Prelude

Quick Start Guide

Protein
Technologies, Inc.

Prelude

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Protein Technologies, Inc.

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

WARNING COLLECTION VIALS MUST BE IN PLACE AT ALL TIMES.

WARNING SYNTHESIS WILL HALT IF WASTE CONTAINER IS FULL.

WARNING DO NOT ATTEMPT TO MOVE THE PRELUDE 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.

Contents

Introduction

Thank you for purchasing your new Prelude peptide synthesizer from Protein Technologies, Inc. The Prelude is a fully automated peptide synthesizer with six reaction vessels that operate simultaneously. Twenty-seven amino acid bottles and eight solvent/reagent bottles allow for maximum flexibility. Extra features include E-mail updates, one-shot deliveries, and automated cleavage.

I.1 About This Book

This book helps you get started with the Prelude.

- Chapter 1, **Instrument Layout**, describes the basic layout of the Prelude.
- Chapter 2, **Accessories**, describes the accessories offered for the Prelude.
- Chapter 3, **Setup Instructions**, describes the basic software and procedures for setting up a synthesis on the Prelude
- Chapter 4, **Post-Synthesis Operations**, describes the procedures for cleaning the Prelude after a synthesis.

I.2 About The Company

Protein Technologies, Inc. (PTI) was founded in 1985 by researchers affiliated with the University of Arizona, and launched its first peptide synthesizer, the PS3, in 1990. Since then, PTI has manufactured and sold the world's finest solid-phase synthesizers. PTI's products are supported by a dedicated field service team, and we are proud of our reputation for reliability. Today, we are growing and innovating to serve the needs of the solid-phase synthesis market. We are here to help. If you have any questions concerning your PTI synthesizer, please feel free to contact us:

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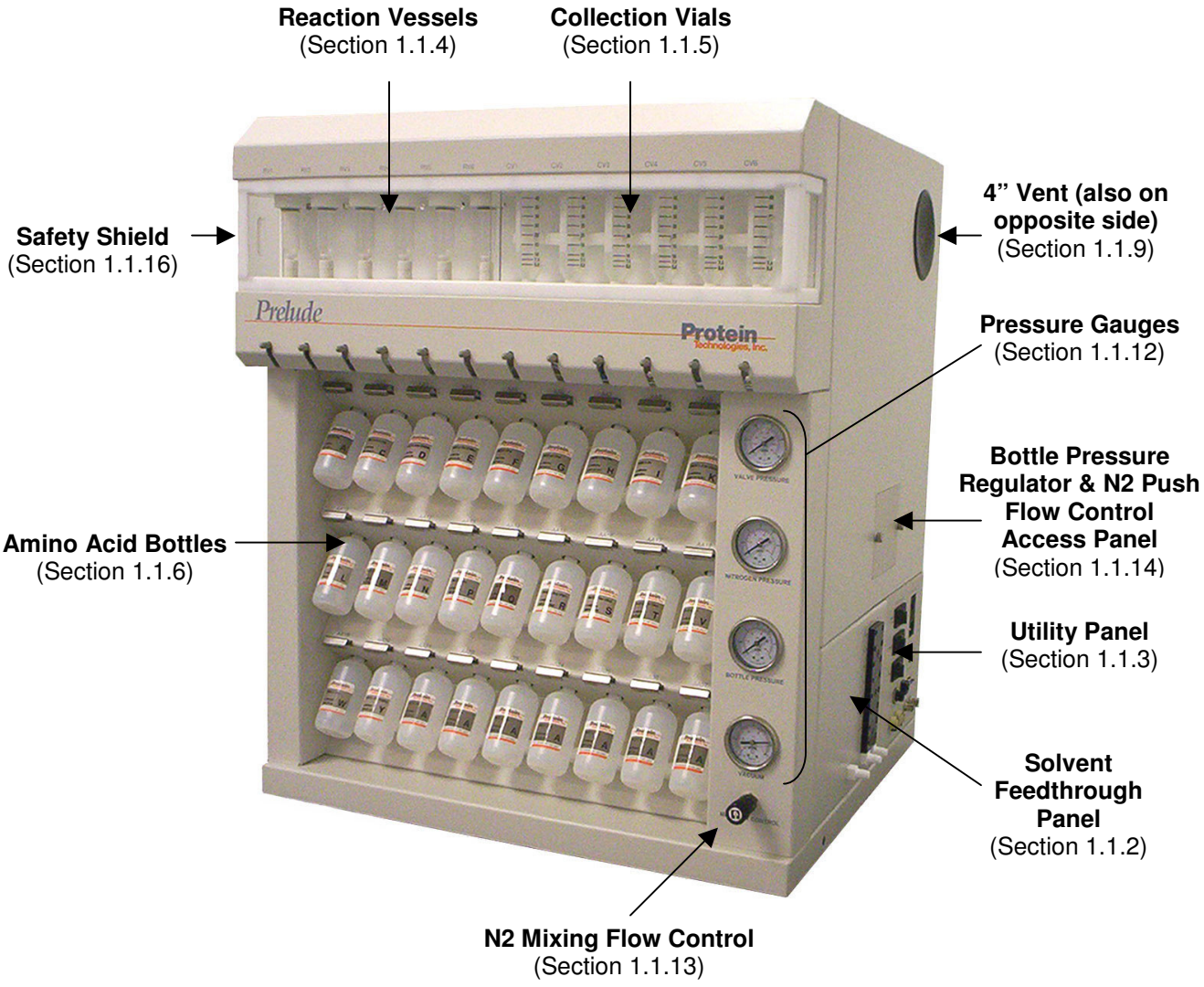
I.3 Common Abbreviations

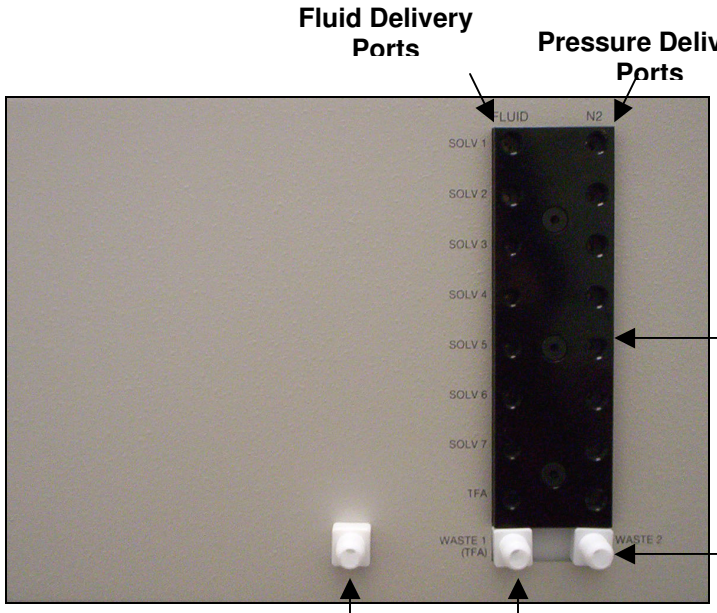
AA – Amino Acid
Act – Activator or Action
Dep – Deprotection Solution or Deprotected
DMF – Dimethylformamide
Fmoc – 9-Fluorenylmethyloxycarbonyl
GLP – Good Laboratory Practice
HBTU – 2-(1H-Benzotriazol-1-yl-)-1,1,3,3-tetramethyluronium hexafluorophosphate
In Hg – Inches of Mercury
M – Molarity (moles/liter)
µL – Microliters
mL – Milliliters
MW – Molecular Weight
NMM – *N*-Methylmorpholine
Psi & Psig – pound(s) per square inch gauge
Reag – Reagent
RV – Reaction Vessel
Solv – Solvent
TFA – Trifluoroacetic Acid
Vac – Vacuum

Chapter 1: Instrument Layout

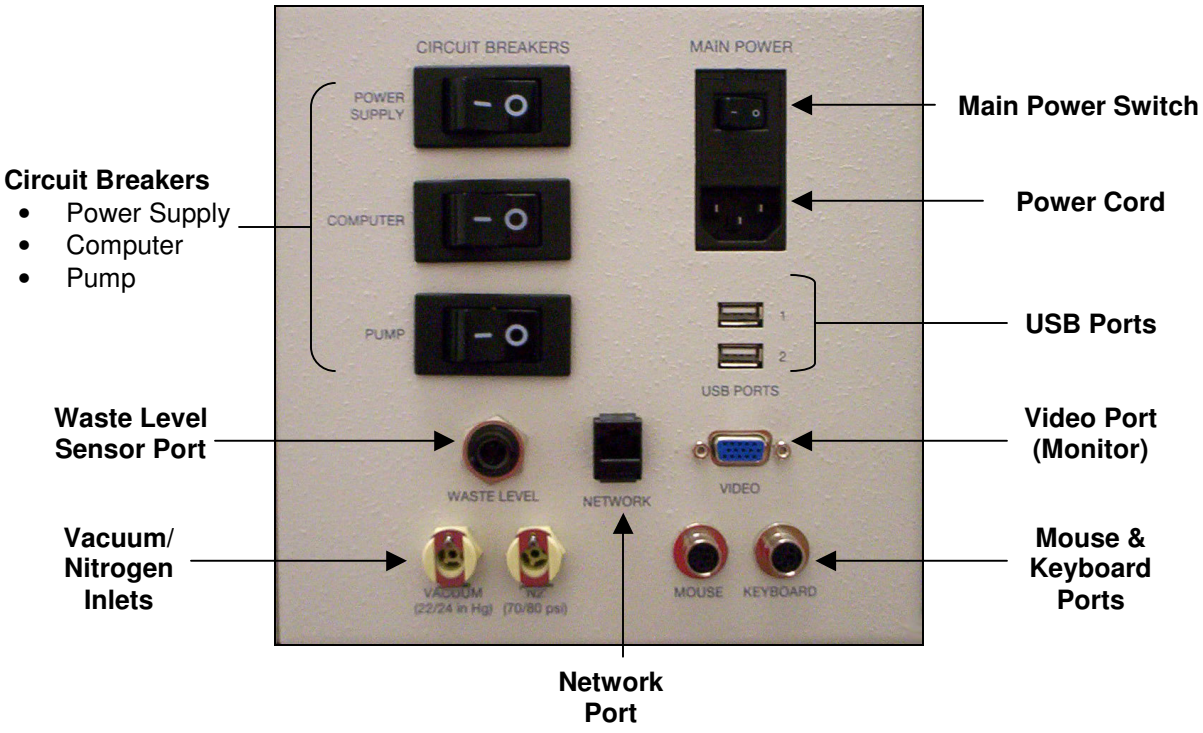
1.1 Prelude Front



1.2 Solvent Feedthrough Panel



1.3 Utility Panel



Chapter 2: Accessories

2.1 Reaction Vessels & O-Rings

10 mL
Cat#: PPS-R10-030, Pkg. of 30
Cat#: PPS-R10-090, Pkg. of 90
Cat#: PPS-R10-180, Pkg. of 180

40 mL
Cat#: PPS-R40-030, Pkg. of 30
Cat#: PPS-R40-090, Pkg. of 90
Cat#: PPS-R40-180, Pkg. of 180

Reaction Vessel O-Rings:
- Bottom: Cat#: 2720001, 1 ea.
- Top: Cat#: 2720002, 1 ea.

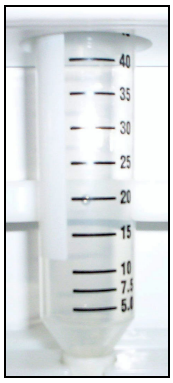
2.2 Amino Acid Bottles

10 mL One Shot
Cat#: 3030001,
1 ea.

120 mL
Cat#: SMP-VX-20, Pkg. of 20
Cat#: AMP-VX-100, Pkg. of 100

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

2.3 Collection Vials



50 mL
Cat#: 3110004,
Pkg of 50

2.4 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 for peptide synthesis on the Prelude (See Appendix A.3 for listings).

2.5 Replacement Parts/Accessories

Protein Technologies, Inc. supplies replacement parts for the Prelude, as well as various accessories, including solvent/reagent 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 3: Setup Instructions

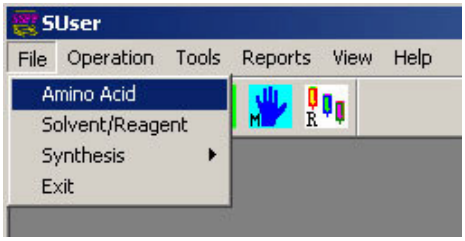
3.1 Check Gauges

The gauges on the front of the Prelude should read as follows:

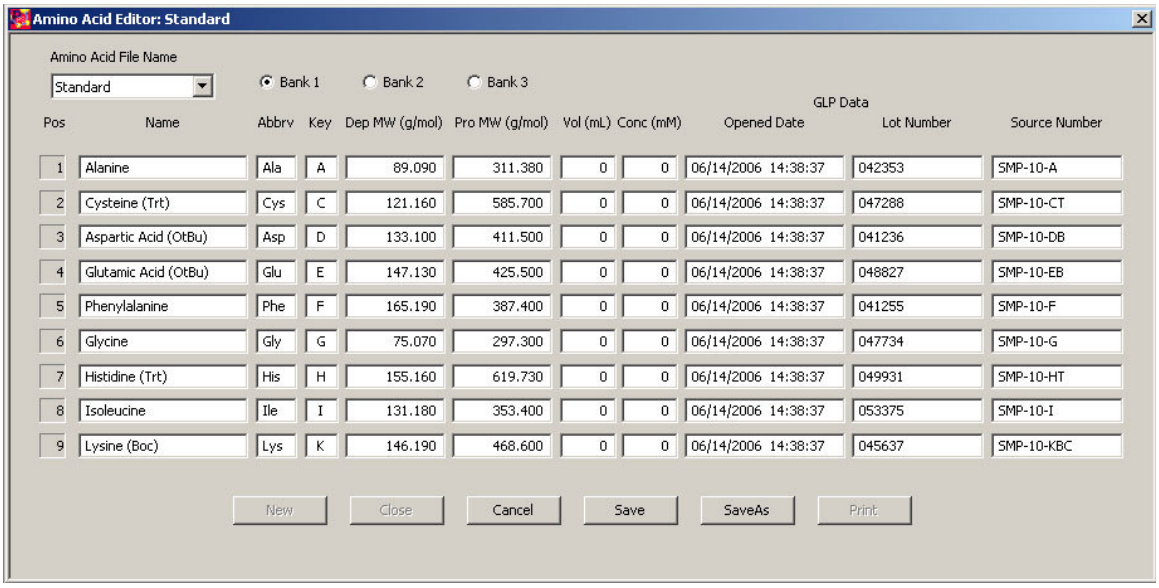
1. **Valve Pressure** – 25-35 psi
2. **Nitrogen Pressure** – 5 psi
3. **Bottle Pressure** – 9 psi
4. **Vacuum** – 17-22 in Hg

3.2 Create A New Amino Acid File

1. To create a new amino acid file, click on the **File** menu and select **Amino Acid.**



2. The **Amino Acid File Manager** will open as a new screen. Click the **New** button. This will open a new amino acid file in the **Amino Acid Editor**.



3. By default, a new file contains the names and molecular weight values of the 20 standard amino acids. Double click in a box to change the value in the following columns:

    a. **Name** – Name of amino acid (or other chemical)

    b. **Abbrv** – Three-letter abbreviation for amino acid (or other chemical)

    c. **Key** – One-letter abbreviation

    d. **Dep MW (g/mol)** – Molecular Weight without protecting groups

    e. **Pro MW (g/mol)** – Molecular Weight with protecting groups

    a. **GLP Data**

        i. **Vol (mL)** – Volume used for synthesis (in mL).

        ii. **Conc (mM)** – Concentration of amino acid solution used for synthesis (mM).

        iii. **Opened Date** – Date (and time) solution was opened.

        iv. **Lot Number** – Lot number.

        v. **Source Number** – Source information (catalog number, company, etc.).

4. Select Bank 1 to access positions 1-9. Select Bank 2 to access positions 10-18. Select Bank 3 to access positions 19-27.

5. Click the **Save** button to save the changes.

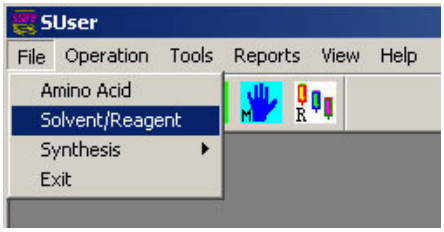
6. Use the **Cancel** button to remove any changes since the last **Save**.

7. To print the open amino acid file, click the **Print** button. It will go automatically to the printer.

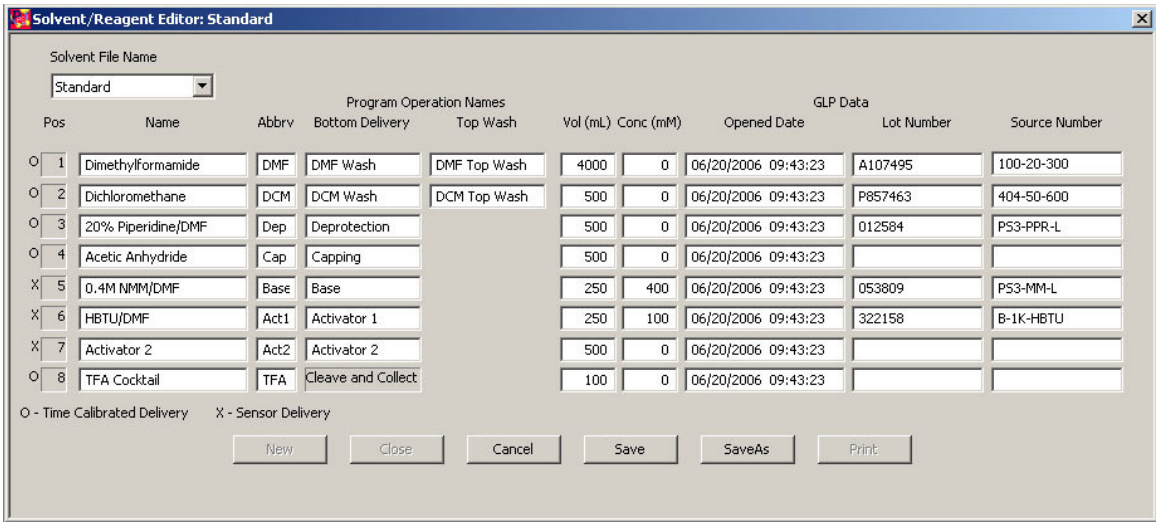
8. To close the window, click the **X** in the upper right corner.

3.3 Create a New Solvent/Reagent File

1. To create a new solvent/reagent file, click on the **File** menu and select **Solvent/Reagent**.



2. The **Solvent/Reagent File Manager** will open as a new screen. Click the **New** button. Enter a name for the new file, and click **OK**. This will open a new solvent/reagent file in the **Solvent/Reagent Editor**.



3. By default, a new file contains the standard solvents and reagents. Double click in a box to change the value in the following columns:

    a. **Name** – Name of solvent or reagent

    b. **Abbrv** – Abbreviation for solvent or reagent

    c. **Program Operation** – Wording displayed on synthesis log to describe delivery of solvent or reagent

        i. **Bottom Delivery** – Solvent or reagent is delivered from the bottom of the RV

        ii. **Top Wash** – Solvent or reagent is delivered from the top of the RV followed by drying with nitrogen

    d. **GLP Data**

        i. **Vol (mL)** – Volume used for synthesis (in mL).

ii. **Conc (mM)** – Concentration of reagent solution used for synthesis (mM).

iii. **Opened Date** – Date (and time) solution was opened.

iv. **Lot Number** – Lot number.

v. **Source Number** – Source information (catalog number, company, etc.).

NOTE To the left of each row is an "O" or an "X." "O" indicates volumes for that bottle position are measured by timed deliveries, while "X" indicates volumes for that bottle position are measured by sensor-controlled deliveries using a metered loop.

4. Click the **Save** button to save the changes.

5. Use the **Cancel** button to remove any changes since the last **Save**. A screen will appear reading, "Abandon All Changes?" The **Yes** button will permanently remove the changes; the **No** button will leave the changes.

6. To print the open solvent/reagent file click the **Print** button. It will go automatically to the printer.

7. Click the **Close** button to exit the **Solvent/Reagent Editor** screen. The **Close** button activates after the file is saved.

3.4 Create a New Program

Two programs are recommended to run a synthesis on the Prelude:

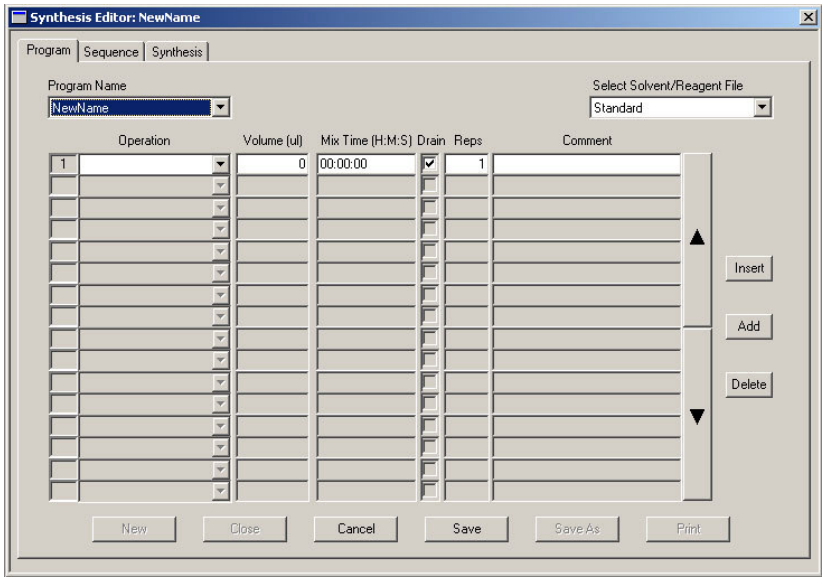
1. **Swelling Program** (Synthesis program with extended initial wash times to swell the resin during the first step)

2. **Synthesis Program**

If automated cleavage is desired, a **Cleavage Program** is also necessary.

1. To create a program, click on the shortcut button:

The **Program File Manager** opens as a new screen. Click the **New** button. Enter a name for the new file, and click **OK**. This opens a new program file in the **Program Editor**.



2. Select a solvent/reagent file by using the pull-down menu next to the **Select Solvent/Reagent File** box. The selected solvent/reagent file determines what operations are available under the **Operations** column pull-down menu.

3. For each step of the synthesis, input:

    a. **Operation** – Use the pull-down menu to select an operation for each step.

    b. **Volume (ul)** – Enter a volume in microliters. The Prelude delivers in 150, 500, and 1000 µL aliquots, so entries are rounded up to the nearest value possible with a minimum volume of 150 µL and a maximum volume of 10000 µL.

    c. **Mix Time (H:M:S)** – Enter the mix time in Hours:Minutes:Seconds. The maximum mix time is 99:59:59.

    d. **Drain** – Click to check or uncheck the box. When checked, the reaction vessel drains at the end of the program step. When unchecked, the reaction vessel is not drained at the end of the program step.

NOTE It is important to leave **Drain** unchecked when delivering amino acid so the amino acid remains in the reaction vessel for the activator delivery.

CAUTION Always deliver amino acid to the reaction vessel before activator to avoid guanidinium formation.

e. **Reps** – Enter the number of times to repeat the step. The maximum number of repetitions is 9.

f. **Comment** – Record comments for the step.

4. Use the buttons to the right to insert, add or delete a step. The buttons are:

   a. **Insert** – To insert a step, click in the comments section of a step and click the **Insert** button. A new step is inserted above that step.

   b. **Add** – To add a step to the bottom of the program, click the **Add** button.

   c. **Delete** – To delete a step, move the cursor to that step and click the **Delete** button.

5. Click the **Save** button to save the changes.

6. Use the **Cancel** button to remove any changes since the last **Save**. A screen will appear reading, "Abandon All Changes?" The **Yes** button permanently removes the changes; the **No** button leaves the changes.

7. To print the open program file, click the **Print** button. It goes automatically to the printer.

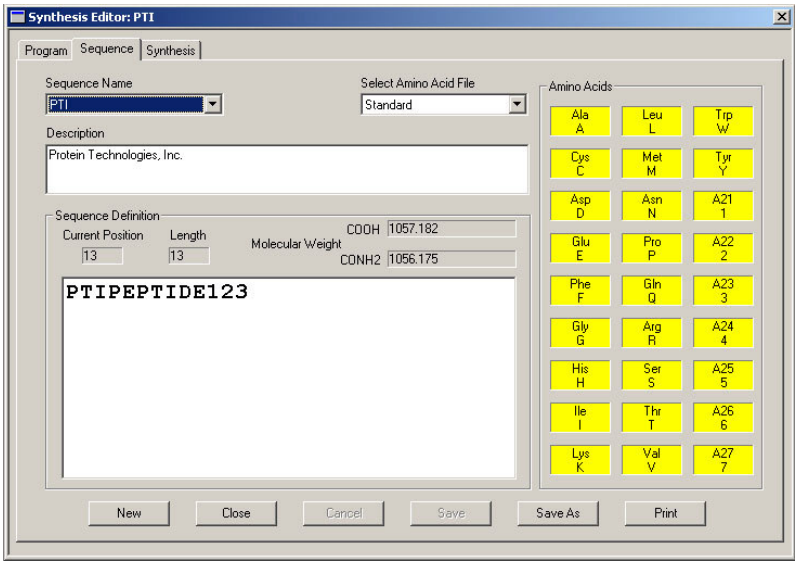
8. Click the **Close** button to exit the **Program Editor** screen. The **Close** button activates after the file is saved.

NOTE The Prelude software has standard swelling, synthesis, and cleavage program files called "Standardsw," "Standard," and "Standard_Cleave," respectively, that can be opened and viewed by selecting a file and clicking **Open** instead of **New** in the **Program File Manager**. These files are standard programs installed by Protein Technologies, Inc.

3.5 Create a New Sequence

1. To create a sequence, click on the shortcut button:

The **Sequence File Manager** opens as a new screen. Click the **New** button. Enter a name for the new file, and click **OK**. A new sequence file opens in the **Sequence Editor**.



2. Select an amino acid file by using the pull-down menu next to the **Select Amino Acid File** box. The values from the selected amino acid file are displayed on the buttons in the **Amino Acids** section to the right.

3. Enter a sequence by clicking the amino acid buttons with the mouse. Alternatively, click in the large white box to place the cursor and use the keyboard to enter or modify the sequence.

4. Enter comments in the **Description** box.

5. Click the **Save** button to save the changes.

CAUTION Sequence characters must match those available in the current amino acid file, or the file will not be saved.

6. Use the **Cancel** button to remove any changes since the last **Save**. A screen will appear reading, “Abandon All Changes?” The **Yes** button permanently removes the changes; the **No** button leaves the changes.

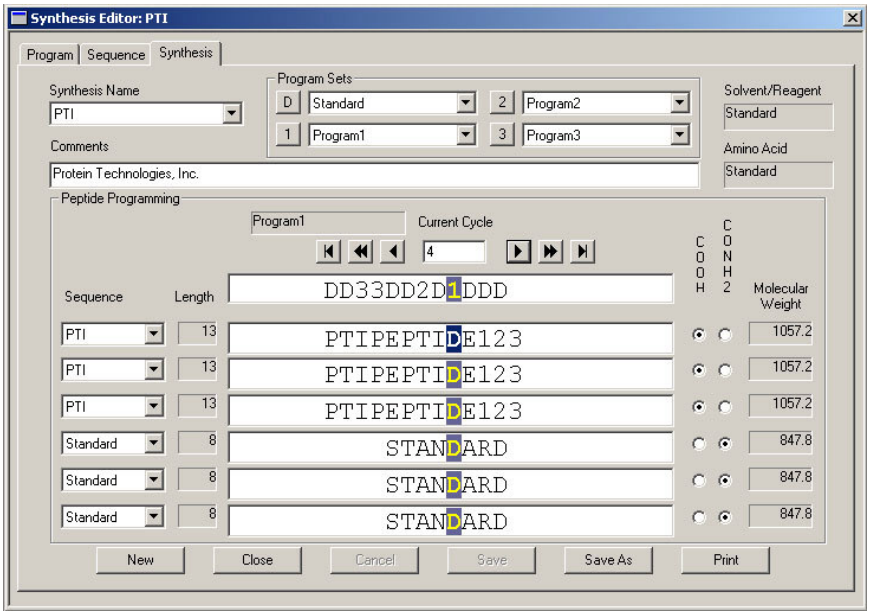
7. To print the open sequence file click the **Print** button. It goes automatically to the printer.

8. Click the **Close** button to exit the **Sequence Editor** screen. The **Close** button activates after the file is saved.

3.6 Create a New Synthesis

1. To create a synthesis, click on the shortcut button:

2. The **Synthesis File Manager** opens as a new screen. Click the **New** button. Enter a name for the new file, and click **OK**. This opens a new synthesis file in the **Synthesis Editor** under the **Synthesis** tab.



3. In the **Synthesis Editor** under the **Synthesis** tab, select a synthesis using the pull-down menu in the **Synthesis Name** box, or create a new synthesis by clicking the **New** button.

4. Assign programs in the **Program Sets** section using the pull-down menus. Make sure your default program is assigned to **D**. The default program will initially be assigned to all cycles of the synthesis.

5. Enter synthesis comments in the **Comments** box.

6. Assign a sequence to each of the six RVs using the pull-down menus in the **Sequence** column. The first row corresponds to RV1, the second row corresponds to RV2, and so on down to RV6.

NOTE The amino acid and solvent/reagent files assigned to the first sequence chosen defines those files for the synthesis. Only sequences with amino acid and solvent/reagent files that match those of the first sequence chosen are allowed in the synthesis. To run a sequence with a different amino acid or solvent/reagent file, a different synthesis file must be created.

7. Assign an acid (COOH) or amino (CONH2) C-terminus to each sequence by clicking in the circle under the corresponding column to the right of the sequence.

NOTE Be sure to use preloaded resin if an acid terminus is selected, and non-preloaded resin if an amino terminus is selected.

8. To assign a program to a cycle, select a cycle using the arrow keys on the keyboard or the arrow buttons on the screen. Then click on a program button (**D**, **1**, **2**, or **3**) in the **Program Sets** section to assign a program to the highlighted cycle.

9. Click the **Save** button to save changes, or click the **Save As** button to save those changes to a new file. Enter the new name and click **OK**.

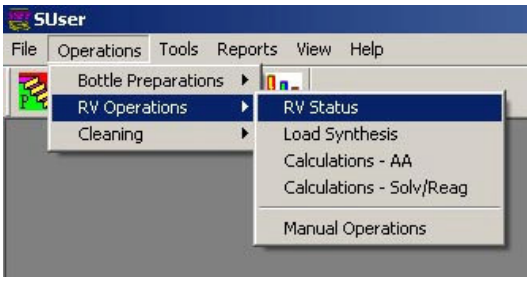
10. Use the **Cancel** button to remove any changes since the last **Save**. A screen will appear reading, “Abandon All Changes?” The **Yes** button permanently removes the changes; the **No** button leaves the changes.

11. To print the open synthesis file click the **Print** button. It will go automatically to the printer.

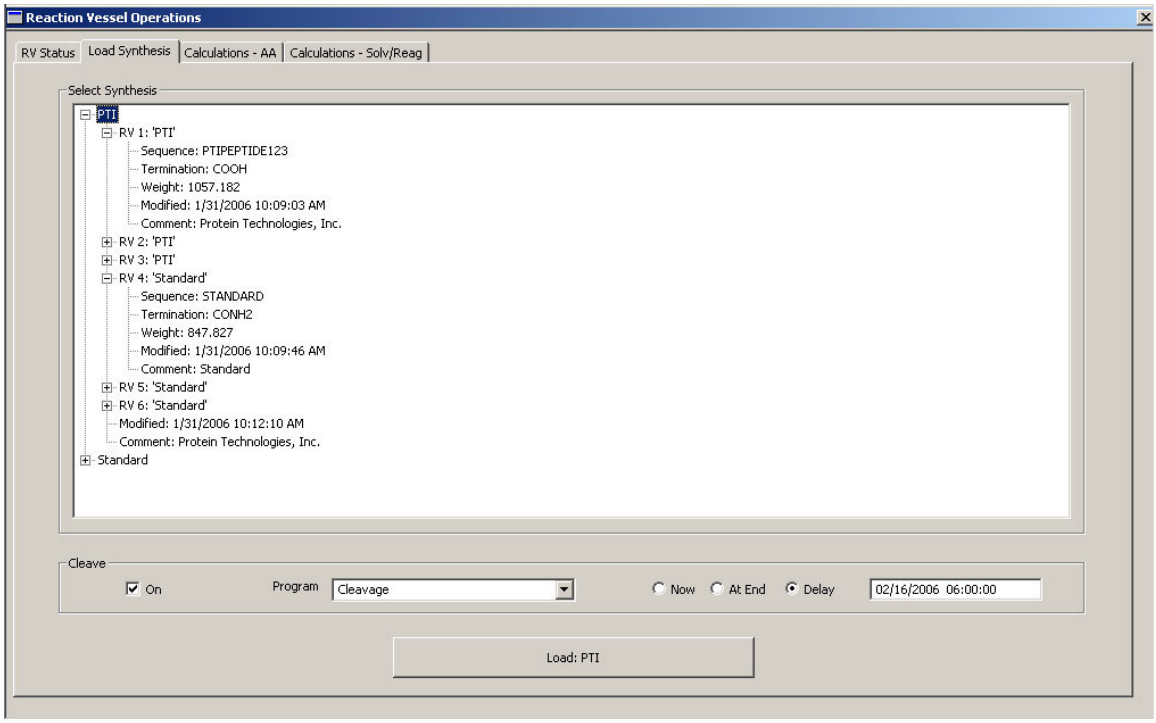
12. Click the **Close** button to exit the **Synthesis Editor**. The **Close** button activates after the file is saved.

3.7 Load Synthesis

1. To load a synthesis, click on the **Operations** menu, select **RV Operations**, and then select **Load Synthesis**.



2. The **Reaction Vessel Operations** screen opens to the **Load Synthesis** tab.



3. In the **Select Synthesis** section, click on a synthesis name to select it. The **Load** button at the bottom of the screen shows the name of the selected synthesis.

4. If on-instrument cleavage is desired, turn on the cleave option by clicking in the **On** box. When the box is checked, the functions in the **Cleave** section activate.

5. Select a cleavage program using the pull-down menu in the **Program** box.

6. Set the cleavage start time by selecting one of three options:

    a. **Now** – To start the cleavage immediately, click the circle next to **Now**.

    b. **At End** – To start the cleavage at the end of the synthesis click the circle next to **At End**.

    c. **Delay** – To delay the start of the cleavage program, click the circle next to **Delay**, then enter a date and time to start the cleavage program. There is no need to delete or backspace in the date box. Type over the date and time to change the numbers.

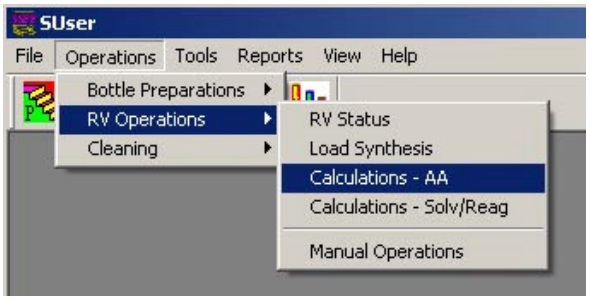
7. Click the **Load** button. This loads the synthesis onto the **RV Status** screen and opens the **Calculations - AA** screen.

3.8 Calculations

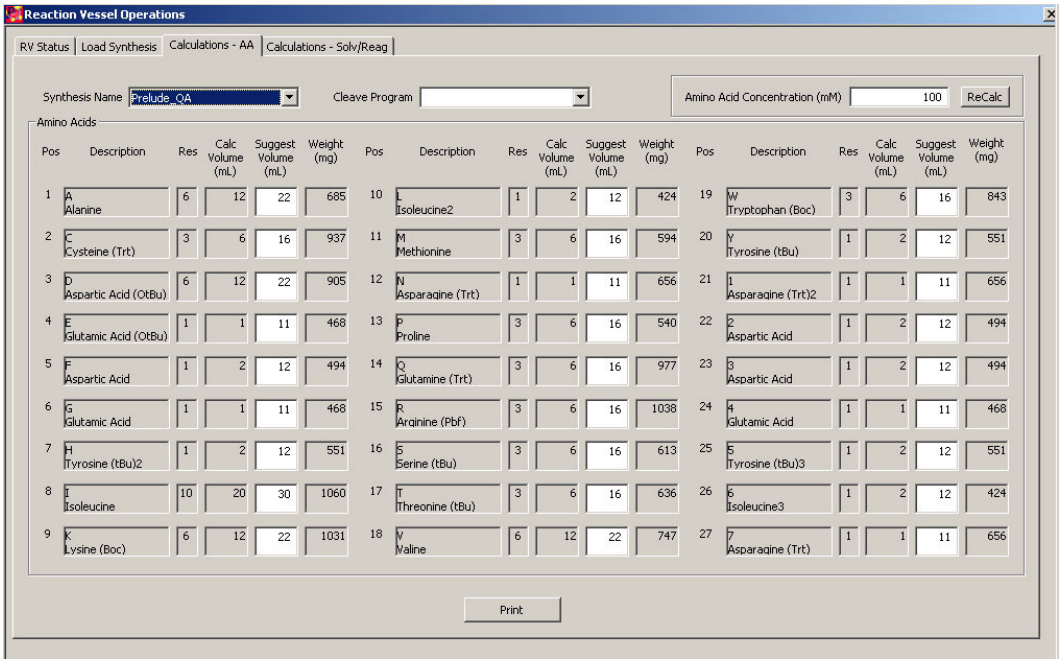
To aid the preparation of the synthesis solutions and resin, use the calculations screens as described below.

To calculate amino acid amounts:

1. Open the **Calculations – AA** screen by clicking on the **Operations** menu, selecting **RV Operations** and then selecting **Calculations – AA**:



or load a synthesis in the **Load Synthesis** screen and the **Calculations – AA** screen opens automatically.



2. Select the desired synthesis using the pull-down menu in the **Synthesis Name** box.

3. Input the desired amino acid concentration (in mM) in the **Amino Acid Concentration (mM)** box.

4. Click the **Recalc** button. The software calculates the minimum volume necessary for the synthesis and displays the value in the **Calc Volume (mL)** column.

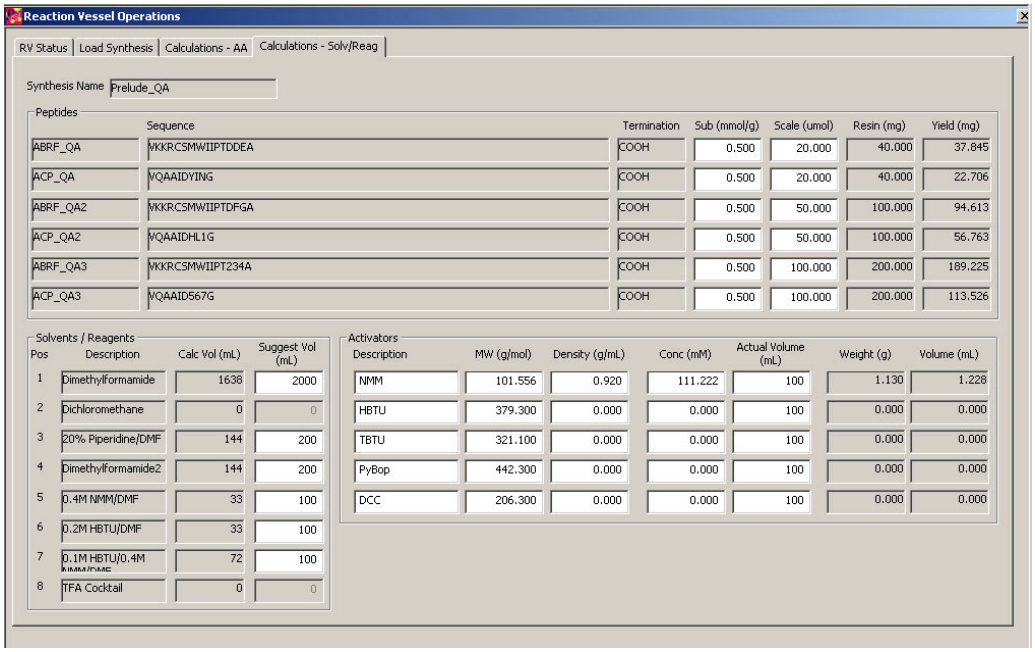
5. The suggested volume for the synthesis is displayed in the **Suggest Volume (mL)** column. Double click in this column to change the values.

CAUTION It is not recommended to use less than the minimum volume. Amino acid bottle may run out of fluid during the synthesis.

6. The software calculates the amount of dry amino acid needed to make up the solutions based on the **Suggest Volume (mL)** and **Amino Acid Concentration (mM)** inputted values. These amounts are displayed in mg in the **Weight (mg)** column.

To calculate resin amounts:

1. Open the **Calculations – Solv/Reag** screen by clicking on the **Calculations – Solv/Reag** tab in the **Reaction Vessel Operations** screen, or if the **Reaction Vessel Operations** screen is closed, using the shortcut button, or the **Operations** menu as described at the beginning of this section.



2. The **Synthesis Name** box will display the synthesis selected in the **Calculations – AA** screen.

3. Input the substitution of the resin (in mmol/g) used for each RV in the **Sub (mmol/g)** column in the **Peptides** section. Input the synthesis scale (in umol) for each RV in the **Scale (umol)** column.

4. The software calculates the resin amount (in mg) and expected peptide yield (in mg) for the synthesis and displays the values in the **Resin (mg)** and **Yield (mg)** columns, respectively.

To calculate solvent/reagent/activator amounts:

1. In the **Solvents/Reagents** section, the software calculates the minimum volume (in mL) needed for each solvent/reagent, and displays the value in the **Calc Vol (mL)** column. The suggested volume for each bottle is displayed in the **Suggest Vol (mL)** box. Double click in this column to change the values.

CAUTION It is not recommended to use less than the minimum volume. Solvent/reagent bottle may run out of fluid during the synthesis.

2. In the **Activators** section, input the volume (in mL) for the activator solution in the **Actual Volume (mL)** box. Input the concentration of the activator solution (in mM) in the **Conc (mM)** column.

3. The software calculates the volume (in mL) and/or amount of dry activator (in g) needed to make up the activator solutions based on the **Actual Volume (mL)** and **Amino Acid Concentration (mM)** inputted values, and the **MW (g/mol)** and **Density (g/mL)** values. These amounts are displayed in the **Volume (mL)** and **Weight (g)** columns, respectively.

To print calculated values:

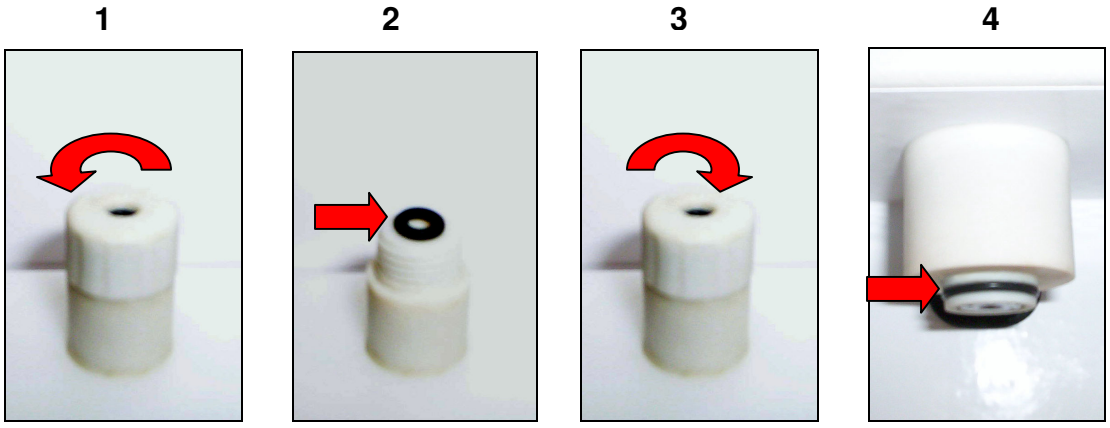
1. Return to the **Calculations – AA** screen by clicking on the **Calculations – AA** tab.

2. Click on the **Print** button to print the calculated values from both the **Calculations – AA** and **Calculations – Solv/Reag** screens.

NOTE Use the **Print** button only after modifying both the **Calculations – AA** and the **Calculations – Solv/Reag** screens to avoid having to print twice.

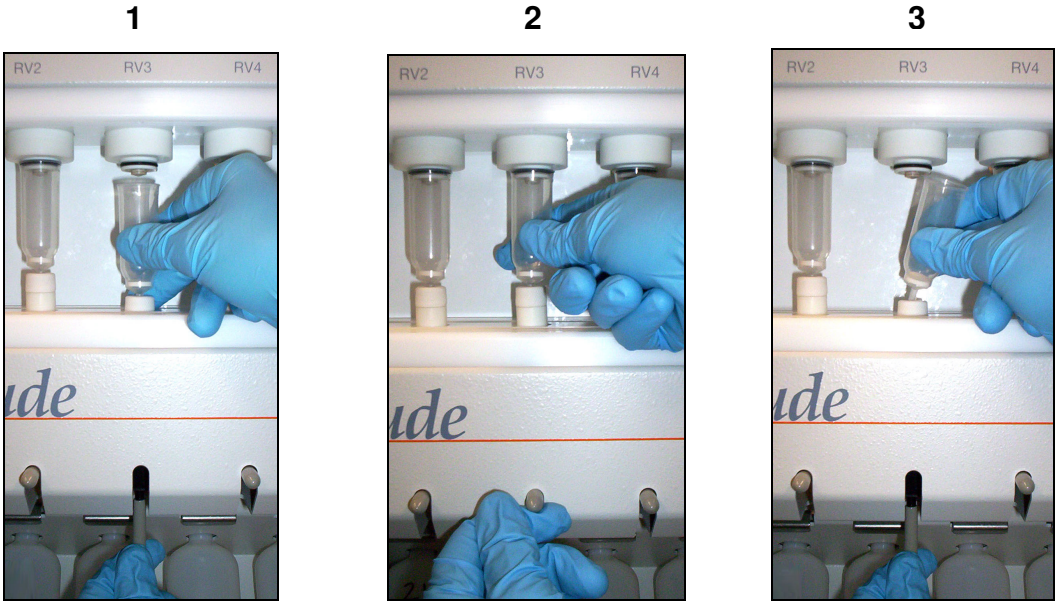
3.9 Instrument Setup

3.9.1 Reaction Vessel O-Ring Installation



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. Tighten the cap over the o-ring.
4. Install a reaction vessel top o-ring on the upper RV seat on the instrument.

3.9.2 Reaction Vessel Installation



1. Lower the cam lever to the vertical position. Push the RV firmly into the lower seat first, then line up with the upper seat.

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

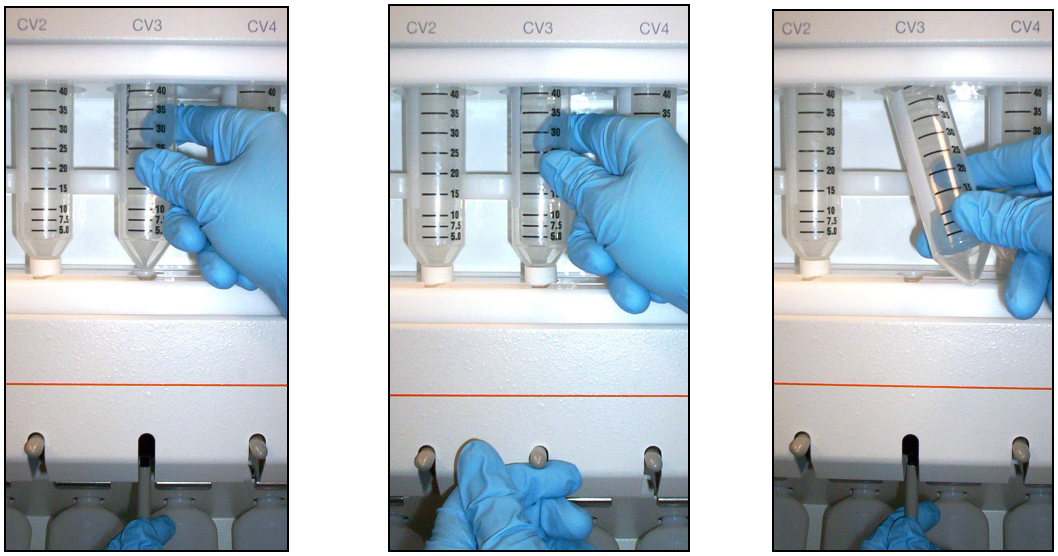
2. Raise the cam lever up into a horizontal position to lock the RV in place.

3. To remove, hold the RV with one hand while moving the cam lever into the vertical position. Pull the top of the RV away from the instrument, then pull the RV up out of the lower seat.

3.9.3 Collection Vial Installation

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

1 **2** **3**



1. Lower the cam lever to the vertical position. Place the uncapped vial into the upper seat first, then line up with the lower seat.

2. Raise the cam lever into the horizontal position to lock the collection vial in place.

3. To remove, hold the collection vial with one hand while lowering the cam lever to the vertical position with the other hand. Tilt the vial and remove from the instrument.

3.9.4 Amino Acid Bottle Installation

To install an amino acid bottle, first make sure the bottle position is vented and backflushed with nitrogen (See **Bottle Preparations** screen, Section 3.10) then:

1 **2** **3**

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

2. The metal slide 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 all of the reagent in the bottle will be used.

3. To release the bottle, make sure the bottle position is vented and backflushed with nitrogen. Then hold the amino acid bottle with one hand while pushing in the metal slide with the other.

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

3.9.5 Solvent/Reagent Bottle Installation

1. Vent the solvent/reagent bottle position (See **Bottle Preparations** screen, Section 3.10).

2. Verify the o-ring is properly installed in the bottle insert and the insert is in the cap. Verify that the solution line has a bottle filter with frit attached.

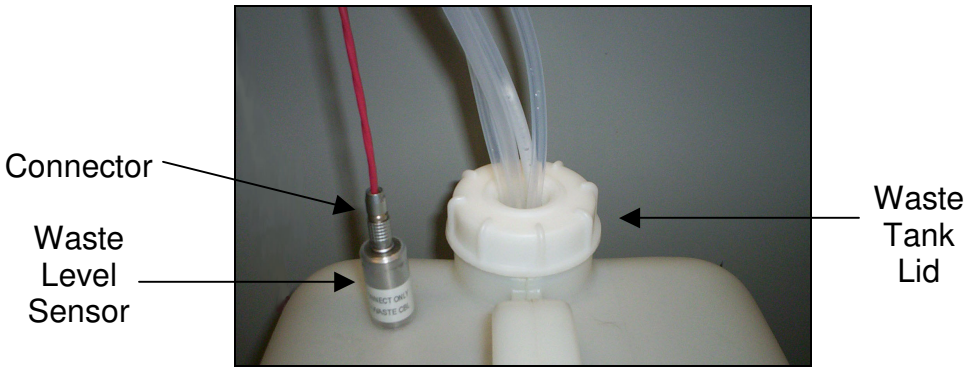
3. Place the bottle in the bottle container. Insert the line so that it is straight and at the bottom of the bottle (FEP tubing can be 'molded' by gentle bending—Do not 'kink' or the tubing integrity will be compromised).

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

To remove the bottle, unscrew the cap while the bottle position is vented.

3.9.6 Waste Container Installation

To install the waste container:



1. Place the waste container into the secondary containment vessel.

2. Plug the connector into the waste level sensor located at the top of the waste container, and plug the other end into the waste level sensor port located on the utility panel on the side of the unit.

3. Connect three 1/4" waste lines to the waste ports on the solvent feedthrough panel and insert them into the waste tank lid.

4. Connect the 1/4" vent line to the exhaust vent and insert the other end into the waste tank lid.

5. Screw the lid onto the waste tank.

To empty:

1. Backflush all bottles.

2. Disconnect the waste level sensor connector by grasping the textured area firmly and pulling directly up. Unscrew the lid.

3. Empty the waste container and place it back into the secondary containment vessel.

4. Screw the lid back on and reconnect the waste level sensor, being careful to line up the two pins before applying pressure.

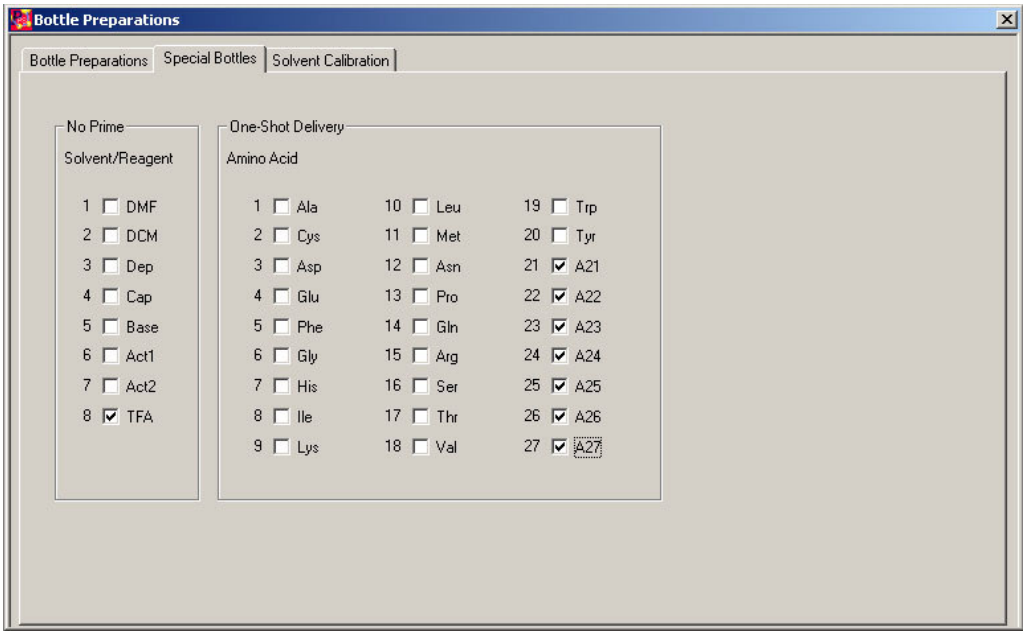
3.10 Bottle Preparations

To pressurize and prime the bottles prior to a synthesis:

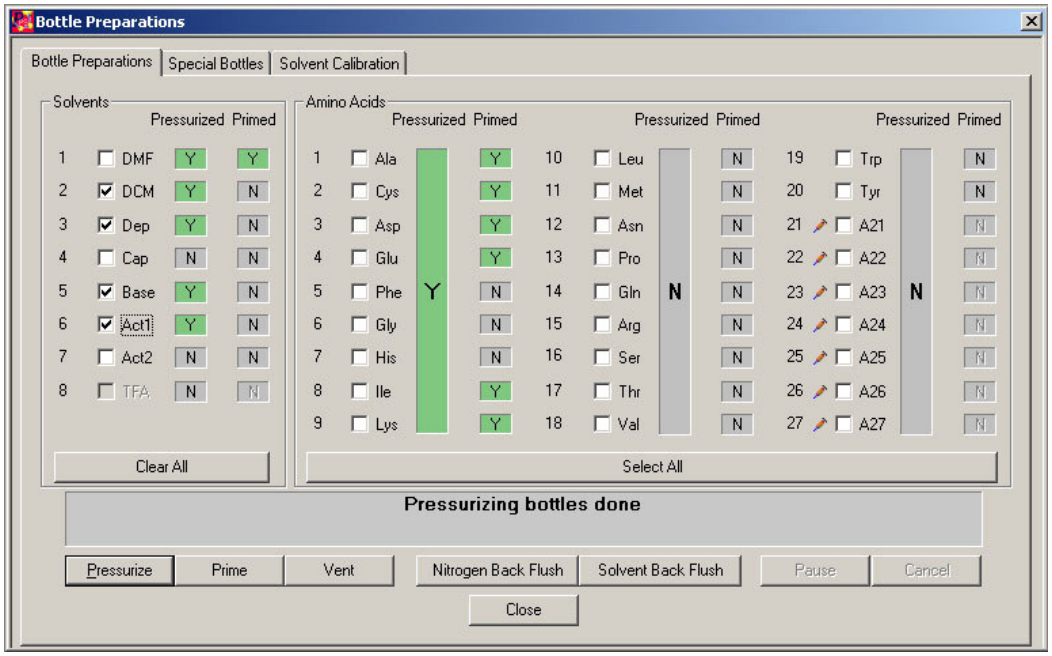
1. Click on the shortcut button:

2. This opens the **Bottle Preparations** screen with the **Bottle Preparations** tab active.

3. If a one-shot delivery is desired, click on the **Special Bottles** tab. Then, click in the box(es) to place a checkmark in the appropriate amino acid bottle position(s).



4. In the **No Prime** section, make sure only the TFA bottle is selected. Return to the **Bottle Preparations** screen by clicking on the **Bottle Preparations** tab.



5. The TFA bottle should be inactive, and a syringe icon should appear next to amino acid bottle positions selected for one-shot deliveries.

6. Select the bottle positions necessary for the synthesis (except the TFA bottle).

7. Click on the **Prime** button to pressurize and prime all selected bottles. Because amino acid bottle positions 1-9, 10-18 and 19-27 share common pressure manifolds, when one bottle in the row is selected and pressurized, all bottles in the row are pressurized. When pressurization is complete, the **Pressurized** column will display a "**Y**" next to the selected bottles. When priming is complete, the **Primed** column displays a "**Y**" next to the selected bottles. Allow a minute for the pressure inside the bottle(s) to stabilize.

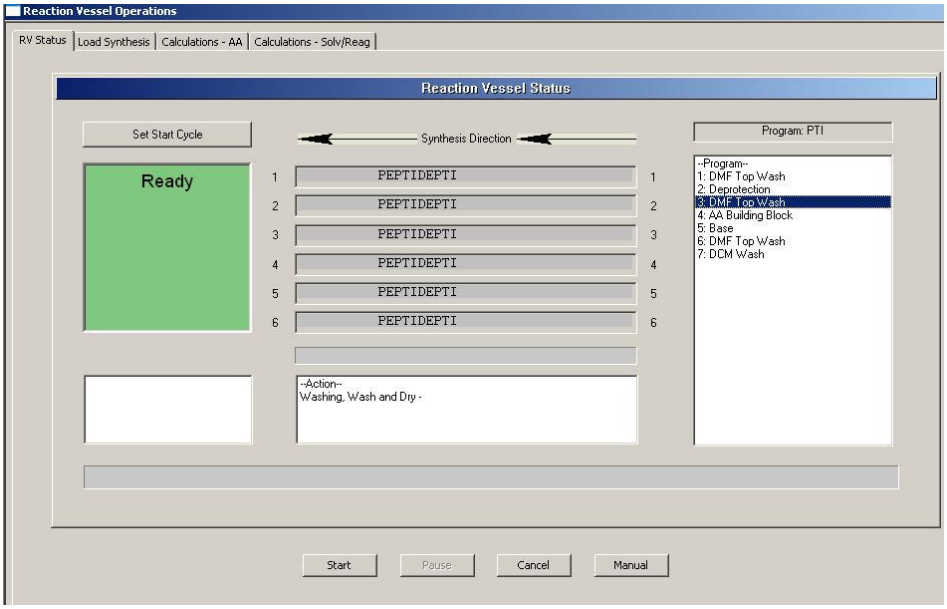
The bottles are now ready for the synthesis.

3.11 Start a Synthesis

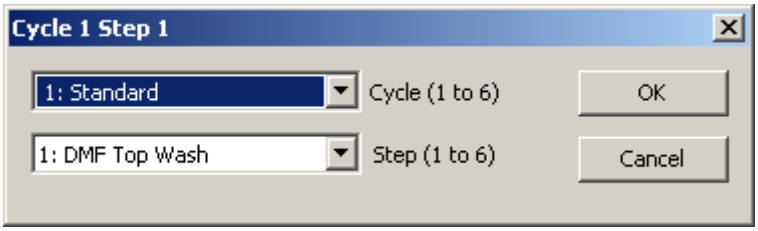
After a synthesis is loaded, and the bottles are pressurized and primed, the synthesis may be started.

1. To start a synthesis, open the **Reaction Vessel Operations** screen by clicking on the shortcut button:

2. The **Reaction Vessel Operations** screen opens with the **RV Status** tab active.



3. By default, the synthesis starts at cycle 1, step 1. To change the starting cycle and step of the synthesis, click on the **Set Start Cycle** button. This opens a new window:



Use the upper pull-down menu to select a starting cycle. Use the lower pull-down menu to select a starting step. Click the **OK** button to accept the changes, or click the **Cancel** button to return to the **RV Status** screen without changing the start settings.

4. After clicking **OK**, a SUser screen appears with the message, “This will set the synthesis to Cycle < # >, Step< # >. Are you sure?” Click the **Yes** button to continue or **No** to cancel.

5. Click the **Start** button to start the synthesis.

6. Click the **Pause** button to pause the synthesis.

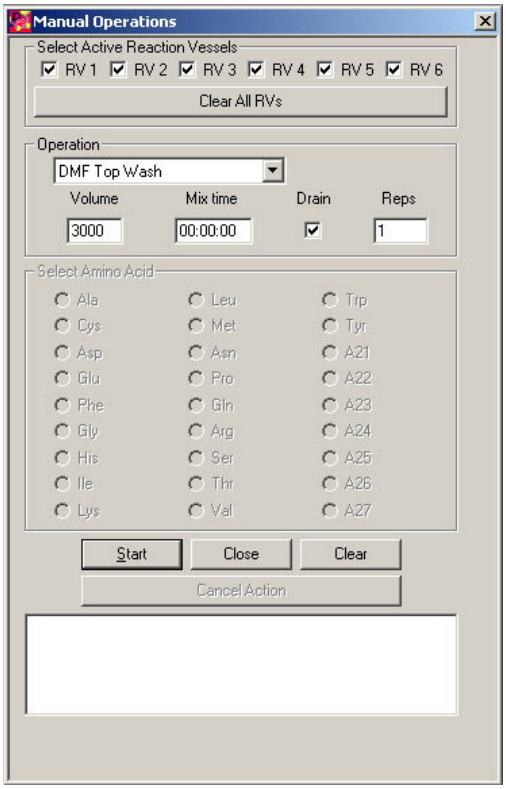
7. Click the **Cancel** button during a pause or before the synthesis is started to delete the synthesis from the **RV Status** screen.

If it is necessary to cancel or make an emergency stop use the **Cancel/E-Stop** button. The **Cancel** button will delete the synthesis from the **RV Status** screen. While a synthesis is running, the **E-Stop** button is active. The **E-Stop** button ends the synthesis immediately and cancels the synthesis.

CAUTION There may be fluid left in the line after using the **E-Stop**. Run a manual DMF wash step to avoid contaminating the next fluid delivery as follows:

1. Click on the shortcut button:

This will open the **Manual Operations** screen.



2. Click on the **Select All RVs** button to select all RVs. When selected, the **Select All RVs** button will change to the **Clear All RVs** button.

3. Use the pull-down menu in the **Operation** section to select **DMF Top Wash**. Input 3000 in the **Volume** box. 00:00:00 should be entered under **Mix time**, the **Drain** box should be checked, and 1 should be entered under **Reps**.

4. Click **Start**. Click the **Cancel Action** to cancel the running operation.

5. When finished, click the **Close** button to close the **Manual Operations** screen.

Chapter 4: Post-Synthesis Procedures

1. Remove collection vials and work up peptides.

2. **Nitrogen Backflush** all bottles used in the synthesis using the **Bottle Preparations** screen (Section 4.1).

3. Discard or store used chemicals.

4. Replace used bottles with empty bottles. Place empty RV's and collection vials on the instrument.

5. Place methanol in Solvent Bottle 2 and perform a **System Clean** (Section 4.2).

6. Empty all bottles and collection vials of rinse solution.

7. **Nitrogen Backflush** all bottles using the **Bottle Preparations** screen (Section 4.1) to remove residual methanol from the system.

8. Empty the waste container.

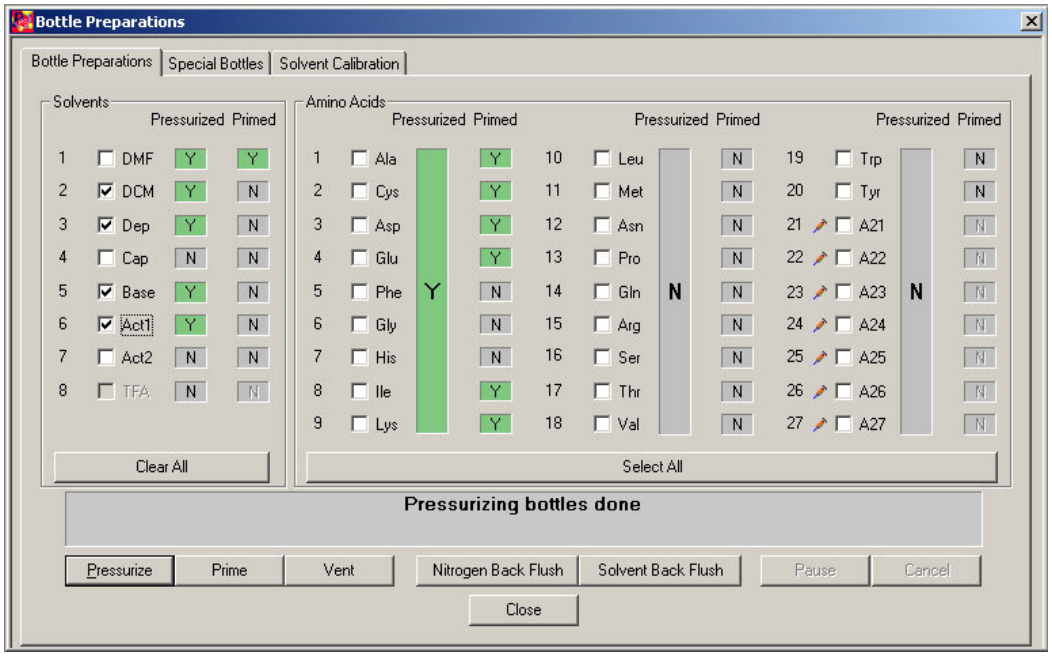
NOTE If time is a concern, DMF may be substituted for methanol. If DMF is used, it is not necessary to **Nitrogen Backflush** all the bottles after the **System Clean**.

4.1 Nitrogen Backflush

To vent and nitrogen backflush all bottles after a synthesis:

1. Click on the shortcut button:

2. This opens the **Bottle Preparations** screen with the **Bottle Preparations** tab active.



3. Select the bottle positions used in the synthesis (except the TFA bottle).

4. Click on the **Nitrogen Back Flush** button to vent and backflush all selected bottles with nitrogen. When venting is complete, the **Pressurized** column will display an "**N**" next to the selected bottles. When nitrogen backflush is complete, the **Primed** column displays an "**N**" next to the selected bottles.

The bottles are now ready to be removed from the instrument.

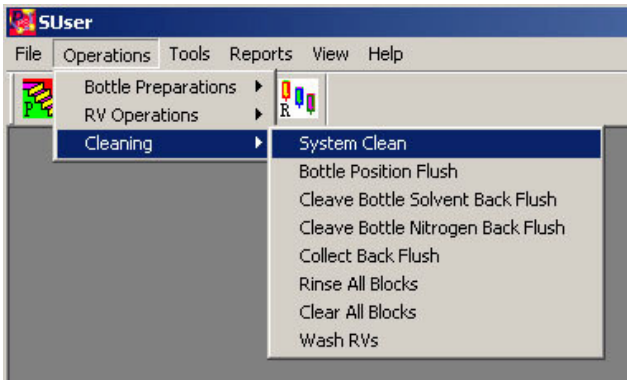
4.2 System Clean

To perform a **System Clean**:

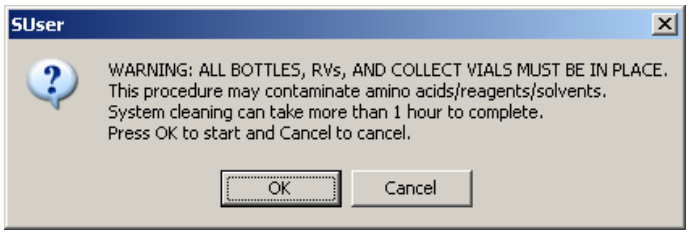
1. Place empty amino acid and solvent/reagent bottles in all positions. Place empty RVs and collection vials in position.

2. Place 1 L of methanol in the Solvent 2 bottle.

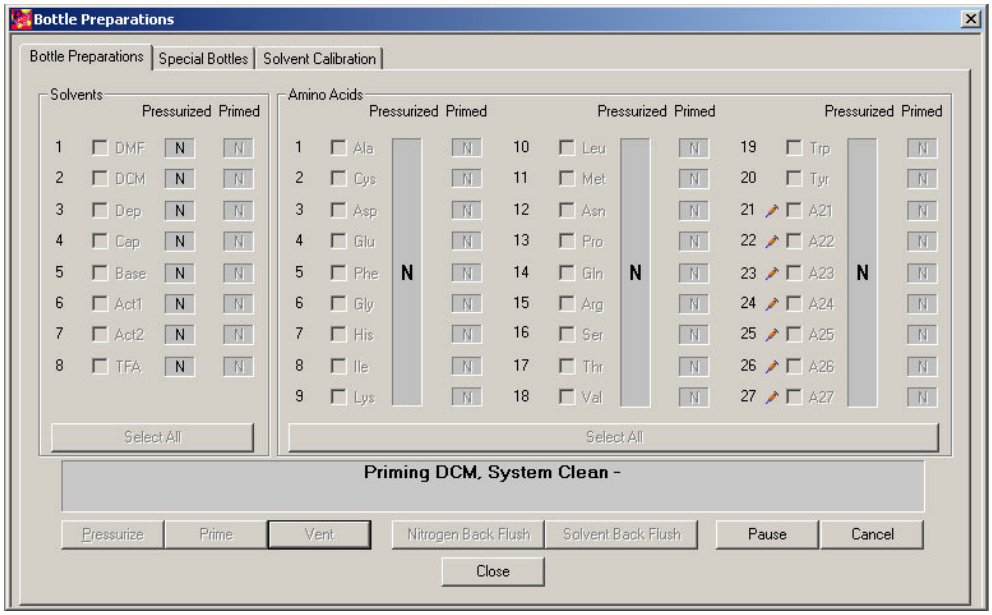
3. Click on the **Operations** menu, select **Cleaning**, and then select **System Clean**.



4. The following warning window will open. Verify all bottles, RVs and collection vials are in place, then click **OK** to start the procedure or **Cancel** to exit the cleaning procedure.



5. During the **System Clean**, the **Bottle Preparations** screen will open.



The status of the operation will be displayed in the status bar. Click the **Pause** button to pause the operation. Click **Resume** to resume a paused operation. Click the **Cancel** button to cancel the operation. Click the **Close** button to close the **Bottle Preparations** screen.

6. After the cleaning operation is complete, remove the bottles and collection vials, dispose of the rinse solution, and replace the bottles and collection vials with clean ones for the next synthesis.

NOTE The **System Clean** takes 1 hour to complete.

CAUTION Remove all chemicals and resins from the Prelude before the **System Clean** because they will be contaminated with methanol.

Appendix

A. Reagents for Peptide Synthesis

A.1 Prelude Bottles Containing N-Fmoc-Protected Amino Acids, Preweighed

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

Prelude Bottles Containing N-Fmoc-Protected Amino Acids, Preweighed, cont.

Catalog No.	Description	Quantity
SMP-05-M SMP-10-M SMP-20-M	FMOC-L-Met	5 mmol 10 mmol 20 mmol
SMP-05-F SMP-10-F SMP-20-F	FMOC-L-Phe	5 mmol 10 mmol 20 mmol
SMP-05-P SMP-10-P SMP-20-P	FMOC-L-Pro	5 mmol 10 mmol 20 mmol
SMP-05-SB SMP-10-SB SMP-20-SB	FMOC-L-Ser(tBu)	5 mmol 10 mmol 20 mmol
SMP-05-TB SMP-10-TB SMP-20-TB	FMOC-L-Thr(tBu)	5 mmol 10 mmol 20 mmol
SMP-05-W SMP-10-W SMP-20-W	FMOC-L-Trp	5 mmol 10 mmol 20 mmol
SMP-05-WBC SMP-10-WBC SMP-20-WBC	FMOC-L-Trp(Boc)	5 mmol 10 mmol 20 mmol
SMP-05-YB SMP-10-YB SMP-20-YB	FMOC-L-Tyr(tBu)	5 mmol 10 mmol 20 mmol
SMP-05-V SMP-10-V SMP-20-V	FMOC-L-Val	5 mmol 10 mmol 20 mmol

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

Catalog No.	Description	Quantity
B-25-A B-100-A	FMOC-L-Ala	25 g 100 g
B-25-RP B-100-RP	FMOC-L-Arg(Pmc)	25 g 100 g
B-25-RBF B-100-RBF	FMOC-L-Arg(Pbf)	25 g 100 g
B-25-NT B-100-NT	FMOC-L-Asn(Trt)	25 g 100 g
B-25-DB B-100-DB	FMOC-L-Asp(OtBu)	25 g 100 g
B-25-CT B-100-CT	FMOC-L-Cys(Trt)	25 g 100 g
B-25-EB B-100-EB	FMOC-L-Glu(OtBu)	25 g 100 g
B-25-QT B-100-QT	FMOC-L-Gln(Trt)	25 g 100 g
B-25-G B-100-G	FMOC-L-Gly	25 g 100 g

Bulk N-Fmoc-Protected Amino Acids, Preweighed, cont.

Catalog No.	Description	Quantity
B-25-HT B-100-HT	FMOC-L-His(Trt)	25 g 100 g
B-25-I B-100-I	FMOC-L-Ile	25 g 100 g
B-25-L B-100-L	FMOC-L-Leu	25 g 100 g
B-25-KBC B-100-KBC	FMOC-L-Lys(Boc)	25 g 100 g
B-25-M B-100-M	FMOC-L-Met	25 g 100 g
B-25-F B-100-F	FMOC-L-Phe	25 g 100 g
B-25-P B-100-P	FMOC-L-Pro	25 g 100 g
B-25-SB B-100-SB	FMOC-L-Ser(tBu)	25 g 100 g
B-25-TB B-100-TB	FMOC-L-Thr(tBu)	25 g 100 g
B-25-W B-100-W	FMOC-L-Trp	25 g 100 g
B-25-WBC B-100-YB	FMOC-L-Trp(Boc)	25 g 100 g
B-25-YB B-100-YB	FMOC-L-Tyr(tBu)	25 g 100 g
B-25-V B-100-V	FMOC-L-Val	25 g 100 g

A.3 Reagents & Kits

Catalog No.	Description	Quantity
	Reagents:	
PS3-PPR-L	20% Piperidine/DMF (Dep)	0.9 L
PS3-MM-L	0.4 N-Methylmorpholine/DMF (Act)	0.9 L
B-100-HBTU B-500-HBTU B-1K-HBTU	HBTU	100 g 500 g 1 kg
	Start-Up Kits:	
PPS-TEST	FMOC Amino Acid Start-up Kit for the PRELUDE. Contains 27 amino acids; 0.9 L Deprotectant; 0.9 L 0.4M NMM; 100 g HBTU; 200 mg Fmoc-Ala-Wang resin; 200 mg Fmoc-Gly-Wang resin.	1 ea.

B. Replacement Parts & Accessories

Catalog No.	Description	Quantity
	Accessories:	
PPS-R10-030 PPS-R10-090 PPS-R10-180	Reaction Vessel, 10 mL PP	Pkg of 30 Pkg. of 90 Pkf of 180
PPS-R40-030 PPS-R40-090 PPS-R40-180	Reaction Vessel, 40 mL PP	Pkg. of 30 Pkg. of 90 Pkg. of 180
2720001 2720002	O-Ring, Reaction Vessel Bottom O-Ring, Reaction Vessel Top	1 ea. 1 ea.
3030001	Bottle, 10 mL One Shot AA	1 ea.
SMP-VX-20 SMP-VX-100	Bottle, 120 mL AA	Pkg. of 20 Pkg. of 100
AAR-400-I AAR-400-X	Bottle, 400 mL AA	1 ea. Pkg. of 10
SMP-270044	Seal, AA Bottle	Pkg. of 20
3110004	Collection vial, 50 mL	Pkg. of 50
	Replacement Parts:	
3000008 3000004	Bottle, 1 L Amber Bottle, 4L Amber	1 ea. 1 ea.
SMP-260205	Cap for 1 and 4 L Bottles	1 ea.
2700042	O-Ring for 1 and 4 L Bottles	1 ea.
SMP-RF-100 SON-270060	Bottle Filter, 1/8 OD Tubing Bottle Filter, 3/16 OD Tubing	1 ea. 1 ea.
2600187 2600262	Bottle Filter Housing, 1/8 OD Tubing Bottle Filter Housing, 3/16 OD Tubing	1 ea. 1 ea.
PPS-271002-12	Gasket, Collect Assembly	Pkg. of 12
0100096	Waste Tank Assembly	1 ea.
3500004	Ferrule, 1/8 OD Tubing	1 ea.
3500041	Ferrule, 1/16 OD Tubing	1 ea.
3500071	Ferrule, 3/16 OD Tubing	1 ea.
3510001	Fitting Nut, 1/8 OD Tube Headless	1 ea.
3510002	Fitting Nut, 1/16 OD Tube Headless	1 ea.
3500070	Fitting Nut, 3/16 OD Tube	1 ea.
4830003	Fuse, 4A, 250VAC, AGC Type	1 ea.
4830001	Fuse, 5A, 250VAC, AGC Type Fast Acting	1 ea.
4830002	Fuse, 2A, 250VAC, AGC Type	1 ea.

Catalog No.	Description	Quantity
	Tools:	
0100129	Meter, Nitrogen Flow	1 ea.
6800018	Fixture, Vacuum Test	1 ea.
6800019	Fixture, Pressure Test	1 ea.
6800023	Tool, 3/16 Tube Fitting Nut	1 ea.
6800027	Tool, 1/8 Tube Headless Fitting Nut	1 ea.

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