times
2. **Cancel** – Cancels changes
3. **Print** – Prints operation times
4. **Load Defaults** – Overwrites all operation times with default values
5. **Help** – Displays the **Operation Times** Help screen
6. **Return** – Return to previous screen

7. **Main Menu** – Returns to **Main Menu**

2.7.8 Settings

The **Settings** screen allows the user to activate special features on the Symphony X. To open the **Settings** screen, select **Setup** from the **Main Menu**, then select **Settings**.

The **Settings** screen is composed of four main sections. In the **Instrument** section are the following two buttons:

1. **Facility** – Allows user to input name of facility
2. **Instrument Name** – Allows user to input name of instrument

The **Collect Mode** section controls the function of the collect vials on the instrument. Select one of two modes:

1. **Cleavage Mode** – Collect vials are used as receptacles for the cleaved peptide. In this mode, DCM must be installed in solvent bottle 7 and TFA must be installed in solvent bottle 8.
2. **Single Shot Amino Acid Mode** – Collect vials are used as additional amino acid positions. All collect vial positions can only perform Single-Shot™ deliveries, not aliquot deliveries. In this mode, the primary solvent must be installed in solvent bottle 7, and all CV positions must have a collect vial installed.

The **Error Recovery** section controls the **Safe Response** feature. The **Safe Response** feature will perform a drain and rinse the reaction vessel using a top wash in the event of a non-system error during an automated synthesis. To set this feature, use the following four buttons:

1. **Enable** – Turn the feature on or off by selecting **Yes** or **No**, respectively
2. **Solvent** – Choose solvent bottle
3. **Volume** – Choose rinse volume (in microliters)
4. **Reps** – Choose the number of times to perform the rinse (1-9)
5. **Dry Time** – Input the number of seconds to dry

The **E-mail Notification** section allows the user to send an email to the indicated email address. To set this feature, use the following three buttons:

1. **On Error** – Select **Yes** to send an email when an error occurs. Select **No** to disable.
2. **On Cycle** – Select **Yes** to send an email at the beginning of each cycle. Select **No** to disable.
3. **On Done** - Select **Yes** to send an email at the end of the synthesis. Select **No** to disable.
4. **Test** - Select **Send Email** to send a test email to the specified address.
5. **Address** – Input email address

NOTE The user can also send an email in the middle of a synthesis by inserting the **E-mail Notification** action into a synthesis program (see Section 2.7.3).

The buttons on the bottom of the screen are:

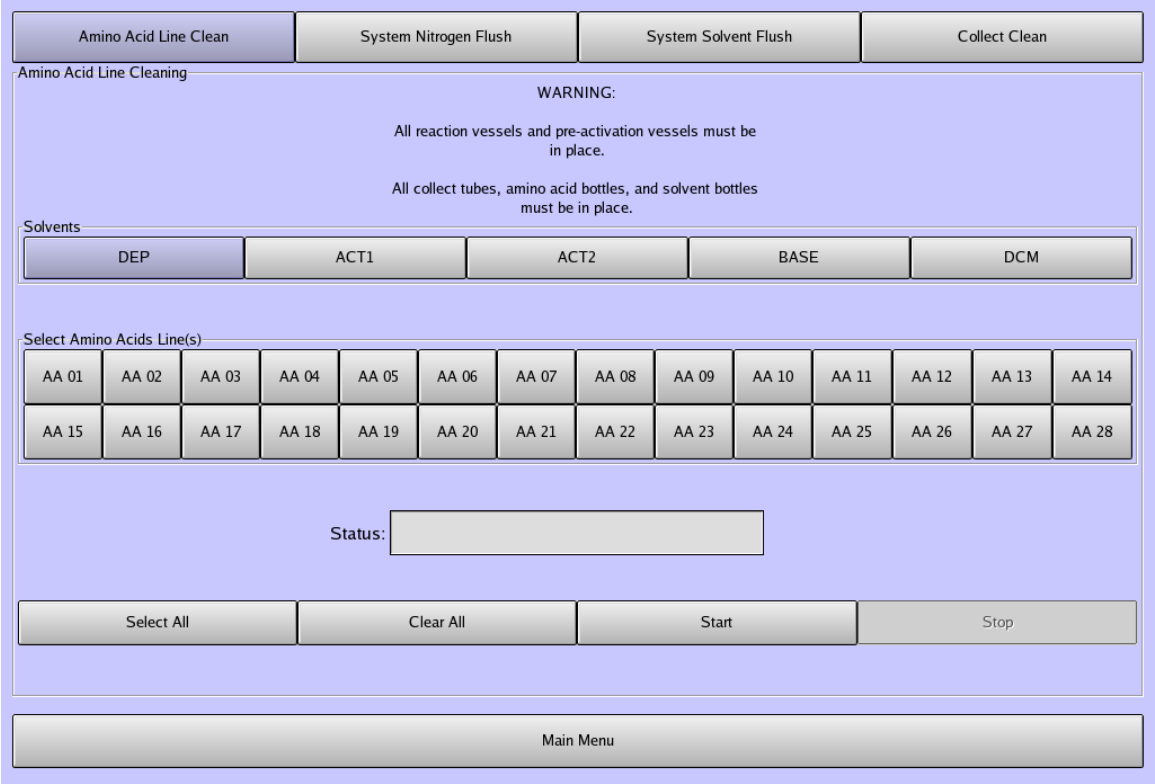
1. **Cancel** – Cancels changes
2. **Save** – Saves changes
3. **Return** – Return to previous screen. Changes must be saved or cancelled before **Return** button becomes active

2.8 Cleaning

Select **Cleaning** from the **Main Menu** to access the **Amino Acid Line Clean**, **System Nitrogen Flush**, **System Solvent Flush** and **Collect Clean** screens. The function of each screen is detailed in the sections below.

2.8.1 Amino Acid Line Clean

The **Amino Acid Line Clean** screen allows you to clean the amino acid bottle lines. To open the **Amino Acid Line Clean** screen, select **Cleaning** from the **Main Menu**, then select the **Amino Acid Line Clean** tab.



In the **Solvents** section, select a solvent to use for cleaning.

In the **Select Amino Acid Line(s)** section, select amino acid position(s) for cleaning.

The **Status** section displays the status of the action.

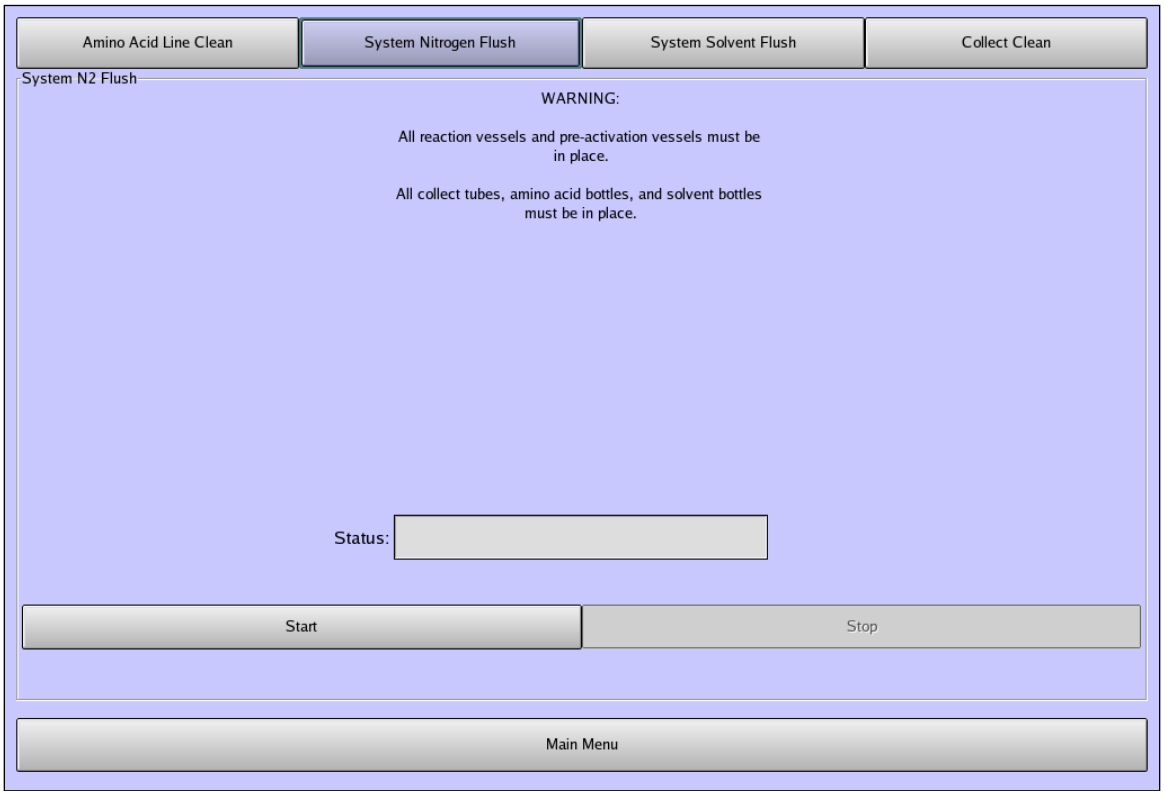
At the bottom of the screen are 4 buttons:

1. **Select All** – Use to select all the amino acid bottle positions for cleaning
2. **Clear All** – Use to deselect all the amino acid bottle positions for cleaning
3. **Start** – Use to start cleaning the selected positions
4. **Stop** – Use to stop the cleaning operation

Select the **Main Menu** button to return to the **Main Menu**.

2.8.2 System Nitrogen Flush

The **System Nitrogen Flush** screen flushes the entire system with nitrogen. To open the **System Nitrogen Flush** screen, select **Cleaning** from the **Main Menu**, then select the **System Nitrogen Flush** tab.



The **Status** section displays the status of the action.

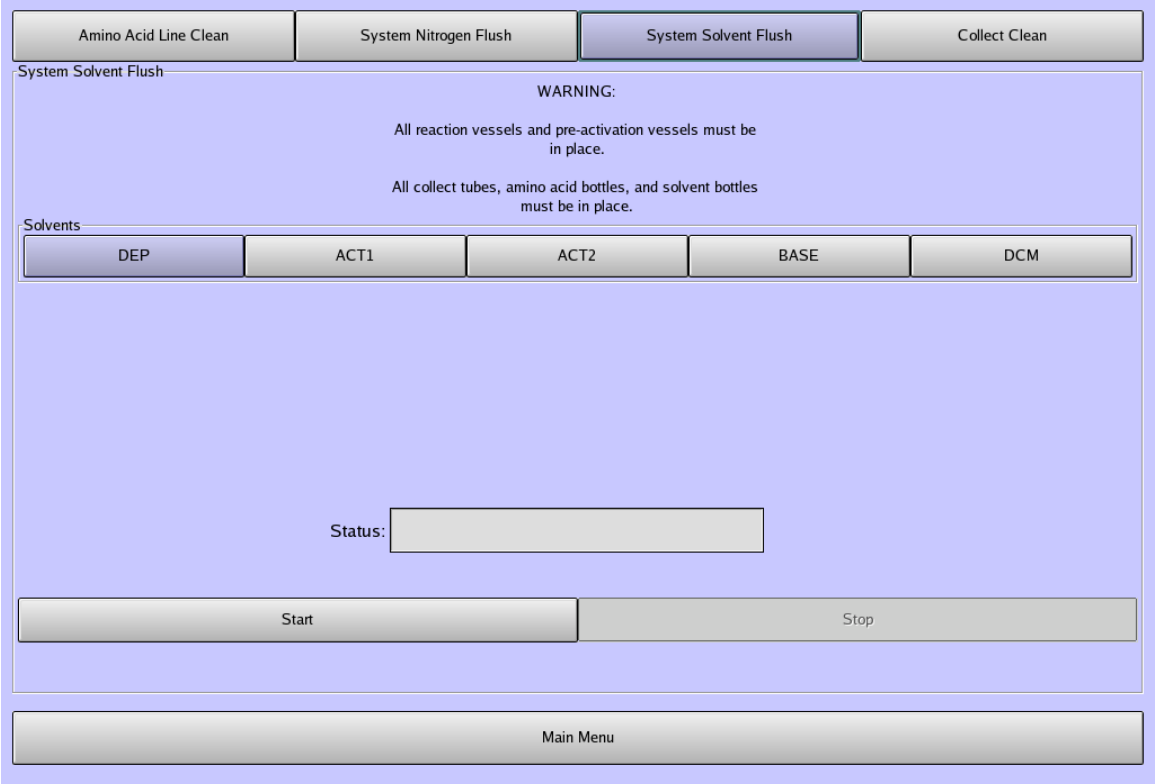
At the bottom of the screen are 2 buttons:

1. **Start** – Use to start the nitrogen flush
2. **Stop** – Use to stop the nitrogen flush

Select the **Main Menu** button to return to the **Main Menu**.

2.8.3 System Solvent Flush

The **System Solvent Flush** screen flushes the entire system with solvent. To open the **System Solvent Flush** screen, select **Cleaning** from the **Main Menu**, then select the **System Solvent Flush** tab.



In the **Solvents** section, select a solvent to use for cleaning.

The **Status** section displays the status of the action.

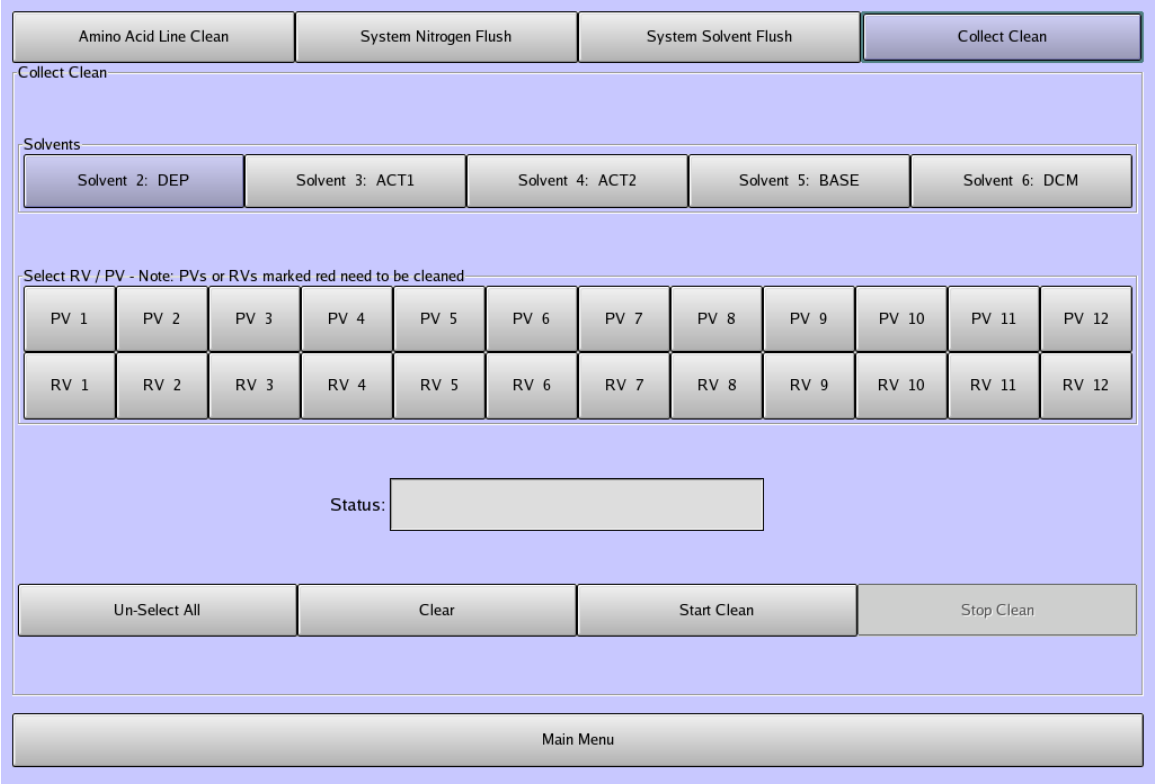
At the bottom of the screen are 2 buttons:

1. **Start** – Use to start the solvent flush
2. **Stop** – Use to stop the solvent flush

Select the **Main Menu** button to return to the **Main Menu**.

2.8.4 Collect Clean

The **Collect Clean** screen allows you to clean the reaction or preactivation vessel and collect vial lines following a collect operation. To open the **Collect Clean** screen, select **Cleaning** from the **Main Menu**, then select the **Collect Clean** tab.



In the **Solvents** section, select a solvent to use for cleaning.

In the **Select RV/PV** section, select an RV or PV position(s) for cleaning. Only one vessel (PV or RV) may be selected per position. RV or PV positions will turn red after a cleavage program was run in that position. When red, a **Collect Clean** operation is required before any operation is allowed in that position. The required **Collect Clean** may be cleared by using the **Clear** button as described below.

The **Status** section displays the status of the action.

At the bottom of the screen are 4 buttons:

1. **Un-Select All** – Use to deselect all positions for cleaning
2. **Clear** – Use to clear a required Collect Clean operation on the selected positions
3. **Start Clean** – Use to start cleaning the selected positions
4. **Stop** – Use to stop the cleaning operation

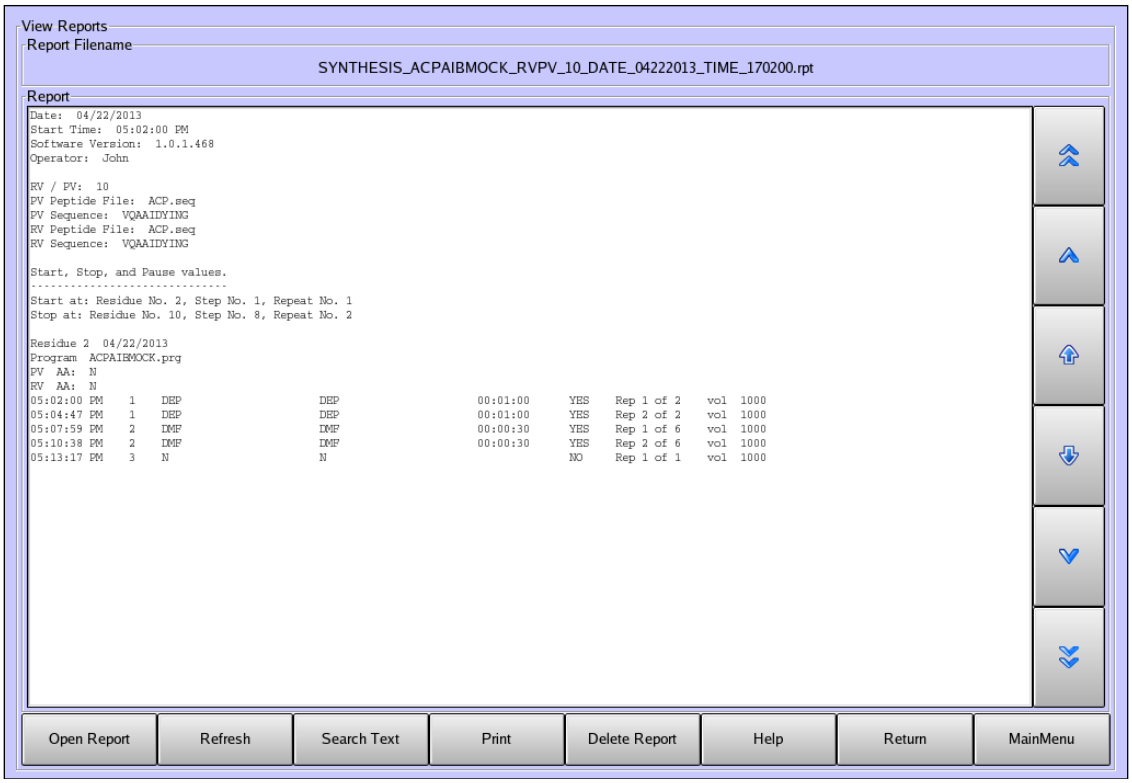
Select the **Main Menu** button to return to the **Main Menu**.

2.9 Reports

The **Reports** menu allows you to access the **View Reports** and **View Log Files** screens. The function of each screen is detailed in the sections below.

2.9.1 View Reports

The **View Reports** screen allows you to print synthesis reports. To open the **View Reports** screen, select **Reports** from the **Main Menu**, then select **View Reports**.



The report filename is listed at the top of the screen, while the content is displayed in the white box. Use the arrow keys to the right to navigate the content.

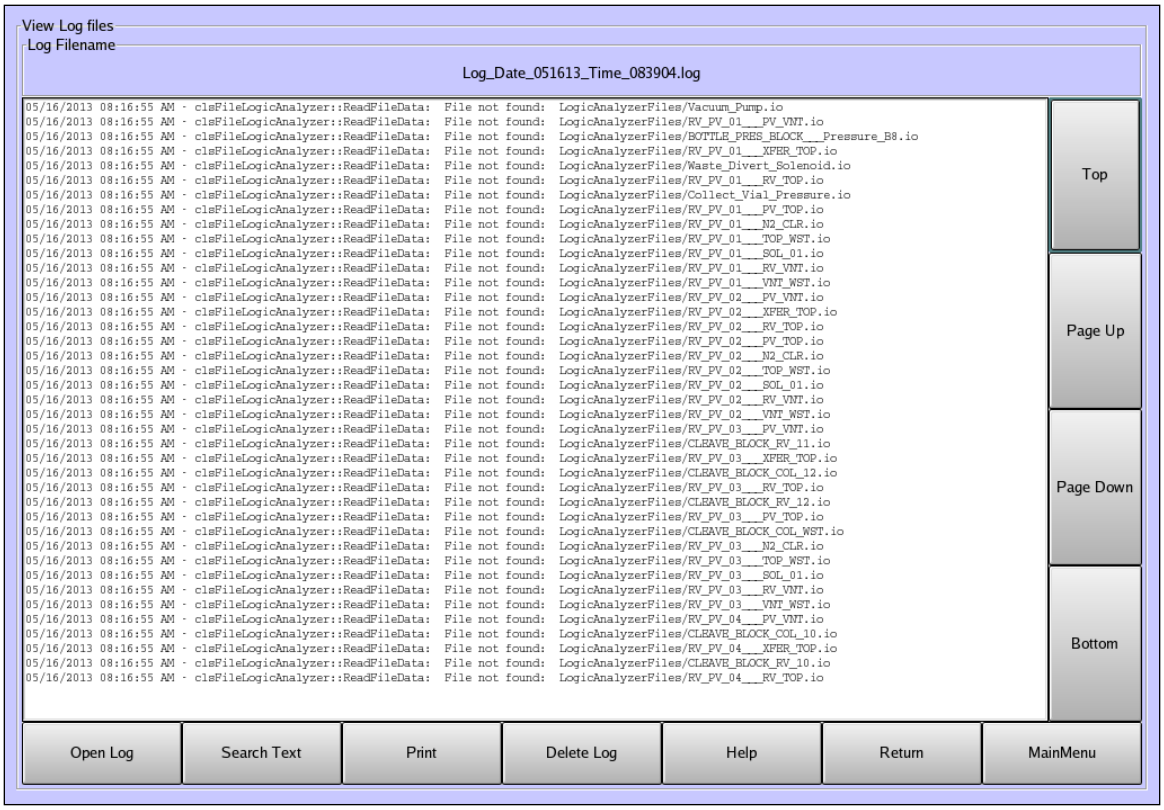
The buttons at the bottom of the screen are as follows:

1. **Open Report** – Select a report to display
2. **Refresh** – Updates report content if synthesis is currently running
3. **Search Text** – Allows user to search report content

4. **Print** – Prints current report
5. **Delete Report** – Deletes selected report
6. **Help** – Displays the **View Reports Help** screen
7. **Return** – Returns to previous screen
8. **Main Menu** – Returns to **Main Menu**

2.9.2 View Log Files

The **View Log Files** screen allows you to print log files. To open the **View Log Files** screen, select **Reports** from the **Main Menu**, then select **View Log Files**.



The log file name is listed at the top of the screen, while the content is displayed in the white box. Use the arrow keys to the right to navigate the content.

The buttons at the bottom of the screen are as follows:

1. **Open Log** – Select a log file to display
2. **Search Text** – Allows user to search log file content
3. **Print** – Prints current log file
4. **Delete Log** – Deletes selected log file
5. **Help** – Displays the **View Log Files Help** screen
6. **Return** – Returns to previous screen
7. **Main Menu** – Returns to **Main Menu**

2.10 Copy Files

The **Copy Files** screen is used to copy synthesis, sequence, program, amino acid, solvent, report, pdf and log files between drives on the Symphony X. First it is important to define the different file types:

1. **Synthesis File** – Created using the **Synthesis Editor** screen. Contains selected synthesis file and associated sequence, program, amino acid and solvent files used in the synthesis. May be saved or loaded from various drives using the **Copy Files** screen.
2. **Sequence File** – Created and deleted using the **Sequence Editor** screen. Contains selected sequence file and associated amino acid file. May be copied between drives on the **Copy Files** screen.
3. **Program File** – Created and deleted using the **Program Editor** screen. Contains selected program file and associated amino acid and solvent files. May be copied between drives on the **Copy Files** screen.
4. **Amino Acid File** – Created and deleted using the **Amino Acid Editor** screen.
5. **Solvent File** – Created and deleted using the **Solvent Editor** screen.
6. **Report File** – Created when a synthesis is run. May be copied between drives on the **Copy Files** screen.
7. **PDF File** – You can create a pdf of any file type by selecting **Print** on the relevant editor, report or log file screen, then selecting **Create a PDF document**. The resulting pdf files can then be copied between drives on the **Copy Files** screen.
8. **Log File** – New file is created at 12:00am daily by the Symphony® X. Records every event (action, user intervention or error) that occurs on the instrument. May be copied between drives on the **Copy Files** screen.

To open the **Copy Files** screen, select **Copy Files** from the **Main Menu**.

There are four available drives:

1. **Local** – Local drive on the Symphony® X internal computer
2. **Network** – Network drive
3. **USB 1** – USB drive in USB 1 port on front of instrument
4. **USB 2** – USB drive in USB 2 port on front of instrument

To copy a file between drives, make a selection from each of the following buttons:

1. **File(s) Source** – Select file source drive from list of active available drives
2. **File(s) Destination** – Select file destination drive from list of active available drives
3. **File Type** – Select file type to copy
4. **File(s) to Copy** – Select one or more files to copy
5. **Copy** - Press the **Copy** button to copy file from source drive to destination drive.

NOTE The **User Workstation Utility** option allows the user to create amino acid, solvent, program, sequence, and synthesis files for the Symphony® X on an external computer and also print log and report files from the Symphony® X. These files may be saved on a USB or network drive and transferred to and from the Symphony® X via one of the two USB ports on the right front panel (Section 1.1.4).

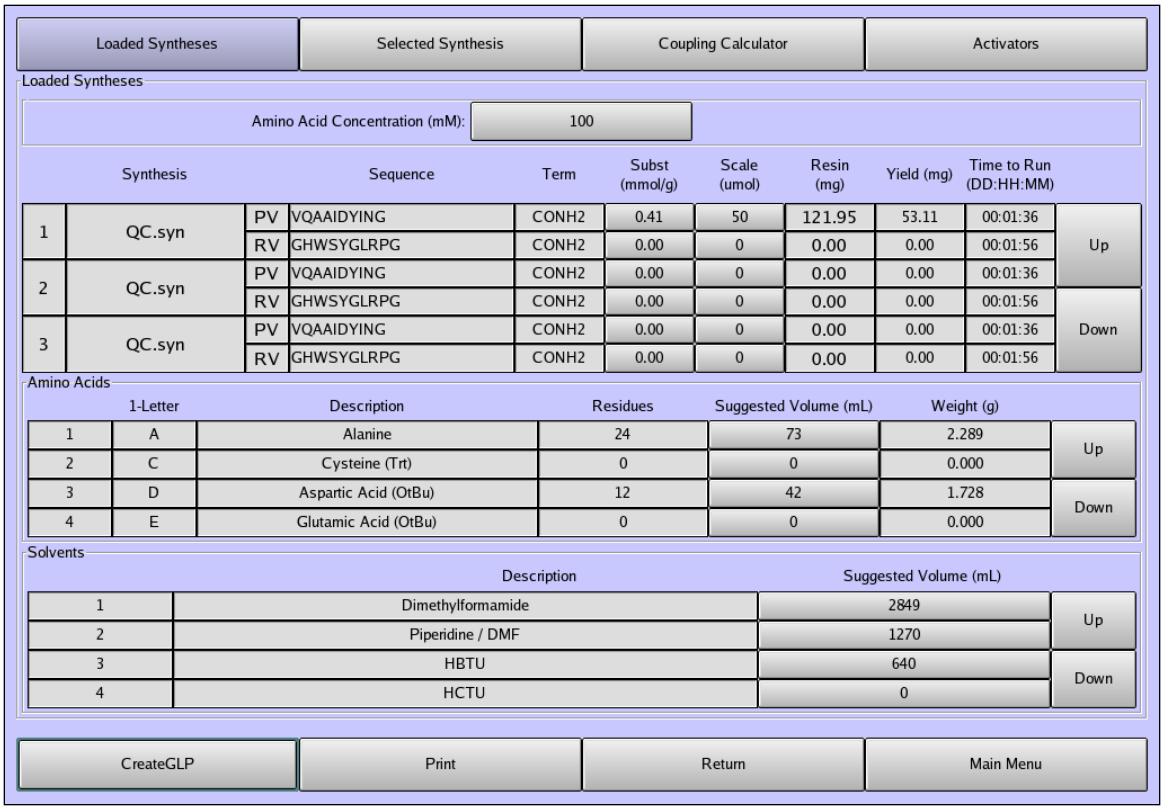
Select the **Main Menu** button to return to the **Main Menu**.

2.11 Tools

The **Tools** menu allows the user to access the **Calculations**, **Machine Status**, and **Diagnostics** screens. The function of each screen is detailed below.

2.11.1 Calculations

The **Calculations** screens allow you to calculate amino acid, activator, and base volumes and concentrations for different scales, ratios and excesses, and displays the volumes and amounts of chemicals required to prepare each amino acid and solvent bottle for a selected synthesis or all loaded syntheses. To open the **Calculations** screens, select **Tools** from the **Main Menu**, then select **Calculations**.



Loaded Syntheses | Selected Synthesis | Coupling Calculator | Activators

Loaded Syntheses

Amino Acid Concentration (mM): 100

	Synthesis		Sequence	Term	Subst (mmol/g)	Scale (umol)	Resin (mg)	Yield (mg)	Time to Run (DD:HH:MM)	
1	QC.syn	PV	VQAAIDYING	CONH2	0.41	50	121.95	53.11	00:01:36	Up
		RV	GHWSYGLRPG	CONH2	0.00	0	0.00	0.00	00:01:56	
2	QC.syn	PV	VQAAIDYING	CONH2	0.00	0	0.00	0.00	00:01:36	
		RV	GHWSYGLRPG	CONH2	0.00	0	0.00	0.00	00:01:56	
3	QC.syn	PV	VQAAIDYING	CONH2	0.00	0	0.00	0.00	00:01:36	Down
		RV	GHWSYGLRPG	CONH2	0.00	0	0.00	0.00	00:01:56	

Amino Acids

	1-Letter	Description	Residues	Suggested Volume (mL)	Weight (g)	
1	A	Alanine	24	73	2.289	Up
2	C	Cysteine (Trt)	0	0	0.000	
3	D	Aspartic Acid (OtBu)	12	42	1.728	Down
4	E	Glutamic Acid (OtBu)	0	0	0.000	

Solvents

	Description	Suggested Volume (mL)	
1	Dimethylformamide	2849	Up
2	Piperidine / DMF	1270	
3	HBTU	640	Down
4	HCTU	0	

CreateGLP | Print | Return | Main Menu

The **Loaded Syntheses** section calculates reagent amounts needed to run all of the syntheses loaded on the **RV Automated Operations** screen. Input the **Amino Acid Concentration (mM)** at the top of the screen. Beneath that are the following columns:

1. **Position** – RV/PV pair position
2. **Synthesis** – Displays loaded synthesis file name
3. **PV/RV** – Indicates PV or RV position
4. **Sequence** – Displays loaded peptide sequence(s)
5. **Term** – Displays the C-terminal functional group (COOH or CONH2)
6. **Subst (mmol/g)** – Input the resin substitution in (mmol/g)
7. **Scale (umol)** – Input the synthesis scale (in micromoles)
8. **Resin (mg)** – The software will calculate the amount of resin (in mg) needed
9. **Yield (mg)** – The software will calculate the theoretical peptide yield (in mg)
10. **Time to Run** – Estimated time for each reaction vessel to complete its synthesis displayed in **DD:HH:MM**.

Use the up and down arrows to the right to scroll through the list.

In the **Amino Acids** section are the following columns:

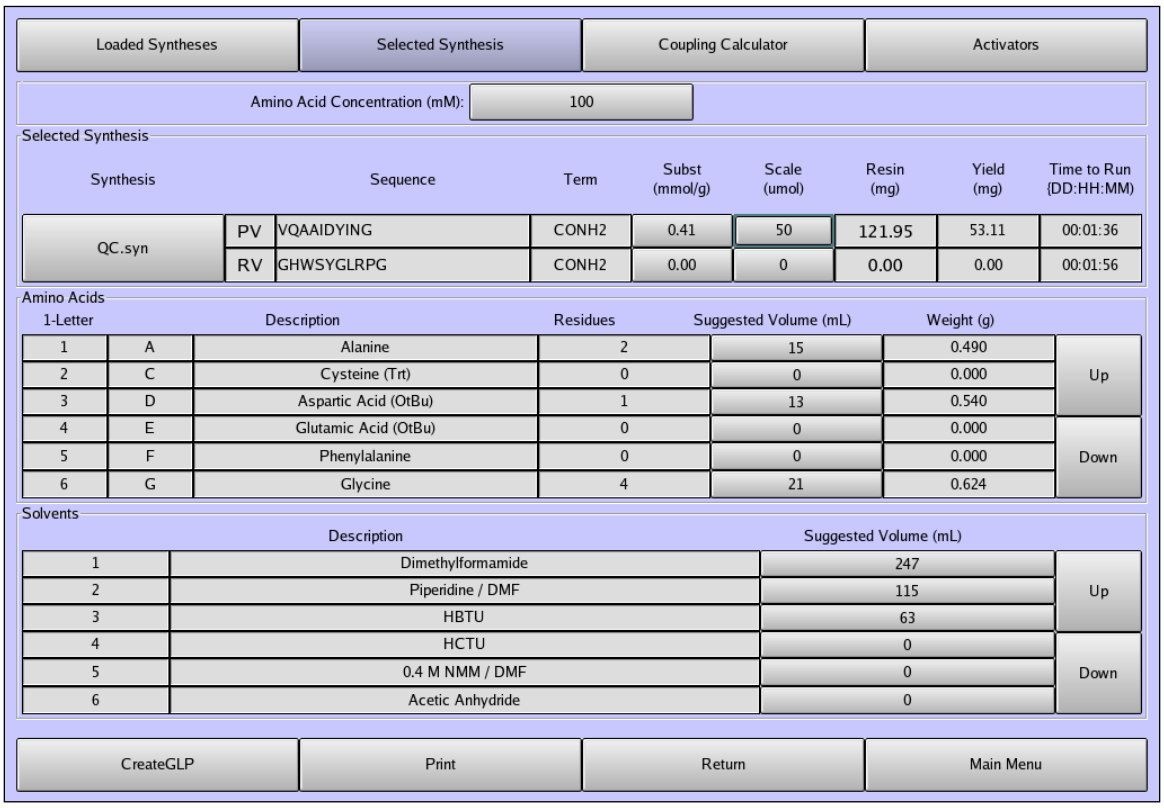
1. **Position** – AA Bottle position
2. **1-Letter** – 1-letter code
3. **Description** – Description of amino acid or reagent
4. **Residues** – Number of times the amino acid is delivered
5. **Suggested Vol (mL)** – Volume suggested for the synthesis. This volume includes calculated and prime volumes and may be modified by the user.
6. **Weight (g)** – Amount (in g) of dry amino acid needed for the amino acid solution based on the **Concentration (mM)** and **Vol (mL)** values.

Use the up and down arrows to the right to scroll through the list.

In the **Solvents** section are the following columns:

1. **Position** – Solvent bottle position
2. **Description** – Description of solvent bottle contents
3. **Suggested Vol (mL)** – Volume suggested for the synthesis. This volume includes calculated, priming and wash volumes and may be modified by the user.

Use the up and down arrows to the right to scroll through the list.



The **Selected Syntheses** section calculates reagent amounts needed to run the selected synthesis. Input the **Amino Acid Concentration (mM)** at the top of the screen. In the **Selected Synthesis** section are the following columns:

1. **Synthesis** – Select a synthesis file
2. **PV/RV** – Indicates PV or RV position
3. **Sequence** – Displays loaded peptide sequence(s)
4. **Term** – Displays the C-terminal functional group (COOH or CONH2)
5. **Subst (mmol/g)** – Input the resin substitution in (mmol/g)
6. **Scale (umol)** – Input the synthesis scale (in micromoles)
7. **Resin (mg)** – The software will calculate the amount of resin (in mg) needed
8. **Yield (mg)** – The software will calculate the theoretical peptide yield (in mg)
9. **Time to Run** – Estimated time for each reaction vessel to complete its synthesis displayed in **DD:HH:MM**.

In the **Amino Acids** section are the following columns:

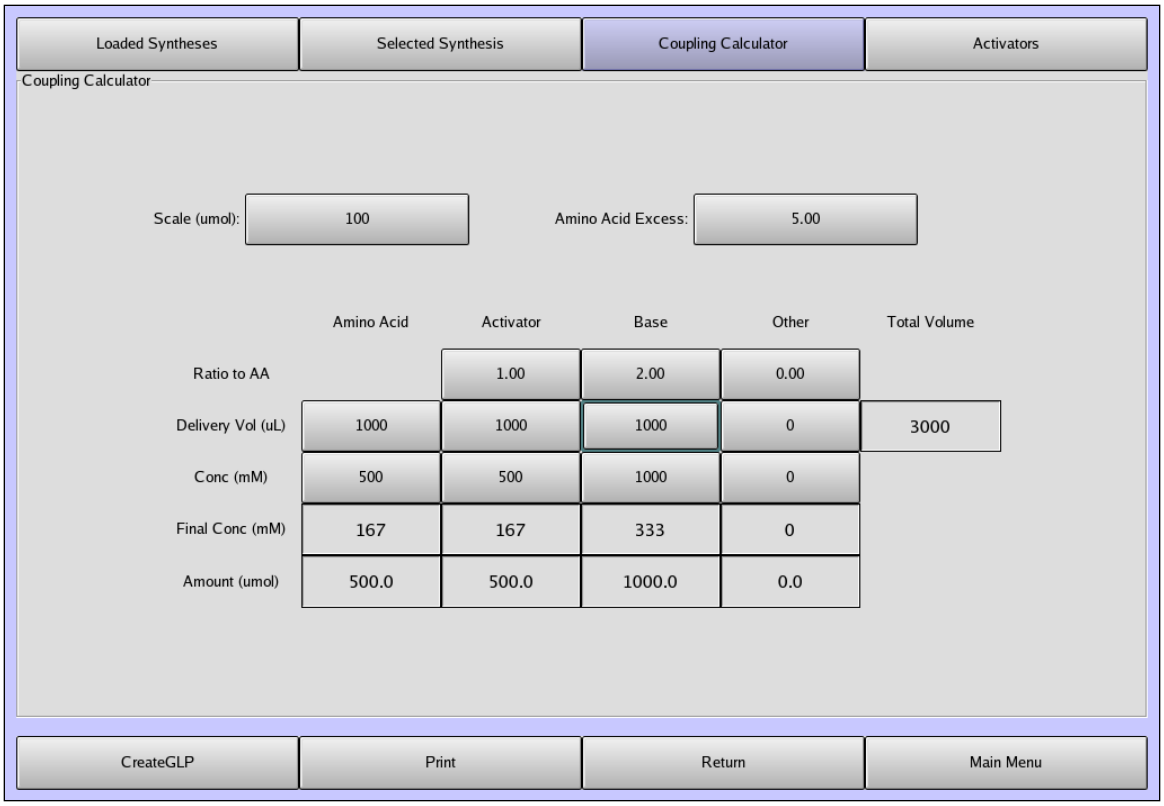
1. **Position** – RV/PV pair position
2. **1-Letter** – 1-letter code
3. **Description** – Description of amino acid or reagent
4. **Residues** – Number of times the amino acid is delivered
5. **Suggested Vol (mL)** – Volume suggested for the synthesis. This volume includes calculated and prime volumes and may be modified by the user.
6. **Weight (g)** – Amount (in g) of dry amino acid needed for the amino acid solution based on the **Concentration (mM)** and **Vol (mL)** values.

Use the up and down arrows to the right to scroll through the list.

In the **Solvents** section are the following columns:

1. **Position** – Solvent bottle position
2. **Description** – Description of solvent bottle contents
3. **Suggested Vol (mL)** – Volume suggested for the synthesis. This volume includes calculated, priming and wash volumes and may be modified by the user.

Use the up and down arrows to the right to scroll through the list.

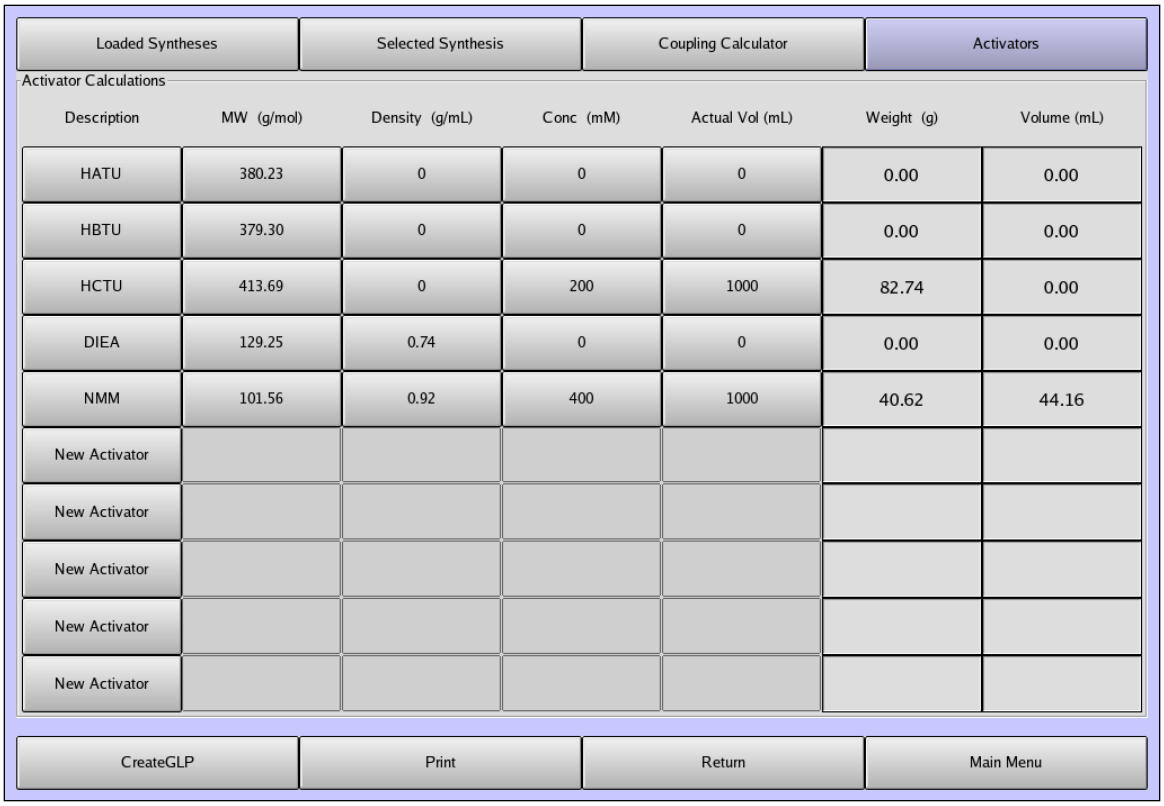


In the **Coupling Calculator** section:

1. **Scale (umol)** – Input synthesis scale (in micromoles)
2. **Amino Acid Excess** – Input the multiple excess of amino acid compared to the resin. Standard is 5x excess.

There are 4 columns below: **Amino Acid**, **Activator**, **Base**, **Other**. In each column, are the following rows:

1. **Ratio to AA** – Input ratio to amino acid (0-100).
2. **Deliver Vol (uL)** – Input delivery volume (in microliters) to calculate the concentration
3. **Conc (mM)** – Input concentration (in mM) to calculate the volume
4. **Final Conc (mM)** – Displays the final concentration of that component in the coupling solution
5. **Amount (umol)** – Displays the amount of that component in the coupling solution.



In the **Activators** section, calculate the amount of activator, base or additives needed for a solution. Input the following:

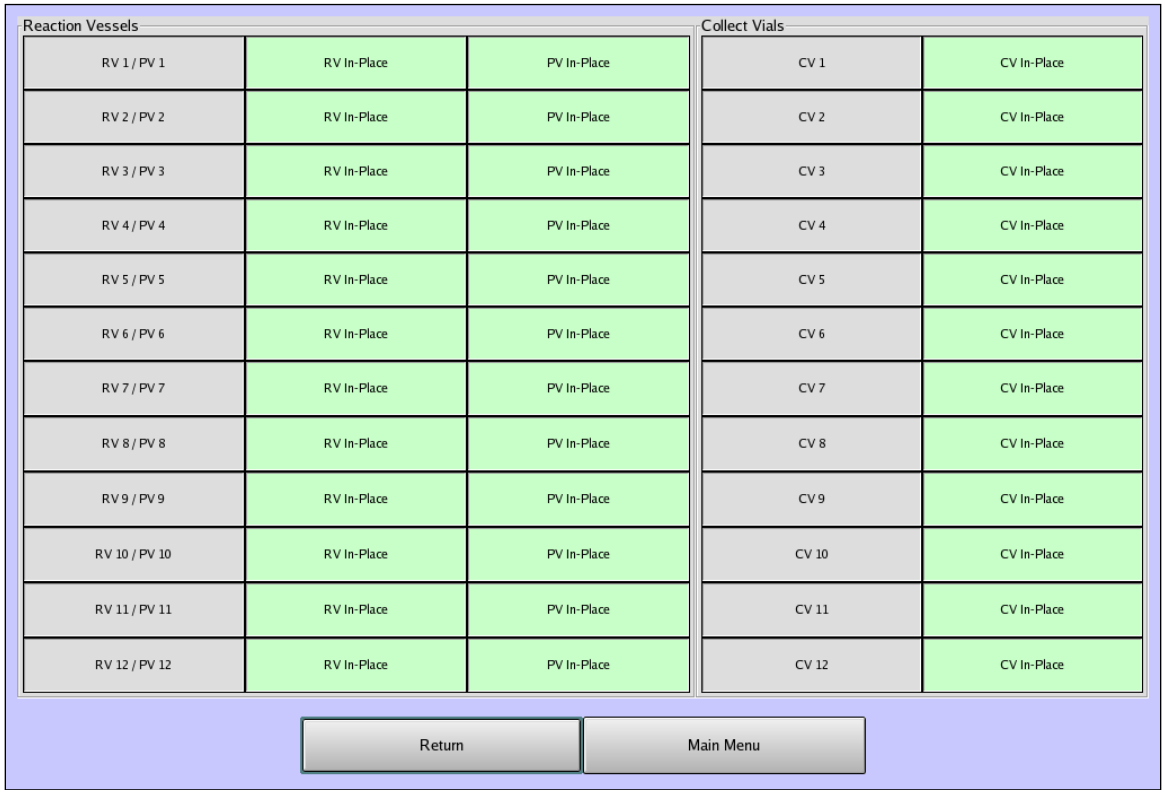
1. **Description** – Input name of reagent.
2. **MW (g/mol)** – Input molecular weight (in g/mol) of the reagent
3. **Density (g/mL)** – Input density (in g/mL) of reagent if liquid
4. **Conc (mM)** – Input desired concentration (in mM) of reagent in solution
5. **Actual Vol (mL)** – Input desired solution volume (in mL)
6. **Weight (g)** – The software will calculate the weight (in g) of reagent needed to prepare the solution if the reagent is a solid
7. **Volume (mL)** – The software will calculate the volume (in mL) of reagent needed to prepare the solution if the reagent is a liquid

At the bottom of the screen are the following buttons:

1. **CreateGLP** – Create a GLP file based on the contents of the **Loaded Syntheses** screen. GLP file can be opened and edited on the **Good Laboratory Practice Editor** screen (See Section 2.7.5)
2. **Print** – Prints values from peptide calculations screen
3. **Return** – Return to previous screen
4. **Main Menu** – Returns to **Main Menu** screen

2.11.2 Machine Status

The **Machine Status** screen shows the current status of the RV and PV positions on the instrument. Green indicates “In-Place.” Grey indicates “Not In-Place.”

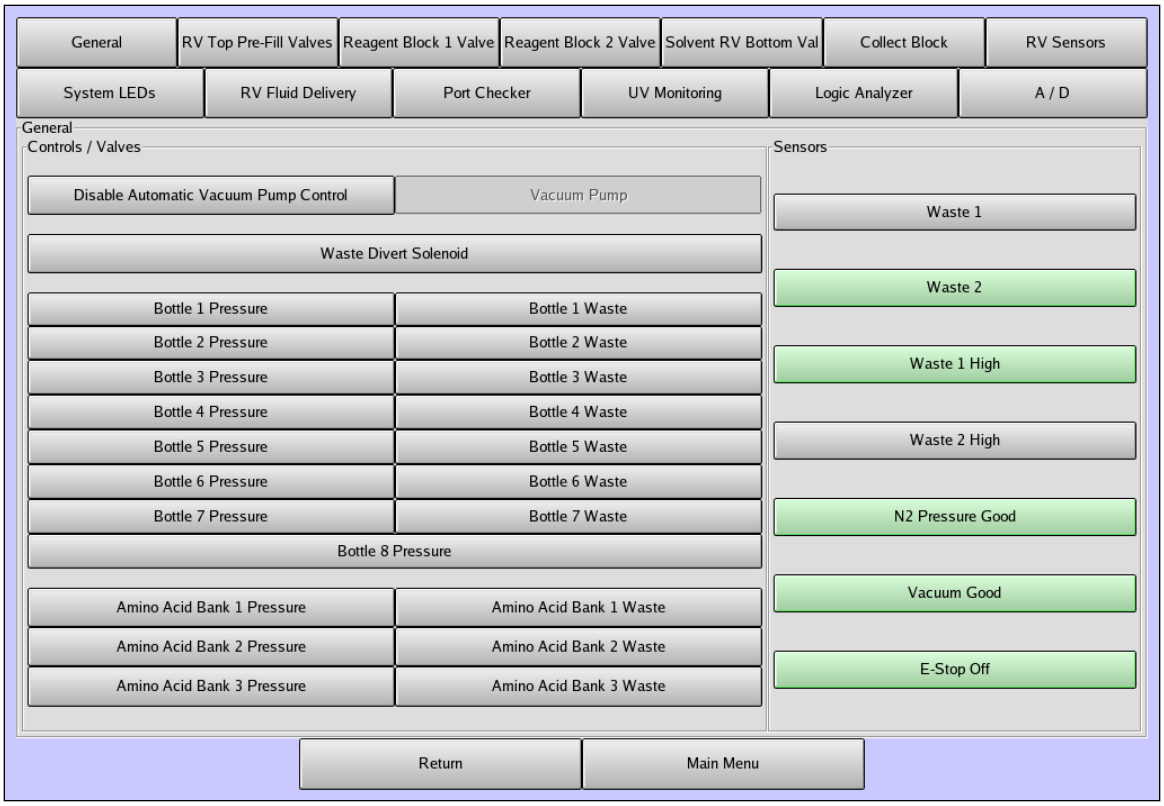


At the bottom of the screen are the following buttons:

1. **Return** – Return to previous screen
2. **Main Menu** – Returns to **Main Menu** screen

2.11.3 Diagnostics

The **Diagnostics** screen displays each valve and sensor and allows each valve to be manually turned on and off. To open the **Diagnostics** screen, select **Tools** from the **Main Menu**, then select **Diagnostics**.



CAUTION Opening some valves may cause leakage and cross-contamination within the instrument. The **Diagnostics** screen should only be operated under supervision from the factory.

The buttons at the bottom of the screen are as follows:

1. **Return** – Return to previous screen
2. **Main Menu** – Return to **Main Menu** screen

Chapter 3: Running A Synthesis

3.1 Basic Synthesis Checklist

Steps for running a synthesis without cleavage (**NC**) or with cleavage (**C**) are shown in the table below.

√	NC	C	Startup & Instrument Check
	●	●	Turn on the *Symphony X* ® Peptide Synthesizer (Section 1.1.5)
	●	●	Check nitrogen supply and gauges (Sections 1.1.3 & 1.1.12)
	●	●	Check vacuum gauge (Section 1.1.4)
	●	●	Check waste level (Sections 1.1.10 & 1.2.6)
	●	●	Change RV/PV upper and lower o-rings, if necessary (every 2 weeks) (Section 1.2.2)
	●	●	Change bottle filters, if necessary (i.e. change of reagent) (Sections 4.3)
√	NC	C	**Software Setup**
	●	●	Create and/or Load amino acid file (Section 2.7.1)
	●	●	Create and/or Load solvent file (Section 2.7.2)
	●	●	Create synthesis program file(s) and optionally create Pre-Synthesis and Post-Synthesis program files (Section 2.7.3)
		●	Create cleavage program file(s) (Section 2.7.3)
	●	●	Create sequence file(s) (Section 2.7.4)
	●	●	Create synthesis file(s) (Section 2.4)
	●		Load synthesis (Section 2.6.1)
		●	Load synthesis & cleavage program (Section 2.6.1)
	●	●	Calculate amino acid/solvent/reagent/resin amounts needed (Section 2.11.1)
√	NC	C	**Instrument Setup**
	●	●	Prepare amino acids/solvents/reagents and load bottles on instrument (Sections 1.2.4-1.2.5)
	●	●	Add resins to RVs and install on instrument (Section 1.3.1)
		●	Install collection vials on instrument (Section 1.3.2)
	●	●	Pressurize and prime all bottles needed for the synthesis (Section 2.5)
√	NC	C	**Run Synthesis**
	●	●	Click on Start in RV Automated Operations Screen for each desired RV position to run synthesis (Section 2.6.1)
	●	●	Adjust nitrogen mix flow control, if necessary (Section 1.1.4)
√	NC	C	**Post-Synthesis Procedures**
		●	Cleave peptides from resin (Section 3.4)
		●	Remove collection vials and work up peptides (Section 3.4)
		●	Perform a Collect Clean on the positions that were used (Section 2.8.4)
	●	●	Perform a N2 Back Flush on all bottles used during synthesis (Section 2.5)
	●	●	Perform System Clean if necessary (every 2 weeks) (Section 2.8.3)
	●	●	Empty the waste container (Section 1.2.6)

3.2 Startup & Instrument Check

To startup the Symphony® X Robotic Peptide Library Synthesizer:

1. Turn on the power switch located on the back of the instrument.

2. Select **Sign In** to sign in (See Section 2.3). Select a user name, type in password and press **Enter**.

3. Check the nitrogen supply and waste level. The nitrogen pressure should be greater than 70 psi, and there should be enough nitrogen in the tank for the synthesis. The gauges on the front of the instrument should read:

   1) Bottle Pressure – 10 psi
   2) Vacuum Gauge – 17-22 in Hg

   The waste tank should be empty.

4. Change bottle filters, if necessary. Bottle filters should be changed in the event of a clogged filter or change of reagent. See Section 4.6 for instructions.

3.3 Write Programs

To create programs, select **Setup / Program Editor** from the **Main Menu**, then choose to create a new program or edit an existing program following the instructions in Section 2.7.3. A typical synthesis uses the following types of programs:

1. Pre-Synthesis Program (optional) – Used before the first cycle, usually to swell the resin
2. Synthesis Program – Main program used throughout the synthesis
3. Pre-Activation Synthesis Program - Used when Pre-Activation is desired
4. Post-Synthesis Program (optional) - Used after the last cycle, usually for the drying the resin
5. Cleavage Program – Program used to cleave the peptide from the resin at the end of the synthesis

Examples of the five types of programs are as follows:

Pre-Synthesis (Swelling) Program:

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Vent Wash	RV	-	-	-	-	1
2	Top Delivery	RV	DMF	3000	0:10:00	Y	3

Synthesis Program (In Situ):

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Top Delivery	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	6
4	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
5	Top Delivery	RV	ACT	1000	0:10:00	Y	1
6	Top Delivery	RV	DMF	3000	0:00:30	Y	1
7	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
8	Top Delivery	RV	ACT	1000	0:10:00	Y	1
9	Vent Wash	RV	-	-	-	-	1
10	Top Delivery	RV	DMF	3000	0:00:30	Y	6

Pre-Activation Synthesis Program:

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Top Delivery	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	6
4	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
5	Top Delivery	PV	ACT	1000	0:02:00	N	1
6	PV to RV	-	-	-	-	-	-
7	Mix	RV	-	-	0:10:00	Y	-
8	Top Delivery	RV	DMF	3000	0:00:30	Y	1
9	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
10	Top Delivery	PV	ACT	1000	0:02:00	N	1
11	PV to RV	-	-	-	-	-	-
12	Mix	RV	-	-	0:10:00	Y	-
13	Top Delivery	PV	DMF	3000	0:00:15	N	1
14	PV to RV	-	-	-	-	-	-
15	Drain Dry	RV	-	-	0:00:15	Y	-
16	Top Delivery	PV	DMF	3000	0:00:15	N	1
17	PV to RV	-	-	-	-	-	-
18	Drain Dry	RV	-	-	0:00:15	Y	-
19	Top Delivery	PV	DMF	3000	0:00:30	Y	2
20	Vent Wash	RV	-	-	-	-	1
21	Top Delivery	RV	DMF	3000	0:00:30	Y	6

Post-Synthesis Program:

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Top Delivery	RV	DCM	3000	0:00:30	Y	6
2	Drain Dry	RV	-	-	0:10:00	Y	-

Cleavage Program:

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Top Delivery	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	6
4	Top Delivery	RV	DCM	3000	0:00:30	Y	6
5	Drain Dry	RV	-	-	0:10:00	Y	-
6	Cleave & Collect	RV	TFA	2500	2:00:00	-	-
7	Cleave & Collect	RV	TFA	2500	0:00:30	-	-
8	Top Delivery	RV	DCM	1000	0:00:30	Y	3
9	Drain Dry	RV	-	-	0:02:00	Y	-

NOTE Cleavage solution is always delivered from solvent position 8.

3.4 Post-Synthesis Procedures

1. Remove collection vials and work up peptides or cleave peptide from resin if on-instrument cleavage was not performed.

2. Perform a **Bottle Position Flush** (Section 2.5) on all bottles used in the synthesis. First perform a **Nitrogen Back Flush** to flush reagent back into the bottles. Replace used bottles with empty bottles, and perform a **Solvent Back Flush** to flush residual reagent from the lines.

3. If a cleavage was performed, do a **Collect Clean** (Section 2.8.4) and a **Cleave Bottle Nitrogen Back Flush** (Section 2.5). Then, replace Solv 8 bottle with an empty bottle and perform a **Cleave Bottle Solvent Back Flush** (Section 2.5).

4. Discard, store, or reuse used chemicals.

5. Empty the waste container.

6. If the instrument will not be used immediately, shutdown the instrument (Section 3.5).

3.5 Instrument Shutdown

It is not necessary to shutdown the ***Symphony X*** ® following each synthesis. Instrument shutdown is only necessary if the instrument needs to be moved or if the instrument will not be in use for an extended period.

To shutdown the ***Symphony X*** ®:

1. Perform a **System Solvent Flush** (Section 2.8.3)
2. Empty all amino acid and solvent/reagent bottles of fluid.
3. Empty the waste container.
4. Shutdown the computer by selecting “Shutdown” from the “Start” menu.
5. Turn off the instrument.
6. Disconnect the nitrogen tank.

Chapter 4: Cleaning and Maintenance

4.1 Cleaning & Maintenance Schedule

Every Synthesis	• **Bottle Position N2 Flush** used bottles (Section 2.5) • **Collect Clean** (Section 2.8.4) (Only After Cleave) • **Cleave Bottle Solvent Back Flush** (Section 2.5) (Only After Cleave)
Every Two Weeks	• **System Solvent Flush** (Section 2.8.3) • Inspect and Replace if necessary: RV upper and lower o-rings (Section 1.2.2)
Annually	• Solv 8 Valve Replacement (Section 4.9) • Amino Acid Bottle Seal Replacement (Section 4.7)
As Needed	• Nitrogen Leak Check (Section 4.5) • Bottle Filter Replacement (Section 4.6) • Amino Acid Bottle Seal Replacement (Section 4.7) • Solvent Bottle Seal Replacement (Section 4.8)

4.2 Amino Acid Line Cleaning

The following procedure is an intensive cleaning routine for cleaning amino acid lines that have precipitated. The Amino Acid Line Cleaning flushes each matrix valve position for the selected Reagent (AA) Line (3X) with the selected cleaning solvent followed by Nitrogen and 3 rinses with Solvent 1, followed again by Nitrogen.

4.3 System Solvent Flush

This is an automated routine to flush all liquid handling lines with solvent followed by clearing with N2. Solvent 1 and a user selected solvent are used to flush the main matrix valve block. Solvent 7 (Methylene Chloride) is used to flush the cleave block. This thorough process is intended for long-term shutdowns, solvent exchange, or cleaning prior to shipping.

4.4 System Nitrogen Flush

An automated routine to flush all liquid handling lines with N2 to simplify short-term shutdowns or preparation for instrument relocation.

4.5 Nitrogen Leak Check

It is recommended to routinely check the sealing of all the reagent supply bottles.

NOTE For all of the following tests, use only one nitrogen supply. Do not allow the nitrogen tank gauge to fall below 75 psi during the test, or the bottle positions that are pressurized for the test will be automatically vented.

Test A: Regulator & QC Test

1. Remove the nitrogen quick-disconnect so that no nitrogen line connected to the unit.

2. Turn off the nitrogen tank valve.

3. Watch the gauge on tank for drop in pressure within 15 minutes. Then turn the tank back on.

4. If the gauge on the nitrogen tank regulator drops, there is a leak. If this is the case, check the tank regulator and tank fitting for leaks with soapy water. Also check the tank regulator outlet fitting and gauges.

5. If the gauge does not drop, there is no leak. If there are no leaks, reconnect the nitrogen quick connect.

Test B: Internal Nitrogen System Test

1. Restart the instrument to ensure all bottles are vented.

2. Connect a nitrogen flow meter between the nitrogen tank and the nitrogen inlet to the unit.

3. If the flow is greater than 25 cc/min, there is a leak in the internal nitrogen system. Call the PTI Technical Service Department at 1-800-477-6834.

4. If the flow is less than 25 cc/min, proceed to Test C.

Test C: Solvent System Test

1. Connect the nitrogen flow meter and pressurize all solvent bottles in the **Bottle Preparations** (2.5) screen.

2. Allow the system to stabilize for 10-15 minutes.

3. Check the nitrogen flow meter to see if there is any flow.

4. If the flow is greater than 25 cc/min, there is a leak in one of the bottles. If the flow is below 25 cc/min, proceed to Test D.

5. To identify the leaky bottle, start at the Solvent #8 position (or #7 if no Solvent #8) and vent the bottle.

6. Check nitrogen flow meter for flow.

7. If the flow is greater than 25 cc/min, move up one bottle and vent Solvent #7 (or #6).

8. Check nitrogen flow meter for flow.

9. If the flow is still greater than 25 cc/min, continue to the next bottle(s) until the flow is below 25 cc/min.

10. When the flow is below 25 cc/min, the last vented bottle has a leak.

11. If a bottle is leaking, inspect the bottle cap, insert, o-ring, and bottle neck. Also check the 1/8” diameter bottle tubing for cracks or leaks.

12. Pressurize the bottle and re-test. If the flow is still above 25 cc/min, call your PTI Technical Service Department representative at 1-800-477-6834. If the flow is below 25 cc/min, proceed to Test D.

Test D: Amino Acid System Test

1. Make sure all 28 amino acid bottles are in place.

2. Pressurize the first amino acid manifold, and let the system stabilize for 5-10 minutes.

3. Check nitrogen flow meter.

4. If the flow is greater than 25 cc/min, vent the system and examine the amino acid bottle seals for solids, cracking, tears or other damage that would interfere with sealing. Pressurize the amino acid manifold again, let stabilize and check the nitrogen flow meter. If the flow is still greater than 25 cc/min, call your local Technical Service Department representative at 1-800-477-6834.

5. If the flow is less than 25 cc/min, proceed to the next manifold. Repeat steps 2-4 for all three amino acid manifolds. If the flow is less than 25 cc/min, the system check is complete.

4.6 Bottle Filter Replacement

The bottle filters should be replaced on a regular basis; the frequency depends upon the quality and concentration of the reagents utilized. Replacing the bottle filters once per month is good practice. Always replace filters for reagents that have precipitated. If a specific reagent cannot be delivered, first try replacing the bottle filter.

The bottle filter consists of a filter housing and a frit. The frit is press fit into the housing. The other side of the housing is partially threaded for the 1/8” diameter bottle tubing. To thread the filter housing onto a bottle tube, gently twist the housing clockwise while pushing it onto the tube. Be certain to thread the assembly completely onto the tubing or bubbles may be introduced between the top of the housing and the tubing. The filter assemblies are easily removed by gently twisting counterclockwise while pulling down. To remove the filter frit, press out the frit from the inside of the housing with a small rod. Alternatively the frit can be removed by lifting it out with a spatula. To replace the frit, put the new frit on a clean, flat surface and press the filter housing firmly over the frit.

CAUTION Always wear protective clothing, safety glasses and gloves when working on the filter assemblies.

Replacement Procedure:

1. From the **Bottle Preparations** screen (Section 2.5), select the positions that need replacement filters.

2. Press the **Nitrogen Back Flush** button to blow out the reagent from the lines.

3. When the operation is complete, remove bottle(s) and wipe exterior reagent off the filter assembly.

4. Unscrew the filter assembly from the tube and remove filter frit from filter housing as described above.

5. Clean and rinse housing with methanol and allow to dry completely.

6. Install new frit by pressing housing over frit.

7. Screw filter assembly back onto tubing.

IMPORTANT When installing the filter assembly onto the tubing, be sure the tube is threaded into the filter housing as far as it will go to prevent nitrogen bubbles from being introduced when the reagent level goes below the top of the filter housing.

NOTE To expedite the replacement procedure, it is best to have extra filter assemblies. The clean filter assembly can be used and the dirty filter can then be cleaned while the instrument is running.

4.7 Amino Acid Bottle Seal Replacement

The amino acid bottle seals should be replaced annually or as needed.

1. To remove, use forceps or tweezers to grab the seal and pull it out of the manifold.

2. To replace, remove filter housing, then put new seal over tube bend the seal into a U-shape. Start by feeding one corner into the manifold.

3. The seal can then be turned and pushed into the manifold in small increments.

4. The metal backing disk 'floats' and can be pressed upward to allow entry of the seal.

5. Replace filter housing and frit.

4.8 Solvent Bottle Seal

The solvent bottle seal consists of an encapsulated o-ring seated in a bottle cap insert. The o-ring can be damaged if not handled properly and should be replaced if a nitrogen leak is noted. Extra caution should be taken not to damage the insert when replacing the o-ring. To remove the o-ring, simply lift the o-ring off the insert with your fingertip. The protective gloves will assist in preventing damage to the inserts by cushioning against fingernail damage.

IMPORTANT Never use sharp or pointed objects to remove the o-rings from the inserts. Even small nicks may cause a nitrogen leak. Never use a razor blade or knife to cut off the o-rings.

CAUTION Always wear protective clothing, safety glasses and gloves when working on bottle seals.

4.9 Solvent 8 Valve Replacement

Due to the extremely aggressive nature of cleavage solutions, annual replacement of the Solv 8 valve on the cleavage block is recommended as preventative maintenance. Please contact your PTI Technical Service representative at 1-800-477-6834 to perform this replacement.

4.10 Procedure for verifying the operation of the waste sensors

As an important safety feature, it is recommended that the operation of the waste sensors be checked on a regular basis to ensure that the sensors are functional and that the system responds properly. As the instrument is equipped with dual waste tanks, it is important to test the operation of the waste switching valve as well. There are two versions of waste tanks depending on the age of the instrument: one with the waste sensor built into the cap assembly and one with

the sensor built into the waste tank. We recommend performing a test whenever the waste tank is emptied or every 30 days, whichever is more frequent. If the system does not register the expected responses listed below, do not use the instrument until the problem has been rectified. Contact PTI Service with any problems or for assistance with these procedures.

Preliminary step:

A. Go to the Bottle Prep screen and vent all the solvent and AA bottles.
B. Locate the waste status indicators in the instrument status bar at the bottom of the page.

For instruments with the waste sensor built into the cap assembly:

1. Disconnect the waste sensor cable from the waste sensor on Waste1. Wait 30 s and verify that the software registers that the Waste1 is FULL. (See pic. 1)
2. Manually fill and drain multiple RV's and ensure that the waste flow is going into Waste2 by observing the fluid moving through the waste tubing going into the Waste2 tank.
3. Reconnect the waste sensor cable to the sensor. Wait 30 s and verify that the software registers that the Waste1 is not full.
4. Wearing appropriate safety equipment, open the lid for the Waste1 container and lift the lid up to reveal the waste sensor under the cap. **NOTE** – Some fluid may still come out of the waste tubing lines. Take appropriate precautions.
5. At the bottom of the shaft for the waste sensor is a small float. Ensure that the float can move freely on the shaft of the sensor. (See pic. 2)
6. Lift the float and hold it up against the collar at the top of its range until the software registers that the Waste1 is FULL. (See pic. 3)
7. Reinstall the waste tank cap on the Waste1 tank.
8. Repeat steps 4 – 7 with Waste2 instead of Waste1.

For instruments with the waste sensor built into the waste tank:

1. Disconnect the waste sensor cable from the waste sensor on Waste1. Wait 30 s and verify that the software registers that the Waste1 is FULL. (See pic. 1)
2. Manually fill and drain multiple RV's and ensure that the waste flow is going into Waste2 by observing the fluid moving through the waste tubing going into the Waste2 tank.
3. Reconnect the waste sensor cable to the sensor. Wait 30 s and verify that the software registers that the Waste1 is not full.
4. Wearing appropriate safety equipment, remove the lid for the Waste1 container and set the lid into a beaker or other container that can catch any fluid that may come out.
5. Look inside the Waste1 tank and locate the small float at the bottom of the shaft for the waste sensor. Using something that is long enough to reach

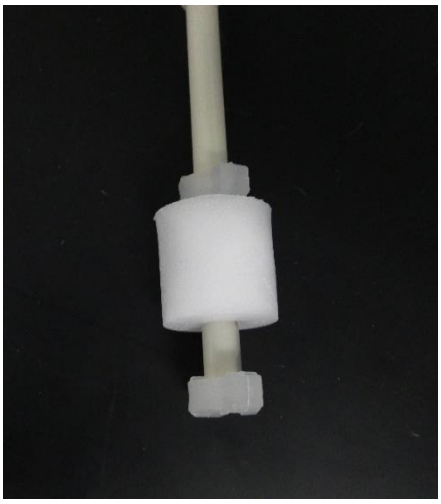
the float as a tool and ensure that the float can move freely on the shaft of the sensor. (See pic. 2)

6. Using the tool selected, lift the float and hold it up against the collar at the top of its range until the software registers that the Waste1 is FULL. (See pic. 3)
7. Reinstall the waste tank cap on the Waste1 tank.
8. Repeat steps 4 – 7 with Waste2 instead of Waste1.

Pic. 1 Waste sensor cable connector

Pic. 2 Waste sensor float

Pic. 3 Waste sensor float in FULL position



4.11 Procedure for backing up Symphony X folder

This section describes the process for backing up the Symphony X folder on the Symphony X instrument. This will allow the folder to be archived and moved off the instrument if desired. Later, the file can be extracted, and the folder restored.

Archiving the Symphony_X folder

- *Shut down the Symphony X software and exit to the desktop*
  "Exit to O.S.".

- Connect the keyboard and mouse to the Symphony X using the USB connections on the front of the instrument.

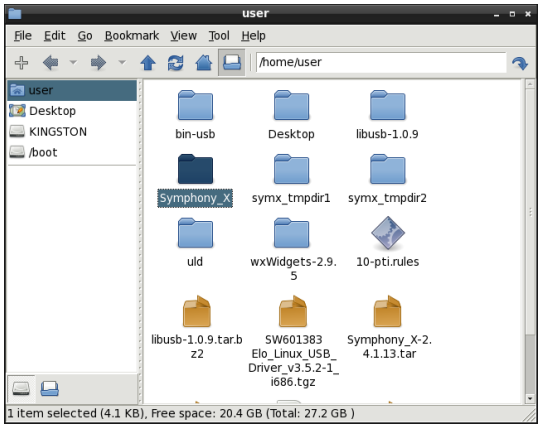
- A USB hub will allow you to connect the USB Memory Stick, keyboard and mouse to the front of the instrument at the same time.

- Alternatively, you may use the rear USB port for the memory stick or alternate between the keyboard and mouse and connect them when needed.

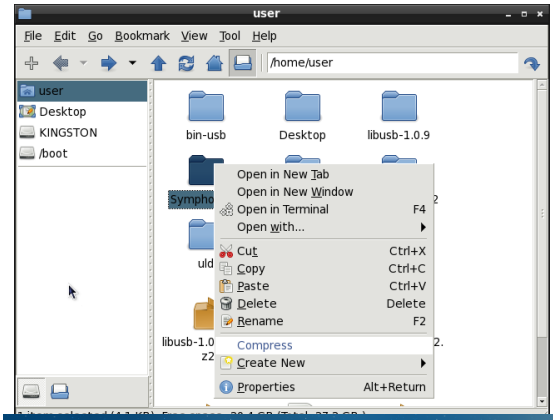
- Unmount any network drives mounted in any folder in the Symphony X folder other than the Net folder.

Double Click on the
My Documents folder
on the desktop

Select the Symphony_X folder

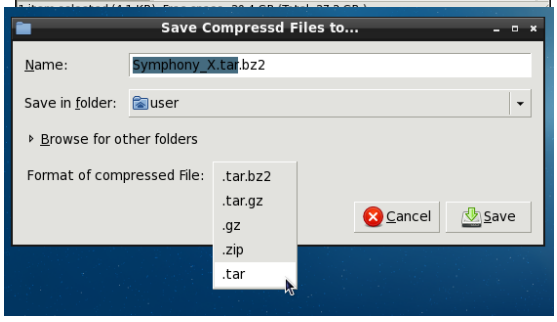


Right Click on the folder and
select Compress



Choose “tar” to be the type of
compressed file.

At the bottom of the list.



Change the name of the compressed file to be:

“Symphony_X_TodaysDate_backup.tar” (without the quotes)

Click Save.

Let the computer perform the compression, this could take some time.

After the folder compression is finished - copy the file to a USB stick for safe keeping.

Restoring the folder:

- *Shut down the Symphony X software and exit to the desktop*
  “Exit to O.S.”.

- Connect the keyboard and mouse to the Symphony X using the USB connections on the front of the instrument.

- A USB hub will allow you to connect the USB Memory Stick, keyboard and mouse to the front of the instrument at the same time.

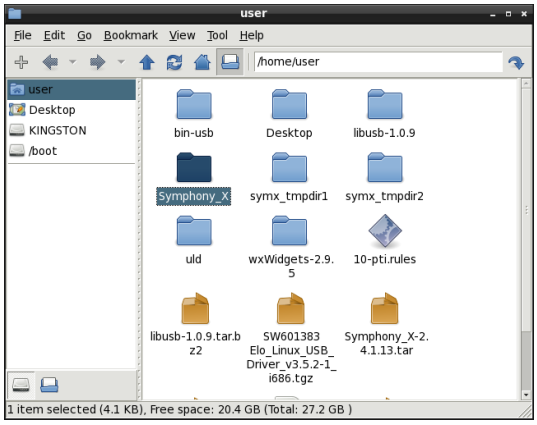
- Alternatively, you may use the rear USB port for the memory stick or alternate between the keyboard and mouse and connect them when needed.

- Unmount any network drives mounted in any folder in the Symphony X folder other than the Net folder.

Double Click on the
My Documents folder
on the desktop

Right click on the Symphony_X folder and select rename, give the current Symphony_X folder an appropriate name such as OldSymphony_X or Symphony_X_before_restore.

Insert the USB stick with the archive, it will show up on the left side of the screen, navigate to the file, right-click on the file and select copy. Go to the menu at the top of the screen and select Edit → Paste. This will copy the archive to the Home/User directory.



Right Click on the archive file in the Home\User directory and select extract here.

This will extract the archive (this may take some time) and you will now have a restored Symphony_X folder.

Go to the desktop and double click the start Symphony X shortcut to start the software.

CHAPTER 5: ERRORS AND RECOVERY

5.1 Common Errors

The following table lists common errors, their cause, and possible corrective actions to take. If the error still persists after all suggested actions have been taken, please contact your PTI Technical Service representative.

Error	Cause	Possible Action(s)
FILL ERROR (pre-fill, small fill, large fill)	Fluid sensor did not sense fluid during a **Fill** operation	• Check for fluid covering the filter in delivery bottle • If many RVs are pausing frequently on the same solvent/reagent, check, clean or replace bottle supply filter at the source. • Check that the bottle pressure gauge is set to 10 psi • Check bottle seal for improper fit/damage/missing parts that may cause a nitrogen leak • Check for nitrogen leaks using external nitrogen flow meter (Section 4.5). • Check for plugs or precipitates and perform **Bottle Position Flush** cleaning operation if required (Section 3.4. #2). • Check waste line for plugs • If only one RV is pausing frequently on numerous solvents, check RV for clogged frits and clean or replace RV if required • Fluid sensor may require service; contact Technical Service Dept.
CLEAR ERROR	The RV fluid sensor senses fluid after the **Clear** operation	• Check that the bottle pressure gauge is set to 10 psi (See Section 1.1.3). • Solvent 7 and 8 only - ensure mix adjustment is not set too low • RV sensor may require service; contact Technical Service Dept.
NOT PRIMED	Sensor does not sense fluid	• Go to **Bottle Preparations** screen and prime bottle (Section 2.5). • Try FILL ERROR Possible Action(s)
NO PRESSURE	The bottle required by the program is not pressurized	• Check nitrogen supply pressure gauge • Go to **Bottle Preparations** screen to pressurize (Section 2.5).
TIME OUT	Operation was not performed in the allotted operation time	• Press **Start** to continue with RV operations.
RV NOT IN PLACE	An RV is removed or not in place when an operation is initiated.	• Ensure RV is pushing the in-place switch push pin up. • Replace RV (Section 1.3.1) and press **Start**.
COLLECTION VIAL NOT IN PLACE	A collection vial is removed or not in place when a cleave or collect operation is initiated	• Replace the required collection vial (Section 1.2.3) and press **Start.**

5.2 System Errors

The following table lists system errors on the Symphony X, their cause, and possible corrective actions to take. The following errors will cause the Symphony X to do one or more of the following:

1. **Vent Bottles** – All solvent bottles are vented and must be pressurized and primed to resume.
2. **Hard Stop** – All actions cease immediately. The current synthesis is canceled and must be reloaded to resume.

CAUTION There may be fluid left in the reaction vessels and/or lines after a Hard Stop. Use the **RV Manual Operations** screen (Section 2.6.3) to drain and/or rinse the RV(s) and wash the valve blocks as necessary to clean the synthesizer and avoid contaminating the next fluid delivery.

NOTE The synthesis must be restarted to resume after a Hard Stop. Select the starting cycle and step using the Start, Stop, Pause function on the **RV Operations** screen, then press **Start**. The synthesis will start at the beginning of the step.

If the problem persists after the suggested actions are taken, please contact your PTI Technical Service representative at 1-800-477-6834.

Error	Cause	Possible Action(s)	Vent Bottles	Hard Stop
Nitrogen Pressure Low	The nitrogen supply switch in the pneumatic inlet assembly senses < 60 psi from the nitrogen supply system.	• Check nitrogen tanks and regulators • Check quick connect fittings for proper fit and/or leaks	√	√
Vacuum Loss	Vacuum supply switch senses < 10 in Hg vacuum after pump stops running.	• Call PTI Technical Service		√
Waste Level High	Waste level sensor indicates the tank is full or not connected to the instrument.	• Empty waste tank(s) and reconnect (Section 1.2.6) • Check/reconnect waste tank connector(s)		√
E-Stop	The E-Stop button was pressed and not released.	• Release E-Stop button (Section 1.1.3) and resume synthesis	√	√

APPENDIX

Appendix A: Reagents For Peptide Synthesis

A.1 Symphony X Pre-Packed N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Amino Acid	Quantity
SMP-05-A	Fmoc-L-Ala-OH	5 mmol
SMP-10-A		10 mmol
SMP-20-A		20 mmol
SMP-05-RBF	Fmoc-L-Arg(Pbf)-OH	5 mmol
SMP-10-RBF		10 mmol
SMP-20-RBF		20 mmol
SMP-05-NT	Fmoc-L-Asn(Trt)-OH	5 mmol
SMP-10-NT		10 mmol
SMP-20-NT		20 mmol
SMP-05-DB	Fmoc-L-Asp(OtBu)-OH	5 mmol
SMP-10-DB		10 mmol
SMP-20-DB		20 mmol
SMP-05-CT	Fmoc-L-Cys(Trt)-OH	5 mmol
SMP-10-CT		10 mmol
SMP-20-CT		20 mmol
SMP-05-EB	Fmoc-L-Glu(OtBu)-OH	5 mmol
SMP-10-EB		10 mmol
SMP-20-EB		20 mmol
SMP-05-QT	Fmoc-L-Gln(Trt)-OH	5 mmol
SMP-10-QT		10 mmol
SMP-20-QT		20 mmol
SMP-05-G	Fmoc-Gly-OH	5 mmol
SMP-10-G		10 mmol
SMP-20-G		20 mmol
SMP-05-HT	Fmoc-L-His(Trt)-OH	5 mmol
SMP-10-HT		10 mmol
SMP-20-HT		20 mmol
SMP-05-I	Fmoc-L-Ile-OH	5 mmol
SMP-10-I		10 mmol
SMP-20-I		20 mmol
SMP-05-L	Fmoc-L-Leu-OH	5 mmol
SMP-10-L		10 mmol
SMP-20-L		20 mmol
SMP-05-KBC	Fmoc-L-Lys(Boc)-OH	5 mmol
SMP-10-KBC		10 mmol
SMP-20-KBC		20 mmol
SMP-05-M	Fmoc-L-Met-OH	5 mmol
SMP-10-M		10 mmol
SMP-20-M		20 mmol

Catalog No.	Amino Acid	Quantity
SMP-05-F SMP-10-F SMP-20-F	Fmoc-L-Phe-OH	5 mmol 10 mmol 20 mmol
SMP-05-P SMP-10-P SMP-20-P	Fmoc-L-Pro-OH	5 mmol 10 mmol 20 mmol
SMP-05-SB SMP-10-SB SMP-20-SB	Fmoc-L-Ser(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-TB SMP-10-TB SMP-20-TB	Fmoc-L-Thr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-WBC SMP-10-WBC SMP-20-WBC	Fmoc-L-Trp(Boc)-OH	5 mmol 10 mmol 20 mmol
SMP-05-YB SMP-10-YB SMP-20-YB	Fmoc-L-Tyr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-V SMP-10-V SMP-20-V	Fmoc-L-Val-OH	5 mmol 10 mmol 20 mmol

A.2 Bulk N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Description	Quantity
FLA-5-A FLA-25-A FLA-100-A FLA-1KG-A	Fmoc-L-Ala-OH	5 g 25 g 100 g 1 kg
FLA-5-RBF FLA-25-RBF FLA-100-RBF FLA-1KG-RBF	Fmoc-L-Arg(Pbf)-OH	5 g 25 g 100 g 1 kg
FLA-5-NT FLA-25-NT FLA-100-NT FLA-1KG-NT	Fmoc-L-Asn(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-DB FLA-25-DB FLA-100-DB FLA-1KG-DB	Fmoc-L-Asp(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-CT FLA-25-CT FLA-100-CT FLA-1KG-CT	Fmoc-L-Cys(Trt)-OH	5 g 25 g 100 g 1 kg
Catalog No.	**Description**	**Quantity**

FLA-5-EB FLA-25-EB FLA-100-EB FLA-1KG-EB	Fmoc-L-Glu(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-QT FLA-25-QT FLA-100-QT FLA-1KG-QT	Fmoc-L-Gln(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-G FLA-25-G FLA-100-G FLA-1KG-G	Fmoc-Gly-OH	5 g 25 g 100 g 1 kg
FLA-5-HT FLA-25-HT FLA-100-HT FLA-1KG-HT	Fmoc-L-His(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-I FLA-25-I FLA-100-I FLA-1KG-I	Fmoc-L-Ile-OH	5 g 25 g 100 g 1 kg
FLA-5-L FLA-25-L FLA-100-L FLA-1KG-L	Fmoc-L-Leu-OH	5 g 25 g 100 g 1 kg
FLA-5-KBC FLA-25-KBC FLA-100-KBC FLA-1KG-KBC	Fmoc-L-Lys(Boc)-OH	5 g 25 g 100 g 1 kg
FLA-5-M FLA-25-M FLA-100-M FLA-1KG-M	Fmoc-L-Met-OH	5 g 25 g 100 g 1 kg
FLA-5-F FLA-25-F FLA-100-F FLA-1KG-F	Fmoc-L-Phe-OH	5 g 25 g 100 g 1 kg
FLA-5-P FLA-25-P FLA-100-P FLA-1KG-P	Fmoc-L-Pro-OH	5 g 25 g 100 g 1 kg
FLA-5-SB FLA-25-SB FLA-100-SB FLA-1KG-SB	Fmoc-L-Ser(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-TB FLA-25-TB FLA-100-TB FLA-1KG-TB	Fmoc-L-Thr(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-WBC FLA-25-WBC FLA-100-WBC FLA-1KG-WBC	Fmoc-L-Trp(Boc)-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-YB FLA-25-YB FLA-100-YB FLA-1KG-YB	Fmoc-L-Tyr(tBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-V FLA-25-V FLA-100-V FLA-1KG-V	Fmoc-L-Val-OH	5 g 25 g 100 g 1 kg

A.3 Reagents & Kits

Catalog No.	Reagents	Quantity
PS3-PPR-L	20% Piperidine/DMF (DEP)	0.9 L
PS3-MM-L	0.4 N-Methylmorpholine/DMF (ACT)	0.9 L
ACT-100-HBTU ACT-500-HBTU ACT-1K-HBTU	HBTU	100 g 500 g 1 kg
ACT-100-HCTU ACT-500-HCTU ACT-1K-HCTU	HCTU	100 g 500 g 1 kg

Catalog No.	Start-Up Kits	Quantity
SYMX-STARTKIT	Fmoc Amino Acid Start-up Kit for the Symphony X. Contains: 30 x 10 mL RVs, 0.9 L Deprotectant; 0.9 L 0.4M NMM; 2 x 0.1 mmol scale Rink amide resin, 2 x 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles and for running test peptides.	1 ea.
SYMX-STARTKIT-I	Fmoc Amino Acid Start-up Kit for the Symphony X. Contains: 30 x 10 mL RVs, 2 x 0.1 mmol scale Rink amide resin, 2 x 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles for running test peptides.	1 ea.

Catalog No.	Cleavage Kits	Quantity
CLEAVEKIT-U	**PTI Universal Cleavage Kit**. Suitable for cleaving peptides containing all 20 standard amino acids. Contains 95 mL TFA, 2 mL water, 2 mL anisole, 1 mL EDT. Makes 100 mL	1 ea.
CLEAVEKIT-R	**Reagent K Cleavage Kit.** Suitable for cleaving peptides containing all 20 standard amino acids. Contains 82.5 mL TFA, 5 mL thioanisole, 5 mL water, 5 g phenol, and 2.5 mL EDT. Makes 100 mL	1 ea.

Appendix B: Replacement Parts & Accessories

Catalog No.	Accessories	Quantity
PPS-GRV40-1	40 mL Reaction Vessel, Glass	1 ea.
PPS-R1.3-180	1.3 mL Reaction Vessel, PP	Pkg. of 180
PPS-R10-030 PPS-R10-090 PPS-R10-180	10 mL Reaction Vessel, PP	Pkg. of 30 Pkg. of 90 Pkg. of 180
PPS-R45-030 PPS-R45-090 PPS-R45-180	45 mL Reaction Vessel, PP	Pkg. of 30 Pkg. of 90 Pkg. of 180
CLV-050-030 CLV-050-090 CLV-050-180	Collection Tube, 50 mL	Pkg. of 30 Pkg. of 90 Pkg. of 180

Catalog No.	Replacement Parts	Quantity
0100096	Assembly, Waste Tank	1 ea.
0100105	Pressure Vessel, 20 L	1 ea.
3010001	Safety-Coated Bottle, 1 L Amber	1 ea.
3010002	Safety-Coated Bottle, 4L Amber	1 ea.
2700042	O-Ring for 1 and 4 L Bottles	1 ea.
2600187	Bottle Filter Housing, 1/8 Tube	1 ea.
SMP-RF-100	Bottle Filter Frit, 1/8 Tube	Pkg. of 100
3500004	Ferrule, 1/8 Tube Yellow	1 ea.
3500043	Ferrule, 1/16 Tube Clear	1 ea.
3500041	Ferrule, 1/16 Tube Blue	1 ea.
3500040	Fitting Nut, 1/16 Tube PP	1 ea.
3500061	Fitting Nut, 1/8 Tube PP	1 ea.
3500072	Fitting Nut, 1/8 Tube PPS	1 ea.
3510002	Fitting Nut, 1/16 Tube Headless PPS	1 ea.
3510001	Fitting Nut, 1/8 Tube Headless PPS	1 ea.

Catalog No.	Tools	Quantity
0100129	Assembly, Flow Meter & Tubing	1 ea.
6800023	Tool, 3/16 Tube Fitting Nut	1 ea.
6800027	Tool, 1/8 Tube Headless Fitting Nut	1 ea.

Appendix C: Infared (IR) Heating System

Infrared heating is the latest breakthrough in heating technology. With the fastest time to temperature of any heating technology, infrared heating is the most powerful heating tool you can use to assist your peptide synthesis. The Symphony®X Multiplex Peptide Synthesizer can be field-upgraded in your facility to a unit with infrared (IR) heating (Cat. #: SYMX-IR-OPT) and/or a unit with UV-monitoring (SYMX-UV-OPT).

C.1 History of Heat in SPPS

Heat has been used to aid in the syntheses of difficult peptides for the last 30 years. First used in 1984 by Janda and colleagues, heating methods range from a simple oil bath, to specially designed heated reaction blocks, to infrared heating today. Below is a brief history of heating methods used in SPPS:

1985 - Tam synthesized TGFα using an oil bath
1986 – Barlos synthesized leucine enkephalin using an oil bath
1991 – Foutch synthesized AT III and other peptides using a recirculating oil bath
1992 – Wang synthesized ACP (65-74) using a domestic microwave
2002 – Erdelyi and Gogoll synthesized TVI and others using a microwave synthesizer
2012 – PTI introduces the first peptide synthesizer to use infrared (IR) heating and synthesizes ACP (65-74), Aib enkephalin, and others on the Tribute®-IR peptide synthesizer.
2013 – PTI introduces the Symphony® X Multiplex Peptide Synthesizer available with infrared (IR) heating

C.2 Advantages of the IR Heating System

PTI's infrared heating system gives you:

1. Fastest time to temperature of any heating technology
2. Accurate temperature sensing
3. Doesn't overshoot
4. Doesn't overcorrect
5. Just steady, stable temperature control
6. Vortex mixing to ensure even temperature profiles

7. Potential for scalability

But that's just the heating system! The best part is this is the only rapid heating system that comes with the patented PTI fluidics system giving you maximum up-time, minimum solvent-usage and worry-free operation that lasts for years!

C.3 Recommended Use

PTI recommends synthesizing all peptides at room temperature first. The large majority of peptides produced worldwide are successfully synthesized at room temperature using conventional methods. When a peptide does not come out well in the first try, it is possible to further optimize the synthesis using more efficient activators, using lower-loaded resins or more hydrophilic solid supports, adding pseudoproline dipeptides or Dmb or Hmb dipeptides, or adding heat (See Sections C.3.2-C.3.5). In the same way that it does not make sense to use pseudoprolines or more expensive hydrophilic solid supports for every synthesis, it is also not recommended to use heat to synthesize all peptides. In the case of the former, it is a needless expense, in the case of the latter, it is to prevent the acceleration of unwanted side reactions. Heat is best used selectively, ideally just for the specific cycles in which it is needed! Difficult cycles can be identified using the UV monitoring feature on the Symphony® X, and protocols can be modified accordingly.

Heat works by accelerating reactions. In certain cases, the use of heat promotes various side reactions. In these cases, there can be some work arounds, but the best results are usually obtained by removing the heat altogether. Specifically, heat should not be turned on during the coupling of cysteine or histidine to minimize racemization or during the coupling of arginine residues to prevent gamma-lactam formation. During the synthesis of phosphopeptides, the heater must be turned off during all deprotection reactions once the phosphate group has been incorporated or it will cleave the phosphate group. Care must also be used when synthesizing peptides containing aspartic acid as heat can accelerate aspartimide formation during the deprotection step. Finally, the heater should be turned off during the coupling of amino acids to C-terminal proline residues to prevent diketopiperazine formation. (See Section C.4)

C.3.1 Starting Protocol

PTI recommends synthesizing all unknown peptide sequences at room temperature, then adding enhancements as needed from there. All reagents listed below are available in our Chemical Catalog. PTI's recommended starting protocol is:

Resin: Rink amide MBHA resin or preloaded Wang-polystyrene resin (loading ~0.5 mmol/g)

Deprotection: 2 x 5 minutes, 20% piperidine/DMF

Coupling: 2 x 10 minutes, 1:1:2 100 mM AA/100 mM HCTU/200 mM NMM in DMF, 5x excess

Washing: 6 x 30 seconds, DMF

Cleavage: 95:2:2:1 TFA/water/anisole/EDT for 2 hours (works for all sequences)

Most peptides using the standard 20 amino acids can be made using the above protocol. Additional tools may be used for difficult sequences. PTI recommends using PTI peptide predictor software and/or UV-monitoring to identify difficult cycles. Difficult sequences are caused by aggregation or steric hindrance. Aggregation occurs when hydrophobic side chains clump together causing a single peptide chain to fold in on itself, or neighboring peptide chains to interact with one another, obscuring the reactive group at the end of the growing chains. The presence of highly sterically hindered side chains can also prevent the facile formation of bonds at the end of the growing peptide chain as well. The sections below list different strategies you can use to improve the outcome of difficult cycles.

C.3.2 Low-Loaded Resin

The most powerful thing you can do is decrease the loading of your polystyrene resin to 0.2-0.3 mmol/g. The reason this is so effective is it effectively eliminates interchain aggregation by keeping neighboring peptide chains too far apart from one another to interact in the first place. At PTI, we typically try not to use resin with loadings much higher than 0.5 mmol/g. The added expense of having to use a little more low-cost polystyrene resin is much less expensive than using a significantly more expensive hydrophilic resin (ChemMatrix or other PEG-based resins), even with higher loadings because low-loaded polystyrene typically produces comparable purities to such resins (within 5%), but significantly higher yields – sometimes up to 4 or 5 times the yield of expensive hydrophilic resins. Therefore, the combination of using a little more low-cost polystyrene resin resulting in comparable purity (usually within 5%), but 4-5 times the yield will result in significantly more purified peptide for the lowest cost.

C.3.3 Coupling reaction

1) **Try increasing the amino acid excess and/or concentration.** Performing the coupling reaction at a higher concentration can significantly improve the coupling efficiency. This can be accomplished simply by increasing the amino acid excess from 5x to 10x, or by

decreasing the overall coupling reaction volume (assuming the resin can be sufficiently covered for good mixing).

2) **Try increasing the reaction times, or the number of couplings.** For very sterically hindered amino acids such as Aib, it was found that 4 x 90 minute couplings with HATU were necessary to incorporate them at high efficiency within the sequence (VQ-Aib-Aib-IDYING-OH; 89% final peptide crude purity), although the other amino acids were able to be coupled for just 2 x 1 minute each.
3) **Try dissolving the difficult amino acid in DMSO.** Dissolving hydrophobic, sterically hindered amino acids has been found to improve the coupling efficiency in some cases. In the synthesis of ACP (VQAAIDYING-OH), dissolving the final valine in DMSO so its coupling occurred in a 1:1 mixture of DMF/DMSO virtually eliminated the valine deletion peak from the HPLC of the crude peptide.
4) **Try a more efficient activator.** If HCTU was insufficient to do the job, try HATU. In rare cases, using an activator that operates via a different mechanism (i.e. PyBOP or PyClock) can improve results for specific sequences (i.e. C-peptide: H-EAEDLQVGQV ELGGGPGAGS LQPLALEGSL G-OH). PTI offers these high-efficiency coupling reagents and others in our chemical catalog.
5) **Try increasing the temperature.** (See below)

The following four items must be in balance in order to maximize coupling efficiency:

1. Activator Efficiency
2. Reaction Time
3. Temperature
4. Sequence Difficulty

Increasing each of the first three items alone can increase the coupling efficiency. However, increasing all three for an easy sequence may actually allow side reactions to occur, resulting in a lower purity peptide. Therefore, when adding heat, it is important to balance it with the other factors. In general, heat should be used with lower efficiency activators for short amounts of time (i.e. DIC/HOBt or HBTU for ~5 minutes at 75°C). If a coupling is extremely difficult (as in the case of Aib or N-methylated amino acids), it may be necessary to use a higher efficiency activator like HATU, and multiple couplings along with elevated temperatures.

C.3.4 Deprotection Reaction

Typically, when a difficult sequence presents itself, it is the coupling reaction that is causing the problem. Occasionally, however, it is possible to improve results by optimizing the deprotection reaction. **Try adding 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to 20% piperidine/DMF.** However, if you do this, be sure to decrease your reaction times to 2 x 30 seconds, or at

most 2 x 1 minute because prolonged exposure to DBU can promote aspartimide formation in Asp-containing sequences.

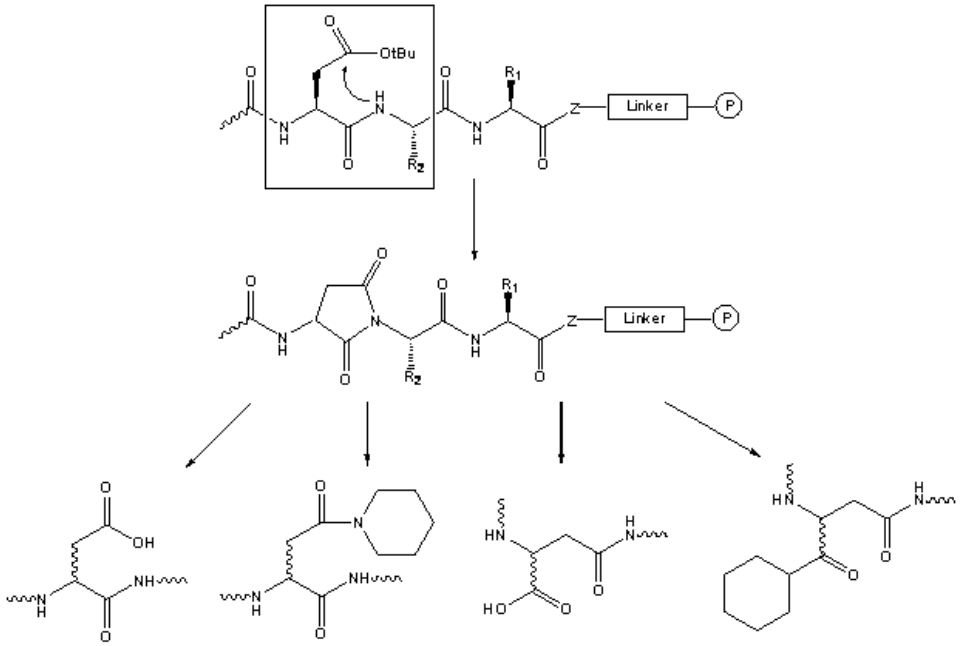
C.3.5 Pseudoproline Dipeptides, Hmb & Dmb Amino Acids and Dipeptides

The addition of a proline can break up aggregation up to 6 residues down the growing peptide chain. Pseudoproline dipeptides, and Hmb and Dmb amino acids and dipeptides are rigid structures that can break up aggregation in a similar way. Strategic placement of such building blocks in a difficult peptide sequence can significantly improve its synthesis, as in the synthesis of h-amylin (1-37): H-KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH_2 (pseudoproline dipeptides underlined). Pseudoproline dipeptides were necessary to obtain the peptide with a usable purity and yield. One limitation is pseudoproline dipeptides must contain a serine or threonine, and can therefore only be used in sequences containing those amino acids. Hmb and Dmb amino acids are less restricted, but are not available for all 20 standard amino acids.

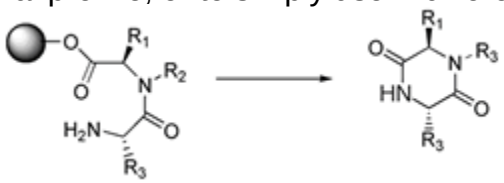
C.4 Side Reactions Accelerated by Heat

---

1. **Racemization** – Racemization is the partial conversion of a chiral amino acid into its other enantiomeric form. Heat can increase the chances of racemization during the coupling reaction, especially for histidine and cysteine residues. It has been suggested to turn the temperature down to 50°C or below during the coupling of these residues, but the best results can be obtained by leaving the heat off completely to minimize this side reaction.
2. **Aspartimide Formation** – Aspartimide formation occurs when an aspartic acid forms an aspartimide in the presence of an acid and/or a base. Once formed, the aspartimide can reopen into various forms. In Fmoc chemistry, the aspartimide can form piperidides when exposed to piperidine in subsequent deprotection steps. Heat can accelerate this side reaction. It is especially prevalent in peptide sequences containing Asp-Gly, Asp-Ala or Asp-Ser. To minimize this side reaction in aspartimide-prone sequences, HOBt can be added to the piperidine deprotection solution. However, HOBt can interfere with UV-monitoring. If UV-monitoring is being used concurrently with heated protocols, a better solution would be to use an alternative deprotection reagent such as piperazine, or to simply turn the heat off during the deprotection steps after aspartic acid has been incorporated in the sequence. Finally, replacing the amino acid immediately preceding the Asp with the Fmoc-(Hmb)-protected version can also help minimize aspartimide formation.



3. **Diketopiperazine Formation** – Diketopiperazine formation is an example of cyclative cleavage. In peptide synthesis, peptides containing a C-terminal proline attached to the solid support via a Wang linker can undergo diketopiperazine formation during the addition of the next amino acid. This resulting cyclized product is cleaved from the resin, resulting in lower overall yields for the synthesis. Heat can significantly accelerate this side reaction in peptides containing C-terminal prolines attached to the Wang linker, resulting in very low to negligible yields. It is highly recommended to turn the heat off during the addition of the next amino acid after a C-terminal proline, or to simply use 2-chlorotrityl chloride resin.



4. **Gamma-Lactam Formation** – Gamma-lactam formation occurs when the activated ester of the incoming Arg amino acid reacts with its own side-chain and forms a ring. This cyclized product is unable to couple to the growing peptide chain, resulting in arginine deletion sequences. Heat accelerates this side reaction, creating higher levels of arginine deletion in sequences prepared with heat. The best way to prevent this side reaction is to turn the heat off during the arginine coupling step. Double-coupling methods have been reported to aid in the synthesis, however, they include a significant amount of time (25 minutes) at room temperature as part of the first coupling, making it impossible to conclude whether the improved incorporation of Arg was due to the reaction being performed at room temperature before the heat was turned on, or actually double coupling at the higher temperatures.

5. **Phosphate Group Cleavage** – Heat can cleave phosphate groups during the deprotection reaction. Therefore, when synthesizing peptides containing phosphate groups, it is important to perform all deprotection steps (after the phosphate group has been incorporated) with the heat turned off.

Appendix D: IntelliSynth UV Monitoring and UV Extend System

With the UV-monitoring and extend control system, it is possible to monitor the extent of the deprotection reaction, and use that data to control deprotection times and repeats, and extend coupling times.

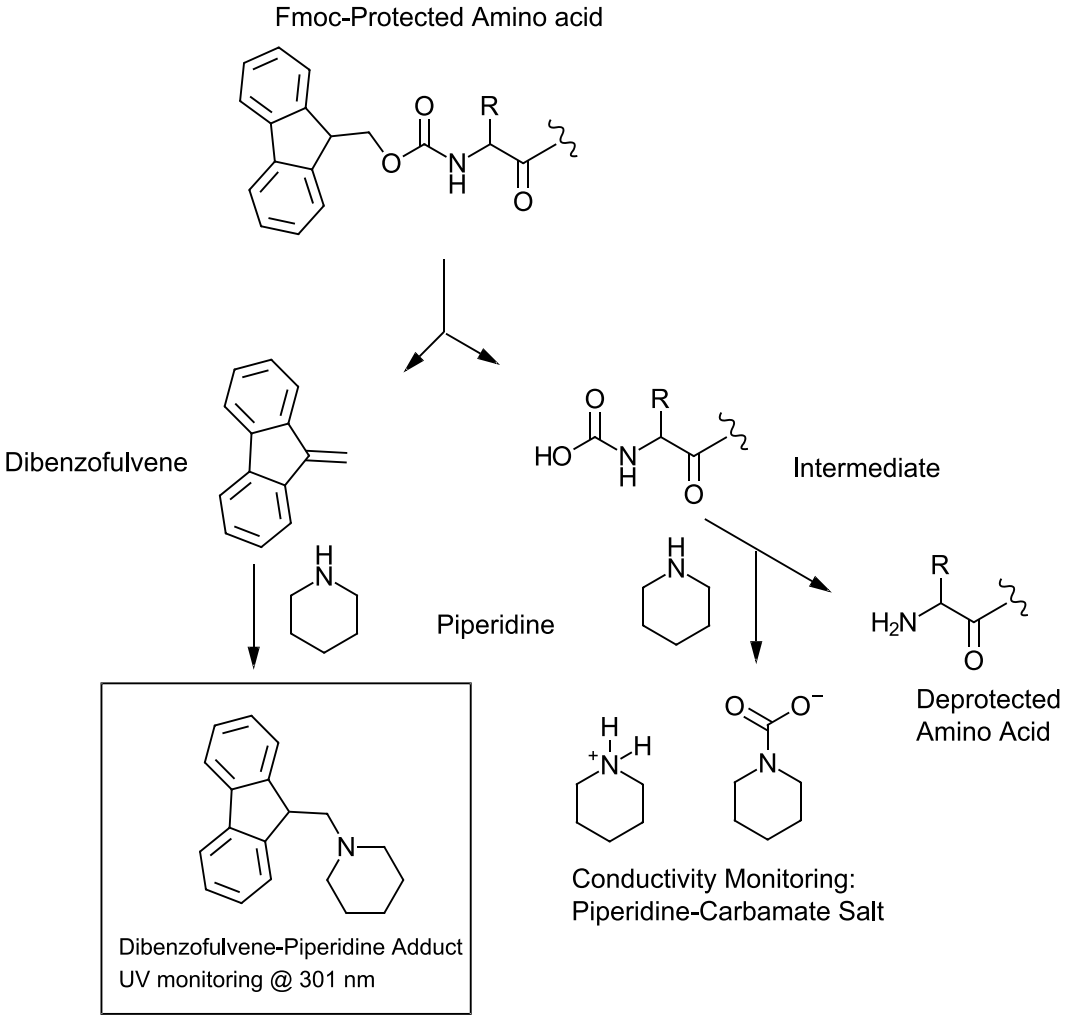
There are 3 main UV-Monitoring operations on the Symphony X.

1. Basic Monitoring – Measures UV absorption of the reaction solution over time to determine the extent of the deprotection reaction, but does not interfere with the synthesis.
2. UV Extend Operations – Measures the UV absorption of the reaction solution to determine the extent of the deprotection reaction and uses that data to control the deprotection reaction times and repetitions.
3. UV Extend Operations and Coupling Feedback – Measures the extent of the deprotection reaction and uses that data to control the deprotection reaction times and repetitions, and extend the coupling times based on the deprotection reaction time.

D.1 How UV Monitoring Works

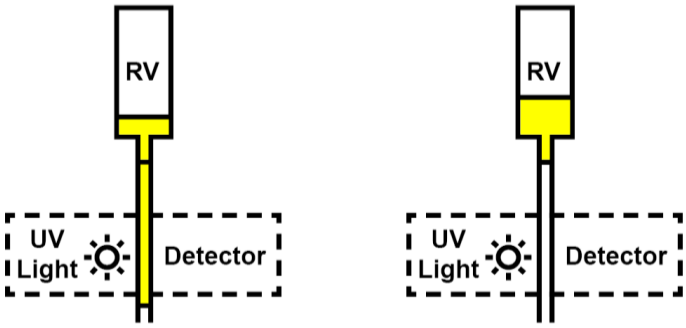
D.1.1 Chemistry

During the deprotection reaction, piperidine removes the Fmoc group and forms a piperidine-dibenzofulvene adduct with the byproduct (See below).



The IntelliSynth UV-Monitoring System monitors the absorbance of this adduct at 301 nm during the deprotection reaction.

D.1.2 The IntelliSynth UV Monitoring System



The IntelliSynth UV Monitoring System consists of a light source and detector encased in a 1 3/8" x 1 3/8" x 1 ¾" (3.5 cm x 3.5 cm x 4.5 cm) housing which measures the absorbance of the fluid in the tubing directly below the reaction vessel. During a mix, part of the fluid is pushed down into the section of tubing exposed to the light source and detector and a measurement is taken (left diagram). The fluid is then pushed back up into the reaction vessel to resume mixing (right diagram). This process occurs once every 10 seconds during a monitored mix.

D.1.3 Advantages of the IntelliSynth UV Monitoring System

By taking a measurement every 10 seconds during a mix, it is possible to determine when the reaction has stopped progressing. This means unlike other UV monitoring systems on the market, the IntelliSynth system can actually determine the shortest deprotection time required for a step rather than just the number of repeats.

D.2 UV Graphs Screen

The **UV Graphs** screen displays the UV absorbance graphs for individual deprotection reactions as well as overall syntheses. The **UV Graphs** screen can be accessed by selecting the **Tools** button at the bottom left of the **Main Menu** (Section 2.2) or **RV Automated Operations** (Section 2.6.1) screen. UV Graphs generate when UV Monitored steps are included in syntheses which are run in RV Automated Operations.

There are two graph types that can be displayed on the UV Graphs screen.

1. Synthesis Graph
2. Individual Mix Graph

The buttons at the bottom of the screen are as follows:

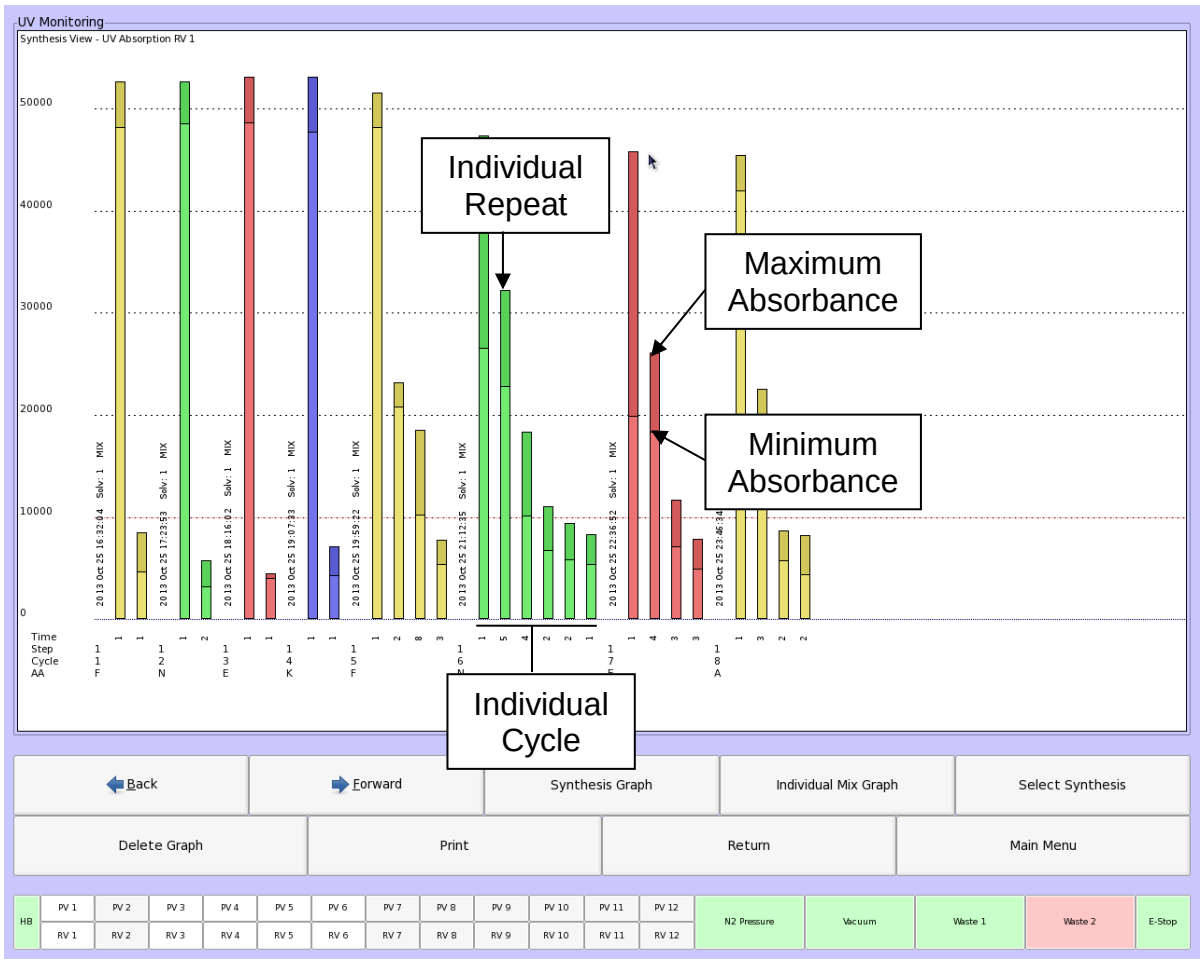
1. Back – Scrolls the graph to the left when full data set does not fit on screen (Synthesis Graph) or toggles to previous data screen (Individual Mix Graph)
2. Forward – Scrolls the graph to the right when full data set does not fit on screen (Synthesis Graph) or toggles to next data screen (Individual Mix Graph)
3. Synthesis Graph – Displays UV monitoring data for latest synthesis.
4. Individual Mix Graph – Displays UV monitoring data for the Individual Mixes of the latest synthesis.
5. Select Synthesis – Use to view UV monitoring data from another synthesis. Opens the Select a Synthesis screen where you can filter by Date of synthesis.
6. Delete – Opens Select a Synthesis screen where you can select a UV Graph (.uvlog) file to delete.
7. Print – Prints graphs from currently selected synthesis.
8. Return – Use to return to the **Tools** menu.
9. Main Menu – Use to return to the **Main Menu**.

UV Graph data updates after a UV Monitored step is complete, giving access to view the Synthesis Graph and Individual Graph data of that step. These semi-real time graphs provide information about difficult deprotection cycles can be viewed during the course of the synthesis or reviewed after the synthesis.

D.2.1 Synthesis Graph

A UV Synthesis Graph displays a summary of the UV absorbance data for a total synthesis. In a Synthesis Graph, each peak represents an individual repeat in a UV Monitored step, where the lighter portion represents the minimum absorbance measured during that repeat, and the darker portion represents the maximum absorbance measured during that repeat. The first peak of each step is labeled with a date and time stamp, and the name of the action that was performed. Peaks are colored according to groups of amino acids with similar properties (red for acidic, blue for basic, yellow for hydrophobic, green for polar, and black for glycine).Below each cycle are the following labels:

1. Time – Time for UV Monitored repeats to complete in minutes, rounded up to nearest whole minute
2. Step – Program step number of monitored step
3. Cycle – Cycle number
4. AA – One letter code of the cycle amino acid based on the sequence

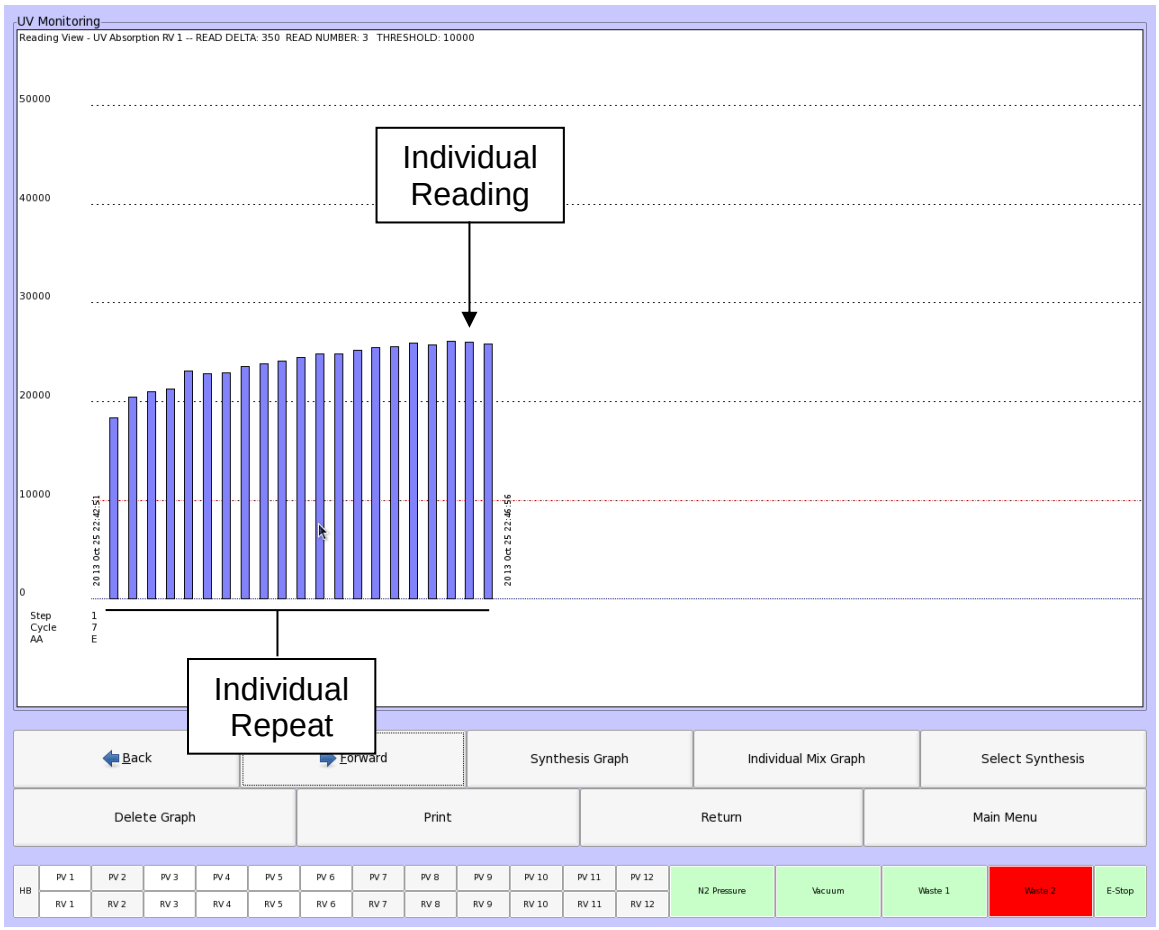


D.2.2 Individual Read Graph

An Individual Read Graph displays UV data for Individual Repeats of UV Monitored steps. A peak is recorded once every 10 seconds when a UV reading is taken.

In an Individual Read graph, each peak represents an individual absorbance reading. Individual readings are shown for each UV Monitored step. The first and last peaks of the step are labeled with a date and time stamp. The top of the Individual Read graph gives information about the operation times being used in the decision making process of the UV operation (if an extend operation). The data included is the Reaction Read Delta, the Read Number and the Absorbance Threshold (Section D.4.2). Below each cycle are the following labels:

1. Step – Program step number
2. Cycle – Cycle number



D.3 Basic Monitoring Mode

D.3.1 Overview

Basic monitoring takes absorbance readings every 10 seconds during the UV Monitored step, but it does not interfere with the synthesis. You can get two types of graphs from this data:

1. Synthesis Graph (D.2.1)
2. Individual Read Graph (D.2.2)

D.3.2 Operation Times

Operation Times do not determine the length of cycles in UV monitored (non-feedback or extend) operations.

D.3.3 Writing a Program

Program steps that perform this type of monitoring include:

1. UV Mix – Mixes fluid present in the RV using the specified mix method for a specified time.
2. UV Top Delivery – Delivers specified volume (μL) of the selected solvent to RV top. Performs UV Mix after solvent delivery.

Related Program steps:

1. UV Attenuate – DMF (Solvent 1) is delivered to RV top and UV measurements are taken. The RV drains at the end of this operation. This step zeroes the detector to the absorbance reading of DMF. This step does not replace calibrating the UV detector (See Section D.6).

NOTE The UV Attenuate step must be included in any synthesis including UV Monitoring steps.

For best results, include a UV Attenuate step as the last wash following deprotection in the Synthesis program and prior to the first deprotection in the Pre-Synthesis program (See Section 2.7.3).

Standard Programs

Standard programs for Pre-Synthesis, Synthesis using In Situ couplings, and Synthesis using Pre-Activation couplings using the Basic UV Monitoring operations are shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis without interfering with the synthesis. There are no UV modifications to the standard Post-Synthesis or Cleave programs (See Section 3.3).

Standard UV Pre-Synthesis (Swelling) Program

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Vent Wash	RV	-	-	-	-	1
2	Top Delivery	RV	DMF	3000	0:10:00	Y	2
3	UV Attenuate	-	-	-	-	-	-

Standard UV Monitored Synthesis Program (In Situ)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Delivery	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
6	Top Delivery	RV	ACT	1000	0:10:00	Y	1

7	Top Delivery	RV	DMF	3000	0:00:30	Y	1
8	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
9	Top Delivery	RV	ACT	1000	0:10:00	Y	1
10	Vent Wash	RV	-	-	-	-	1
11	Top Delivery	RV	DMF	3000	0:00:30	Y	6

Standard UV Monitored Synthesis Program (Pre-Activation)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Delivery	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
6	Top Delivery	PV	ACT	1000	0:02:00	N	1
7	PV to RV	-	-	-	-	-	-
8	Mix	RV	-	-	0:10:00	Y	-
9	Top Delivery	RV	DMF	3000	0:00:30	Y	1
10	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
11	Top Delivery	PV	ACT	1000	0:02:00	N	1
12	PV to RV	-	-	-	-	-	-
13	Mix	RV	-	-	0:10:00	Y	-
14	Top Delivery	PV	DMF	3000	0:00:15	N	1
15	PV to RV	-	-	-	-	-	-
16	Drain Dry	RV	-	-	0:00:15	Y	-
17	Top Delivery	PV	DMF	3000	0:00:15	N	1
18	PV to RV	-	-	-	-	-	-
19	Drain Dry	RV	-	-	0:00:15	Y	-
20	Top Delivery	PV	DMF	3000	0:00:30	Y	2
21	Vent Wash	RV	-	-	-	-	1
22	Top Delivery	RV	DMF	3000	0:00:30	Y	6

D.4 Deprotection with UV Extend Operations

D.4.1 Overview

This mode is the same as the Basic Monitoring mode, however, the data is used to extend the deprotection time and number of repetitions without affecting any other steps in the cycle. The UV Extend Operations alter the deprotection reaction time in two ways.

1. The time of an Individual Repeat can lengthen depending on the observed UV absorbance in an Individual Read Graph. If the slope between a number of Individual Readings is above a set threshold (See D.4.2.2) another Individual Read will be added to the repeat. If the absorbance changes less than the threshold for a set number of reads (See D.4.2.3), the repeat will end. The repeat will end automatically if it has run for a maximum amount of time (See D.4.2.1).

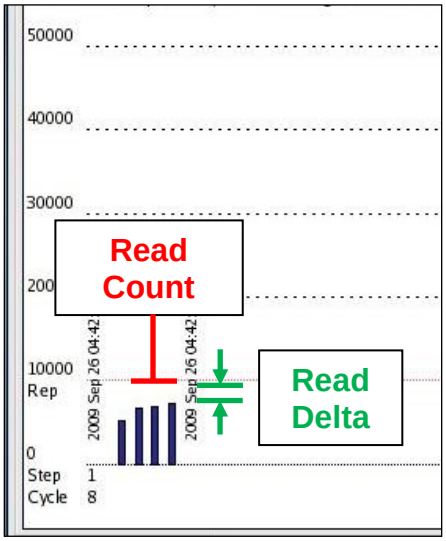
2. The number of repeats can increase depending on the observed UV absorbance in a Synthesis Graph. After a minimum number of repeats is completed (See D.4.2.6), the instrument checks if the absorbance is above a set absorbance threshold (See D.4.2.4). If the absorbance is above the threshold, another repeat is added, and if the absorbance is below the threshold the operation will end. The operation will end automatically if it has run for a maximum number of repetitions (See D.4.2.5).

D.4.2 Operation Times

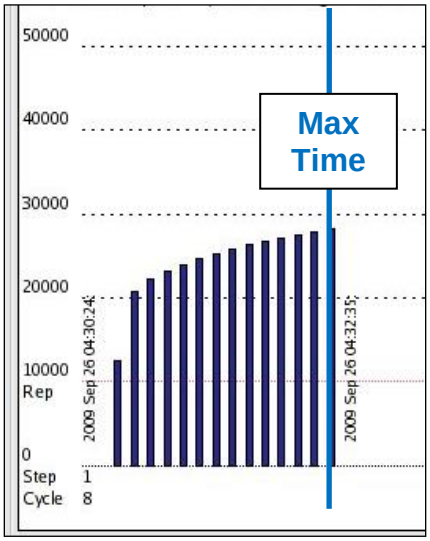
There are several operation times which affect the length of cycles in UV Extend Operations. These operation times are listed here as a reference, but they cannot be altered without access to the Factory user. If you would like to alter an operation time listed here, please contact PTI.

1. Maximum Repetition Time: This is the maximum time a UV Extend operation will take for a single repeat of a UV Extend step. The default is 20 minutes (**1,200,000 ms**).
2. Reaction Read Delta: This is the minimum change in absorbance between concurrent Individual Reads or Individual Repeats necessary to trigger the time to extend. The default is **350**.
3. Reaction Read Count: This is the number of Individual Reads whose slopes between them must be less than the Reaction Read Delta to trigger the end of the repeat. The default is **3**.
4. Absorbance Threshold: This is the minimum absorbance necessary to trigger the instrument to add another repeat. Below this threshold, there is a small enough amount of adduct formation (See D.1.1) that the deprotection step can be considered complete in most cases. The default is **8000**.
5. Maximum Repetition Count: This is the maximum number of repetitions that will be allowed to occur during a single UV Extend operation. The default is **10**.
6. Repetition Threshold Count: This is the minimum number of repeats which must fall below the Absorbance Threshold before the operation will end. The default is **1**.

A repeat will end when:

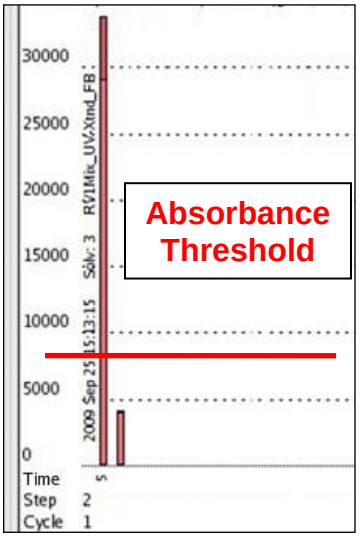


(1) The number of consecutive absorbance readings that fall within the Reaction Read Delta value of each other is equal to the Reaction Read Count.

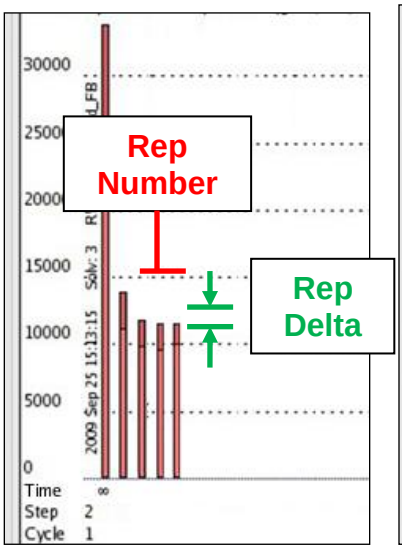


(2) The Maximum Repetition Time is reached.

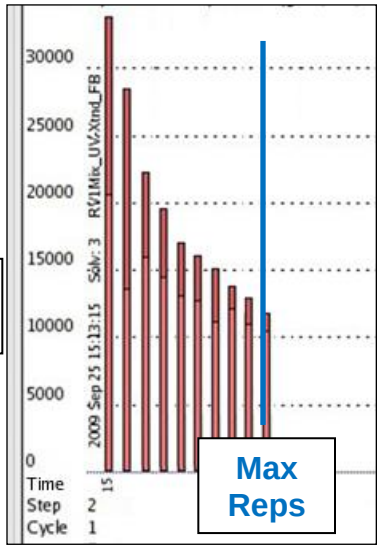
The operation will stop repeating when:



(1) The peak height is below the Absorbance Threshold.



(2) The number of consecutive Individual Repeats that fall below the Absorbance Threshold is equal to the Repeat Threshold Count.



(3) The maximum number of repetitions is reached.

D.4.3 Writing a Program

UV Extend operations to be used in programs include:

1. UV Mix Extend – Performs UV Mix. Extends the time of the step depending on the UV absorbance measurements taken during the mix by increasing the time of a single repeat (See 4.1.1), but does not extend by adding repeats.
2. UV Top Extend – Performs UV Top Delivery. Extends the time of the step depending on the UV absorbance measurements taken during the mix by increasing the time of the repeats (See 4.1.1), and by adding repeats (See 4.1.2). Drains RV when a repeat is added, and restarts the operation from the beginning.

Standard Programs

The standard programs using the UV Extend Operations are shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis. They will control the deprotection reaction times and repetitions. The coupling reaction is unaffected.

NOTE The UV Attenuate step must be included in any synthesis including UV Monitoring steps. The UV Attenuate step re-zeroes the UV detector and takes the place of a DMF wash so as not to waste solvent or time.

For best results, include a UV Attenuate step as the last wash following deprotection in the Synthesis program and prior to the first deprotection in the Pre-Synthesis program (See Section 2.7.3).

Standard UV Pre-Synthesis (Swelling) Program

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Vent Wash	RV	-	-	-	-	1
2	Top Delivery	RV	DMF	3000	0:10:00	Y	2
3	UV Attenuate	-	-	-	-	-	-

Standard UV Deprotect Extend Synthesis Program (In Situ)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Extend	RV	DEP	2000	0:02:30	Y	-
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
6	Top Delivery	RV	ACT	1000	0:10:00	Y	1
7	Top Delivery	RV	DMF	3000	0:00:30	Y	1
8	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
9	Top Delivery	RV	ACT	1000	0:10:00	Y	1
10	Vent Wash	RV	-	-	-	-	1
11	Top Delivery	RV	DMF	3000	0:00:30	Y	6

Standard UV Deprotect Extend Synthesis Program (Pre-Activation)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Extend	RV	DEP	2000	0:02:30	Y	-
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
6	Top Delivery	PV	ACT	1000	0:02:00	N	1
7	PV to RV	-	-	-	-	-	-
8	Mix	RV	-	-	0:10:00	Y	-
9	Top Delivery	RV	DMF	3000	0:00:30	Y	1
10	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
11	Top Delivery	PV	ACT	1000	0:02:00	N	1
12	PV to RV	-	-	-	-	-	-
13	Mix	RV	-	-	0:10:00	Y	-
14	Top Delivery	PV	DMF	3000	0:00:15	N	1
15	PV to RV	-	-	-	-	-	-
16	Drain Dry	RV	-	-	0:00:15	Y	-
17	Top Delivery	PV	DMF	3000	0:00:15	N	1

18	PV to RV	-	-	-	-	-	-
19	Drain Dry	RV	-	-	0:00:15	Y	-
20	Top Delivery	PV	DMF	3000	0:00:30	Y	2
21	Vent Wash	RV	-	-	-	-	1
22	Top Delivery	RV	DMF	3000	0:00:30	Y	6

D.5 Deprotection and Coupling with UV Extend Operations

D.5.1 Overview

This mode is the same as the Deprotection with UV Extend Operations, however the UV data from the deprotection step is also used to extend the coupling time in the cycle.

The coupling reaction time is determined by 2 Operation Times. These operation times are listed here as a reference. If you would like to alter an operation time listed here, please contact your PTI service representative.

1. Maximum Reaction Time – Determines the maximum coupling reaction time. The default is 1 hour (**3,600,000 ms**).
2. Coupling Multiplier – The deprotection time of the first repeat during UV Top Extend or UV Mix Extend is multiplied by the Coupling Multiplier to obtain the coupling time as long as this value falls below the Maximum Reaction Time. The default setting is **2**.

D.5.2 Writing a Program

The UV Extend Deprotection and Coupling Operations used to write programs are the following:

1. UV Coup Extend – Mixes liquid in the RV using specified mix method for a calculated duration of time dependent on the previous extend operations (See D.5.1). The time specified for this step in the program is the minimum coupling time.
2. UV Top Extend – Performs UV Top Delivery. Extends the time of the step depending on the UV absorbance measurements taken during the mix by increasing the time of the repeats (See 4.1.1), and by adding repeats (See 4.1.2). Drains RV when a repeat is added, and restarts the operation from the beginning.
3. UV Mix Extend – Performs UV Mix. Extends the time of the step depending on the UV absorbance measurements taken during the mix by increasing the time of a single repeat (See 4.1.1), but does not extend by adding repeats.

NOTE If UV Coup Extend is included in a program, in order for its reaction time to calculate correctly, it must be included in a program after a UV Top Extend or UV Mix Extend step.

Standard Programs

The standard programs for the Deprotection and Coupling with UV Extend Operations are shown below. These programs will produce a UV graph of the deprotection reactions for the synthesis. They will control the deprotection reaction times and repetitions, and extend the coupling time based on the deprotection time of the first repeat.

NOTE The UV Attenuate step must be included in any synthesis including UV Monitoring steps. The UV Attenuate step re-zeroes the UV detector and takes the place of a DMF wash so as not to waste solvent or time.

For best results, include a UV Attenuate step as the last wash following deprotection in the Synthesis program and prior to the first deprotection in the Pre-Synthesis program (See Section 2.7.3).

Standard UV Pre-Synthesis (Swelling) Program

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	Vent Wash	RV	-	-	-	-	1
2	Top Delivery	RV	DMF	3000	0:10:00	Y	2
3	UV Attenuate	-	-	-	-	-	-

Standard UV Synthesis Extend Program (In Situ)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Extend	RV	DEP	2000	0:02:30	Y	2
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
6	Top Delivery	RV	ACT	1000	0:00:00	N	1
7	UV Coup Extend	RV	-	-	0:05:00	Y	-
8	Top Delivery	RV	DMF	3000	0:00:30	Y	1
9	AA Delivery	RV	Cycle AA	1000	0:00:00	N	1
10	Top Delivery	RV	ACT	1000	0:10:00	N	1
11	UV Coup Extend	RV	-	-	0:05:00	Y	-
12	Vent Wash	RV	-	-	-	-	1
13	Top Delivery	RV	DMF	3000	0:00:30	Y	6

Standard UV Synthesis Extend Program (Pre-Activation)

Step	Operation	RV/PV	Solvent	Volume	Time	Drain	Reps
1	UV Top Extend	RV	DEP	2000	0:02:30	Y	-
2	Vent Wash	RV	-	-	-	-	1
3	Top Delivery	RV	DMF	3000	0:00:30	Y	5
4	UV Attenuate	-	-	-	-	-	-
5	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1

6	Top Delivery	PV	ACT	1000	0:02:00	N	1
7	PV to RV	-	-	-	-	-	-
8	UV Coup Extend	RV	-	-	0:05:00	Y	-
9	Top Delivery	RV	DMF	3000	0:00:30	Y	1
10	AA Delivery	PV	Cycle AA	1000	0:00:00	N	1
11	Top Delivery	PV	ACT	1000	0:02:00	N	1
12	PV to RV	-	-	-	-	-	-
13	UV Coup Extend	RV	-	-	0:05:00	Y	-
14	Top Delivery	PV	DMF	3000	0:00:15	N	1
15	PV to RV	-	-	-	-	-	-
16	Drain Dry	RV	-	-	0:00:15	Y	-
17	Top Delivery	PV	DMF	3000	0:00:15	N	1
18	PV to RV	-	-	-	-	-	-
19	Drain Dry	RV	-	-	0:00:15	Y	-
20	Top Delivery	PV	DMF	3000	0:00:30	Y	2
21	Vent Wash	RV	-	-	-	-	1
22	Top Delivery	RV	DMF	3000	0:00:30	Y	6

D.6 Calibrating the UV Detector

The following is a step by step guide to calibrating the Symphony X UV Detector.

1. Fill Solvent 1 container with DMF. Attach Solvent 1 container using quick connects. Place empty bottles on solvent bottle positions 2-8.
2. Dissolve SMP-20-A bottle using 40mL of DMF and place in AA1 position. Place empty AA bottles in all other positions.
3. From the **Main Menu** select **Bottle Prep** and pressurize and prime Solvent 1 and AA 1 (See Section 2.5).
4. Select **Main Menu** < **Tools** < **Calibrate UV**.
5. Select **Fill RV (DMF)** and **Drop to Sensor**. Select **UV light off** to turn the light on. Adjust sensor POT until UV Reading shows 50,000 ± 500. Watch reading for 1-2 minutes. Reading should not fluctuate more than 200 from set point.
6. Select **UV light on** to turn the light off. Select **Cancel** to end. Select **Drain RV**.
7. Select **Automatic Calibration**. Let the calibration run. The high and low readings will record automatically.
8. Select **Main Menu** < **Bottle Prep**. Select Solvent 1 and **N_2 Flush**. Repeat for AA1. Remove AA1 when finished.

INDEX

GYROS PROTEIN
Technologies

peptides@gyrosproteintech.com
www.gyrosproteintechnologies.com

Worldwide:
4675 South Coach Drive
Tucson, AZ 85714, U.S.A.
Phone: +1-520-629-9626
Toll Free: 800-477-6834
Fax: +1-520-629-9806