

Liberty Blue™

Automated Microwave Peptide Synthesizer

Manual



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Introduction

About the Instrument

This manual is intended for use by both novice and experienced users for operation and maintenance of the Liberty Blue Automated Microwave Peptide Synthesizer. Installation of the Liberty Blue must be completed by a CEM certified service technician. Additional information, including, but not limited to: training notes and videos, application notes, software updates, and parts can be viewed on CEM's website at <http://cem.com/peptide-synthesis>. A registered account is required for certain downloads.

This manual refers to Liberty Blue Software version 2.2 for all software information, including screen shots and technical information.

Common Abbreviations and Units

Abbreviation	Definition
°C	degrees Celsius
AA	amino acid
ACP	acyl carrier protein 65-74
DIC	N,N'-Diisopropylcarbodiimide
DMF	N,N'-dimethylformamide
Fmoc	9-fluorenylmethyloxycarbonyl
g	gram
Hz	Hertz
ID	inner diameter
L	liter
m	meter
mg	milligram

Abbreviation	Definition
mL	milliliter
mm	millimeter
mmol	millimole
MW	molecular weight
OD	outer diameter
Oxyma Pure	ethyl 2-cyano-2-(hydroxyimino)acetate
PN	Part Number
psi	pounds per square inch
RV	reaction vessel
s	seconds
VAC	volts of alternating current
W	Watts

How To Obtain Support

Applications Support

For the latest Liberty Blue applications information, go to <http://www.cem.com/liberty-blue.html>. The CEM Liberty Blue pages contain downloadable applications notes, a listing of recent microwave peptide synthesis publications, and more. A registered account is required for download.

CEM is proud to provide applications support for any peptide synthesis related questions from a team of trained chemists with a complete peptide synthesis lab. For applications support, call (800) 726-3331 (inside the US) or (704) 821-7015 and ask for “Peptide Applications”, or email CEM Liberty Blue applications support at peptide.support@cem.com.

Technical Support

For the latest technical support information, go to <http://www.cemservice.us>. The CEM Service website provides access to the CEM Knowledge Base, which contains helpful troubleshooting information. From the website requests for phone or email support can also be submitted.

CEM is proud to provide technical support for the Liberty Blue from a team of specially trained Service Technicians. For technical support in the US and Canada, call (800) 726-5551 or (704) 821-7015 and ask for “Liberty Blue Service”. For technical support outside the US and Canada, contact your local CEM Subsidiary or Distributor.

Requested Information

When contacting CEM for support, please provide the following information about the instrument:

Liberty Blue Serial Number

Liberty Blue Firmware Version

Discover Serial Number

Discover Firmware Version

Liberty Blue Software Version Number and Build Number

The report for the synthesis that was running when the error occurred

Troubleshooting Bundle (see below)

Firmware versions (both Liberty Blue and Discover) and Liberty Blue software version can be found within Liberty Blue Software from the About Liberty Blue menu (Help Tab). Serial numbers can be found on the silver tags on the rear of the Liberty Blue and on the side of the Discover.

In the event of a crash, the software will automatically create a Troubleshooting Bundle to be emailed to CEM support for diagnosis. A Troubleshooting Bundle can be manually created from the Help Menu.

CEM Corporation Headquarters

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CEM Distributors

For a complete list of distributors of CEM products, including contact information, go to the CEM website (<http://www.cem.com>), select Contact CEM, and then select your region to see a list of distributors by country.

Safety

Safety Notations

This manual uses three safety alert words at points in the documentation where the user should be aware of potential hazards. The safety alerts are shown in color-coded boxes. The three words—NOTE, CAUTION, and WARNING—indicate differing levels of observation or action as described below:

NOTE

A **NOTE** is intended to provide emphasis of procedures that may be misinterpreted or overlooked, or to otherwise clarify confusing situations.

CAUTION

A **CAUTION** is intended to provide essential information and to emphasize procedures which, if not strictly followed, may result in improper instrument operation.

WARNING

A **WARNING** is intended to emphasize dangerous or hazardous conditions which may result in personal injury to the user and damage or destruction of the instrument.

Personal Laboratory Safety

Solvents and chemical reagents used on the Liberty Blue can be hazardous and should only be handled under a fume hood. Personal protective gear, including but not limited to safety glasses, chemical resistant gloves, and a laboratory coat are highly recommended when operating the Liberty Blue. For detailed information on the safety requirements for the chemicals used on the Liberty Blue, refer to the appropriate SDS documents.

Waste Disposal

Waste produced by the Liberty Blue can be hazardous. For detailed information on the safety requirements for the chemicals used on the Liberty Blue, refer to the appropriate SDS documents.

WARNING

Handle all waste under a fume hood, and wear suitable protective clothing such as safety glasses, chemical resistant gloves, and a laboratory coat. Dispose of all waste in accordance with all applicable local, state, and federal health and safety recommendations.

Fume Ventilation

The Liberty Blue operates as a semi-closed system, with minimal venting of any hazardous solvent fumes through the vent lines coming from the side of the unit. The vent lines must be vented into a proper chemical fume hood or exhaust line no longer than six feet (6')/two meters (2 m) from the instrument.

In addition, adequate ventilation should be provided for preparation of reagents and solvents for use on the system. All solvent bottles and the Waste Reservoir should be placed into proper secondary containers to minimize the risk of exposure.

Operating Precautions

The Liberty Blue must be grounded. In the event of an electrical short circuit, grounding reduces the risk of electric shock by providing an escape wire for electric current. This instrument is equipped with a cord having a grounding wire with a grounding plug. The plug must be plugged into an outlet that is properly installed and grounded. Consult a qualified electrician or service technician if the grounding instructions are not completely understood or if doubt exists as to whether the instrument is properly grounded. If it is necessary to use an extension cord, use only a 3-wire extension cord that has a 3-blade grounding plug and a 3-slot receptacle that will accept the plug from the instrument. The marked rating of the extension cord must be equal to or greater than the electrical rating of the instrument.

Do not replace detachable main supply cord with inadequately rated cords.

The possibility of instrument-induced electromagnetic interference (EMI) is minimal if the instrument is operated as outlined in this manual. The instrument should not be placed close to any electrical device susceptible to EMI.

This instrument utilizes high voltages and microwave radiation. Only those trained in repair and maintenance of high voltage and microwave power systems should perform instrument service and repair.

This instrument complies with United States Code of Federal Regulations 21 CFR Part 1030.10 (C) for microwave leakage. This instrument complies with FCC Requirements in the United States Code of Federal Regulations (47CFR Part 18) – Industrial, Scientific and Medical (ISM) Equipment – emissions requirements.

WARNING

Only accessories which meet the manufacturer's specifications shall be used.

Do not attempt to lift or move the Liberty Blue system. Contact CEM Corporation if the Liberty Blue system needs to be moved.

If instrument is used in a manner not specified by CEM Corporation in this manual, then the protection provided by the equipment may be impaired.

System Requirements

Bench and/or Fume Hood Space

The Liberty Blue should be positioned on the bench such that access to the electrical outlets for the system is not restricted.

System Components Space Requirements

Liberty Blue Instrument (Discover Module and Liberty Blue Module):

20 in (50.8 cm) W x 21 in (53.3 cm) D x 30 in (76.2 cm) H

(Depth includes 3" (7.62 cm) clearance behind instrument for unimpeded airflow at rear fan ducts)

CEM Supplied Controller:

15 in (38.1 cm) W x 10 in (25.4 cm) D x 16 in (40.6 cm)

(Height includes 14.1" (35.8 cm) display)

Environmental Conditions

The Liberty Blue is designed for indoor use only.

Temperature Range: 50 °F – 85 °F (10 °C – 29 °C)

Relative Humidity Range: 10 – 85%

Inert Gas Source

The Liberty Blue requires an inert gas source (either high purity grade nitrogen or argon) capable of supplying 25 psi (20 L/min flow) within ten feet (10')/three meters (3 m) of the right side of the instrument.

Electrical Requirements

The Liberty Blue requires electrical power of 120 VAC (60 Hz, 1.7 A) (or 240 VAC [50 Hz, 1.7 A] where applicable). Specific power requirements (120 VAC vs. 240 VAC) can be found on the nameplate affixed to the rear of the Liberty module and on the side of the Discover module.

Three (3) grounded electrical connections providing a total of 10 A are required for all components:

Liberty Blue power cord

Router power cord

Controller (laptop computer) power cord

Fuse Replacement

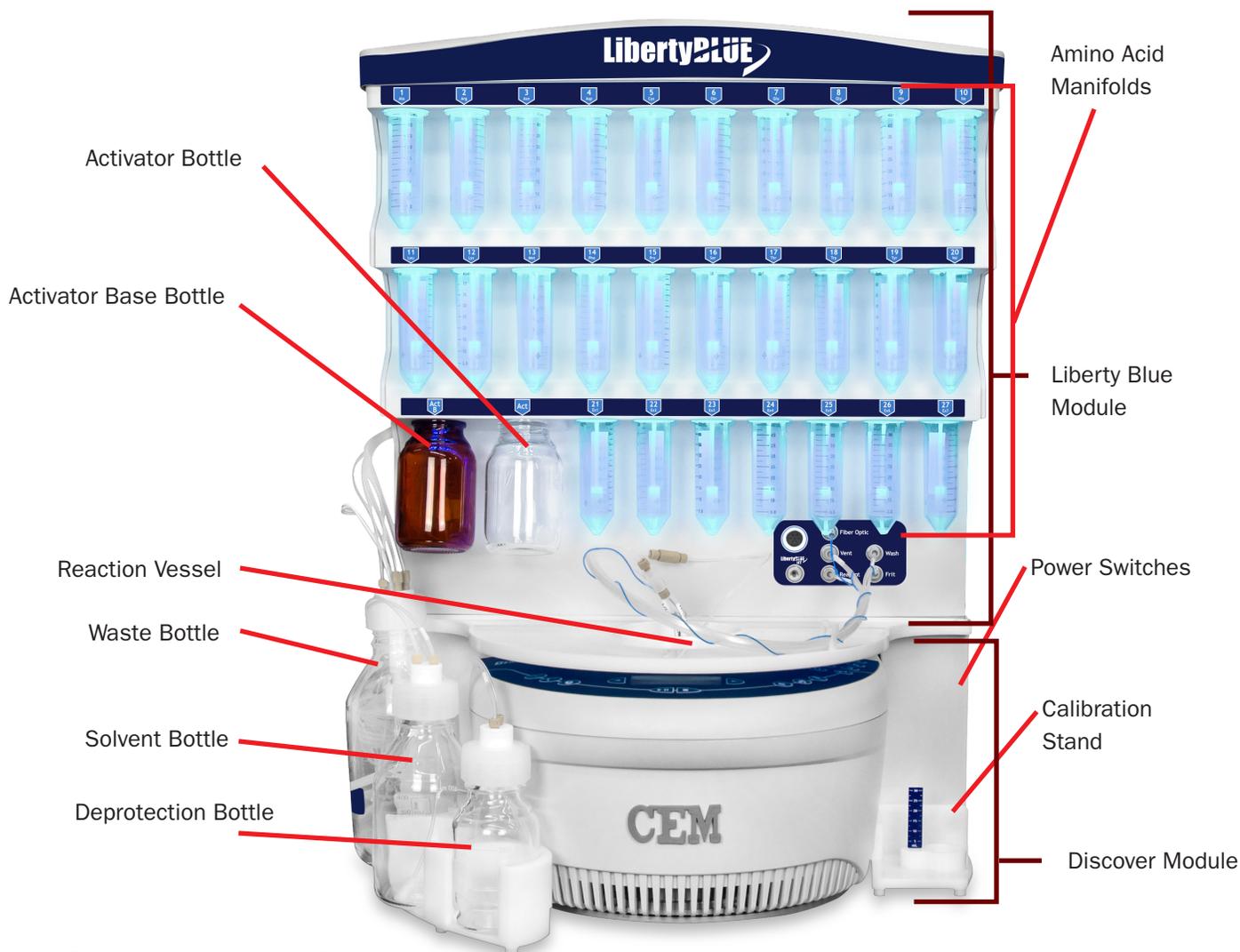
The Liberty Blue has four operator replaceable fuses located in the power inlet module (at the rear of the instrument, where the power cord connects to the Liberty Blue module). The fuses are rated F 250 V 2 A. The top pair of fuses (PN BR198826) are North American style, 5 mm x 20 mm size, and are rated F 250 V 2 A. The bottom pair of fuses (PN 188340) are North American style, 5 mm x 20 mm size, and are rated F 250 V 10 A.

i NOTE

Optionally, the router can be powered directly from the Liberty Blue module without the need for an external connection (using Router-Serial Power Cable, PN 243290).

Introduction To The Liberty Blue

The Liberty Blue is the latest generation of automated microwave peptide synthesizer. Built on CEM's flexible Discover microwave platform, the Liberty Blue is capable of synthesizing peptides faster and more efficiently than conventional synthesizers, thanks to the system's patented circular microwave cavity. The Liberty Blue's efficient Flex-Add™ technology eliminates the need for sample loops and allows infinite volume delivery options for a wide range of synthesis scales.



Components and Parts

Liberty Blue Module: The Liberty Blue handles all of the fluid transfer into and out of the reaction vessel. In addition, the Liberty Blue manages communications with the computer controller and the Discover during peptide synthesis.

Discover Microwave Reactor: The Discover microwave reactor holds the reaction vessel and generates the microwave energy used to irradiate the sample. The Discover is capable of operating independently of the Liberty Blue.

Power Switches: There are two power switches, one for the Discover and one for the Liberty Blue. Both switches must be on to operate the Liberty Blue.

External Bottles: Bottles for waste, main solvent, and deprotection connect to the manifold on the side of the Liberty Blue using GL45 caps and tubing assemblies. The external bottles sit in a bottle holder magnetically.

secured to the front of the Liberty Blue module. Optimal large capacity pressure rated bottles are available (PN 551240, 551245, 551250, 551255).

Amino Acid Manifolds: There are 27 positions on the manifold for amino acids, 20 corresponding to a specific amino acid, and 7 extra positions. Each position is labeled using the position number and 3-letter abbreviations of the amino acids. These positions are designed for use with 50 mL centrifuge tubes for the amino acids and 250 mL bottles for activator and activator base. Optional 120 mL tubes (PN 216560-M, pack of 30) are available for the amino acid positions. Up to one liter GL45 threaded bottles can be used with optional adapters (PN 909465), for the activator and activator base positions.

NOTE

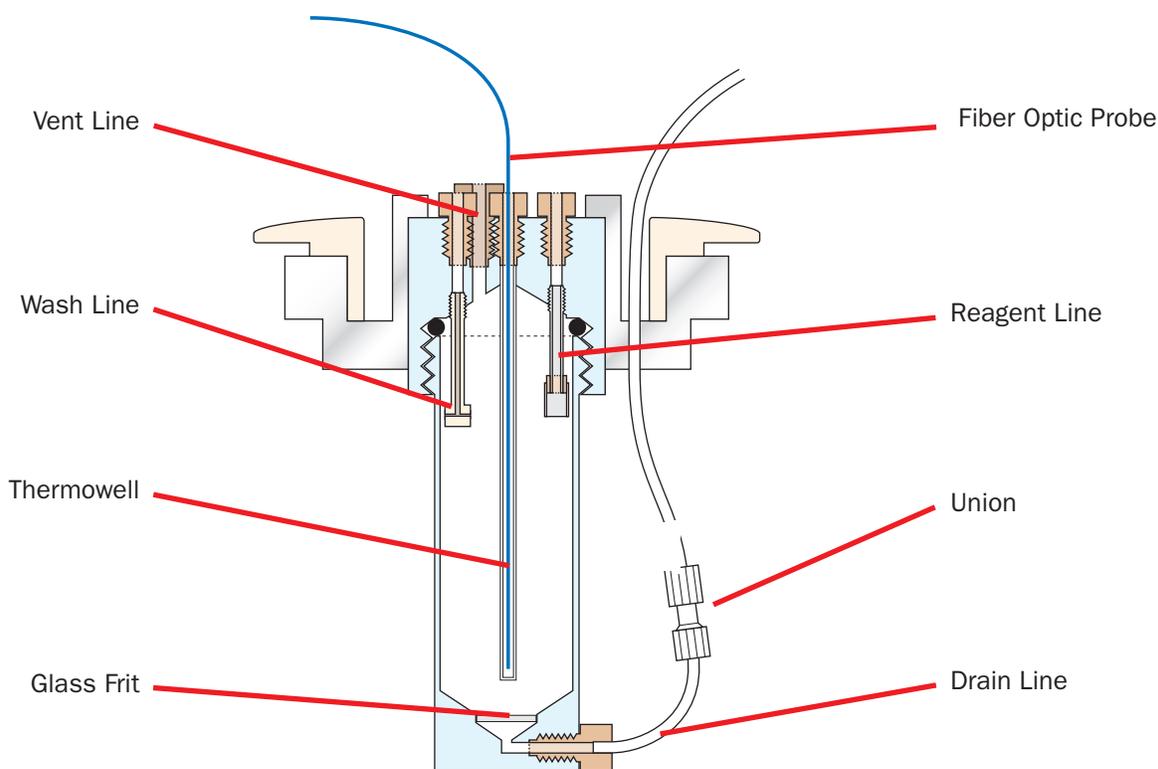
The 7 external positions are not available with the Liberty Lite.

Reaction Vessel: The reaction vessel is where the synthesis takes place. Reagents are added to the vessel and washed out throughout the synthesis of the peptide. There are different size vessels that can be used depending on the scale of the synthesis.

Fiber Optic Temperature Probe: The fiber optic probe is inserted in the top of the reaction vessel. It allows the system to monitor the temperature of the reaction vessel.

Waste Container: The waste lines carry all of the system's waste out into an external waste container. The waste container is equipped with a level sensor and will trigger the system to pause if the container is full. An optional large capacity (20 L) waste container is available (PN 909490).

Reaction Vessel Components



Quick Setup Guide

Create and Load the Liberty Method

i NOTE

To load an existing method, skip to step 9.

Create the Method

1. Open the appropriate  folder or  sub folder in the Methods box for the new method.
2. Click the **+** New Method button.
3. The  method will appear in the appropriate folder. Enter a name for the method and press Enter.
4. Click into the Sequence box. A list of the amino acids in the current bottle setup will drop down. To enter the sequence, either click the appropriate amino acid positions in the drop-down, or type the one-letter abbreviation for the desired amino acids.

i NOTE

The amino acids in the sequence should be entered from N-terminus to C-terminus.

5. Select the scale (0.005 mmol to 5.0 mmol) from the Synthesis Scale box, then select the resin type (Standard or High Swelling) from the Resin Type box.

i NOTE

The scale and resin type must be selected before changing any of the other parameters, as each scale and resin type has specific default options that will be loaded upon selection.

6. The remaining options can now be selected.
 - 6.1. C-Terminus: The C-terminus (acid or amide) is only used to calculate the molecular weight, and does not affect the synthesis conditions.
 - 6.2. Resin Type: The resin type will determine the first coupling step of the reaction. If Preloaded is checked, the software assumes the C-terminal amino acid is already loaded on the resin and therefore skips that coupling. If Not Preloaded is selected, the first coupling will proceed as normal. If Chloride Loading is selected, a chloride coupling step will be used for the C-terminal amino acid coupling.
 - 6.3. Resin Name: The resin name entered in this box will appear in the Method Report, but will not affect the synthesis conditions.
 - 6.4. Resin Loading: The substitution value entered in this box (in meq/g) will appear in the Method Report, but will not affect the synthesis conditions.
 - 6.5. Resin Cycle: The resin cycle determines the swelling conditions used to prepare the resin at the beginning of the synthesis. Make sure the amino acid cycles match the Resin Swelling Cycle (HS vs. non HS).
 - 6.6. Final Deprotection Cycle: By default, the N-terminal protecting group is removed at the end of the synthesis. Selecting an alternate Final Deprotection cycle allows control of this step.
 - 6.7. Bottle Setup: If using a bottle setup other than the standard default, it must be selected prior to entering the sequence.

7. Assign cycles for each residue in the sequence that will not use the default cycle.

NOTE

For long peptides, clicking the Quick Jump button will open a shortcut to view all amino acids in the sequence.

- 7.1. From the Amino Acid Cycles table, click on the amino acid to highlight it, then double-click the cycle to open the drop-down of available amino acid cycles.
- 7.2. From the drop-down, select the desired cycle. This will change the cycle for that highlighted amino acid only. To change the cycle for all amino acids to the selected cycle, right-click the cycle and select "Apply this cycle to all".

NOTE

To select multiple amino acids, hold Ctrl and left-click the desired amino acids. This will highlight multiple rows in the Amino Acid Cycles table. When all the desired amino acids are highlighted, click the Mark Rows button at the top of the Amino Acid Cycles table. The total number of selected rows will be listed next to the  flag icon. When multiple rows are marked (indicated by a blue highlight), any change made to one row will be made to all selected rows. When all changes are made, click the Clear button to deselect all rows.

8. When the method is complete, click Save to save the method, then close the  Liberty Method Editor.

Load the Method

1. From the Liberty Blue application software main screen, open the appropriate  folder in the Methods tab.
2. Click on the desired  method, and drag the method to the resin position. Alternatively, right-click on the desired  method and select "Queue for run".
3. The method name will appear in the Loaded Method indicator. The indicator will turn blue, meaning the method is loaded and ready to run.

Prepare and Load Reagents

Prepare Reagents

1. To determine the amount of each reagent needed and how to prepare them, use the  Usage and  Reagent Calculators.
 - 1.1. From the Calculators tab, select the  Usage Calculator.
 - 1.1.1 If the method is not already loaded, from the Methods box at the bottom left of the Usage Calculator, select the method to be included in the calculation and drag the method into the table on the top left of the Usage Calculator.
 - 1.1.2 The calculator will automatically determine the total usage for each reagent. For amino acids, enter the desired concentration in the Amino Acid Concentration box, and the calculator will automatically determine the mass of each amino acid to weigh out.
 - 1.1.3 If desired, print a Usage Report.
 - 1.2. From the Calculators menu, select the  Reagent Calculator.
 - 1.2.1 Resin: Enter the scale of the synthesis and the resin substitution to determine the mass of resin needed.

1.2.2 Other Reagents: For the other reagents, click on the appropriate tab (Activator, Activator Base, Deprotection) and then select the appropriate reagent from the list. Enter the concentration and final volume needed, and the calculator will update with the mass required.

1.2.3 If desired, print a Reagent Report.

Load the Reagents onto the Liberty Blue

1. Load all reagents onto the Liberty Blue.
 - 1.1. Connect centrifuge tubes with amino acid solution to the appropriate positions. Connect empty centrifuge tubes to all unused positions.
 - 1.2. Connect activator and activator base bottles to the appropriate positions on the system.
 - 1.3. Check the level of solvent remaining in the solvent bottle. If more solvent is required, use the Change Bottle Main Solvent operation to replace the bottle (see Change Bottle Main Solvent in Section 4).
 - 1.4. Check the level of deprotection solution remaining in the deprotection bottle. If more deprotection is required, use the Change Bottle Deprotection operation to replace the bottle (see Change Bottle Deprotection in Section 4).

Load the Resin into the Reaction Vessel

1. Load the resin (100-200 mesh only) into the reaction vessel.

CAUTION

The 30 mL reaction vessel should be used for 0.005 - 0.25 mmol syntheses (up to 1 g of resin). The 125 mL reaction vessel should be used for 0.5 - 5 mmol syntheses (up to 8 g of resin).

- 1.1. Remove the reaction vessel from the microwave cavity.
- 1.2. Unscrew the bottom port of the beige union connector on the vessel drain line. Then, unscrew the vessel body from the attenuator.
- 1.3. Weigh the appropriate amount of dry resin (100-200 mesh only) into the vessel body.
- 1.4. Connect the vessel body to the attenuator, then connect the drain line to the vessel body using the union connector fitting.
- 1.5. Place the vessel back into the microwave cavity, securing the attenuator.

Run the Method

Ensure the Liberty Blue is Ready to Run

1. Verify that the waste container has sufficient capacity.
2. Verify that the fiber optic probe is fully inserted into the thermowell.

WARNING

If the probe is not inserted all the way to the bottom of the vessel the Liberty Blue will not accurately measure the temperature, and significant overheating of the vessel will occur. This will result in poor synthesis quality and/or serious damage to the vessel.

3. Verify that the method is correct by looking at the steps outlined in the Liberty Methods widow, and then load the method from the Methods tab. If there are any errors, make corrections and reload the method.

- 3.1. Clear the method by clicking the small X at the top of the resin indicator.
- 3.2. To correct the method:
 - 3.2.1 Open the  Liberty Method Editor.
 - 3.2.2 Open the  folder where the method was saved, then click on the method to load it.
 - 3.2.3 Click Save to save the method after editing, then close the Liberty Method Editor.
 - 3.2.4 Load the corrected method as described above.

Run the Method

1. Click the Start button at the top of the Liberty Blue Software main screen.
2. The Loaded Method indicator will turn yellow to indicate the method is running.
3. The Liberty Blue will illuminate all required amino acids positions for the method. Ensure that all reagents are connected to the correct reagent positions, empty centrifuge tubes are connected to any unused reagent positions, and that there is sufficient solvent and deprotection solution, then click OK to continue.
4. The Liberty Blue will go through a series of initialization steps and leak checks.
5. During the run, the current step of the method is highlighted in yellow in the Cycle Steps table of the Current Run tab. A status bar in the lower right of the main screen indicates relative progress of the run.
6. When the run is complete, the Loaded Method indicator will turn green, indicating the method was successfully completed.
7. When the run is complete, remove the resin from the reaction vessel.
 - 7.1. Remove the reaction vessel from the microwave cavity.
 - 7.2. Unscrew the bottom port of the beige union connector on the vessel drain line. Then, unscrew the vessel body from the attenuator.
 - 7.3. Using dichloromethane (DCM) or another appropriate solvent, rinse the resin into a clean, empty centrifuge tube.

NOTE

The Liberty Blue is not equipped to perform peptide cleavage. All cleavage must be carried out at either room temperature or in the Razor™ Peptide Cleavage system.

- 7.4. Rinse any resin clinging to the thermowell into the centrifuge tube. Ensure there is no resin on the top of the reaction vessel attenuator.
- 7.5. Connect the vessel body to the attenuator, then connect the drain line to the vessel body using the beige union connector.
- 7.6. Place the vessel back into the microwave cavity, securing the attenuator.
8. The peptide is now ready to be cleaved and analyzed, and the Liberty Blue is ready for its next run.

Operation of the Liberty Blue

The operation of the Liberty Blue is controlled through the Liberty Blue application software. The Liberty Blue includes an external computer controller for running the Liberty Blue Software. This computer is connected to the Liberty Blue and the Discover through an Ethernet connection or wireless router.

Software Terminology

Microwave Method: The specific microwave parameters used in a cycle. Microwave Methods can be created or modified using the Microwave Editor.

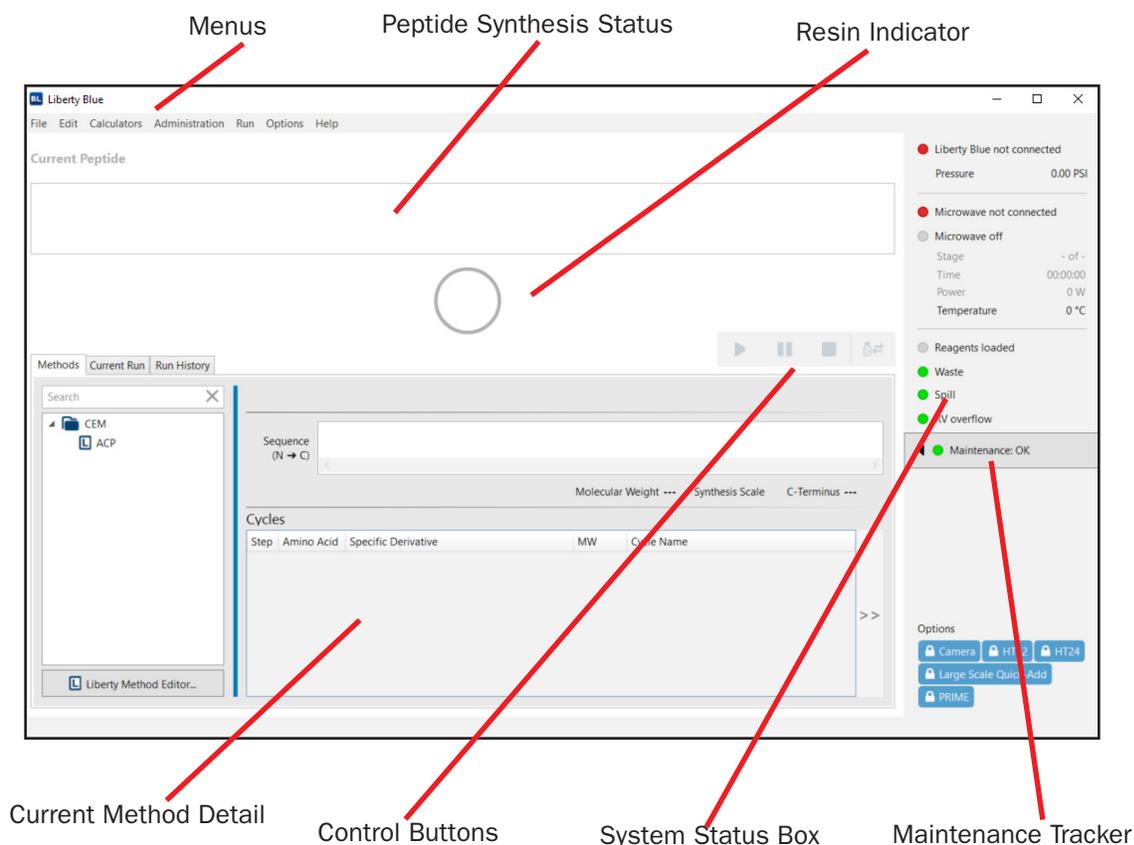
Cycle: The specific steps used for each residue within a given sequence, including deprotection, washing, and coupling. Cycles can be created or modified using the Cycle Editor.

Liberty Method: The specific parameters used to synthesize a peptide. For each Liberty Method, a sequence is entered, and then parameters (individual coupling cycles for each residue, C-terminus type, final deprotection, etc.) are selected. Liberty Methods can be created or modified using the Liberty Method Editor.

Run: A specific instance of a method being loaded and started in Liberty Blue Software. A Run History file is recorded for each run.

Liberty Blue Software Main Screen

Control Buttons



Start/Resume: This button starts the first method in the queue, or resumes a paused method.

Pause: This button pauses the current method.

Stop: This button stops the current method. Liberty Blue Software will finish the operation it is currently performing before stopping the method.

Refill Reagents: This button allows for the replacement of reagent bottles (including amino acids, activator, activator base, deprotection, and main solvent) without stopping the run.

Menus

File: This menu contains the Import and Export commands which will import and/or export a copy of selected methods, associated cycles, and operations from the database. These files can be transferred to another Liberty Blue database or submitted to CEM for troubleshooting purposes.

Edit: This menu contains the Liberty Method, Cycle, and Microwave editors, as well as the Reagent Library and Bottle Setup editors, and the Notification screen.

Calculators: This menu contains Reagent and Usage Calculators.

Administration: This menu contains the View Audit Trail screen. If users are enabled, this menu also allows administrators to create Users and User Groups.

Run: This menu contains the Control Buttons (Start/Resume, Pause, Stop).

Options: This menu contains the Maintenance, Diagnostics, Calibration, and Settings screens, and the Check Data Integrity command (which will verify there are no problems with the Liberty Blue Software database).

Help: This menu contains the About Liberty Blue screen (which displays the software and firmware version numbers), and allows you to create a troubleshooting bundle.

Indicators

Resin Indicator: The Resin Indicator shows the status for the currently loaded method: blue when ready to run, yellow when running, red when stopped, green when complete.

Peptide Synthesis Status: This displays the peptide sequence being synthesized and indicates the status of the synthesis.

Current Method Detail: This window displays the currently selected method.

System Status Box: The System Status Box contains indicators that show Liberty Blue and Discover communication status, current temperature and system pressure readings, microwave method parameters, sensor status for the waste container, spill tray, and reaction vessel overflow sensors, and maintenance tracker.

Tabs



Methods: From this tab, methods are loaded into the resin position.

Current Run: This tab displays the Method that is currently running. The current step of the method is highlighted in yellow. When the “Keep Cycles table synced” box is checked, the cycles tables will automatically move to the next cycle as the method progresses.

Run History: From this tab, detailed logs of each run can be accessed.

Microwave Editor

The  Microwave Editor allows for the control and customization of the microwave steps of any cycle. By editing the power and duration of a microwave step, difficult peptides can be synthesized with higher purity and yield. The  Microwave Editor can be accessed from the Edit menu.

The specific microwave power setting required to obtain acceptable heating rates will vary from unit to unit. As such, care must be taken to optimize the microwave settings.

There are two key times for optimal microwave heating:

- Ramp Time: This refers to the approximate time it takes to heat the reaction mixture from its starting temperature to its desired temperature.
- Total Time: This refers to the total reaction time including both the ramp and hold time.

NOTE

It is important to note that the Liberty microwave methods are not programmed by specifying a set ramp time. This term is only guidance for what should be observed when applying the method. The actual ramp time is controlled by adjusting the MW power within each method. Default power settings are given that serve as a starting point for optimizing the MW power settings.

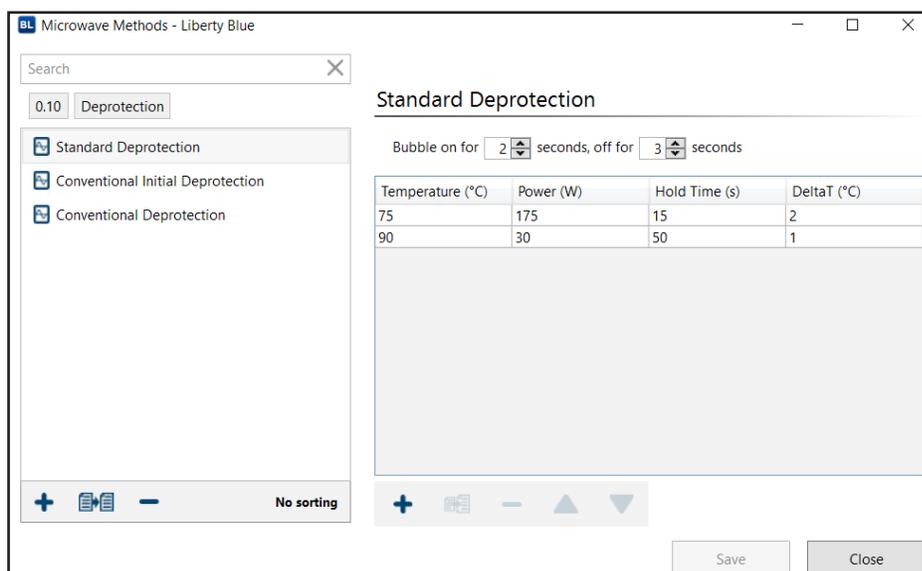
Microwave Method Folders

The  Microwave Editor contains a variety of optimized default microwave methods separated by scale and type (deprotection or coupling).

Creating a New Microwave Method

Microwave Methods allow for detailed control of the microwave heating and reaction time for each step in the synthesis. The  Microwave Editor allows the user to select the microwave time, microwave power, maximum temperature, and sample agitation.

To create a new Microwave Method:



1. Click on the filter buttons at the top of the  Microwave Editor to open the filter list.

i NOTE

A Microwave Method created at a specific scale will only be available for use in the  Cycle Editor for cycles created at the same scale.

2. Select a scale and method type (the currently selected parameters will be highlighted). Click out of the filter list to open the selected cycle type.
3. Click the  New Method button.
4. The new  Microwave Method will appear in the selected folder. Enter a name for the  Microwave Method and press Enter.
5. Enter the temperature (in °C), Power (in W), Time (in s), and Delta T (in °C) for the first step in the method.
6. Add the next step to the method by clicking the  Add Step button, and then enter the power, temperature, and time settings for the second step.
7. Repeat step 5 for each step in the method.
8. Enter the amount of time to bubble and the time between each bubbling pulse (the default setting is on for 2 seconds, off for 3 seconds).
9. Save the method by clicking the Save button.

i NOTE

To delete a step, click the step to highlight it, then click the  Delete Step button. To insert a new step between existing steps, click a step to highlight it, then click the  Insert Step button. The new step will be inserted above the selected step. To move a step up or down, click the step to highlight it, then click the  Move Up or  Move Down buttons.

Editing an Existing Microwave Method

The  Microwave Editor allows for settings on existing methods to be modified and saved as needed. The most common reason to modify an existing method is to increase or decrease the microwave power to optimize peptide synthesis. To modify an existing method:

1. Click on the filter buttons at the top of the  Microwave Editor to open the filter list.
2. Select a scale and method type (the currently selected parameters will be highlighted). Click out of the filter list to open the selected cycle type.
3. Click on the  Microwave Method to be modified.
4. Make any changes as needed.
5. Click Save to save the changes to the method.

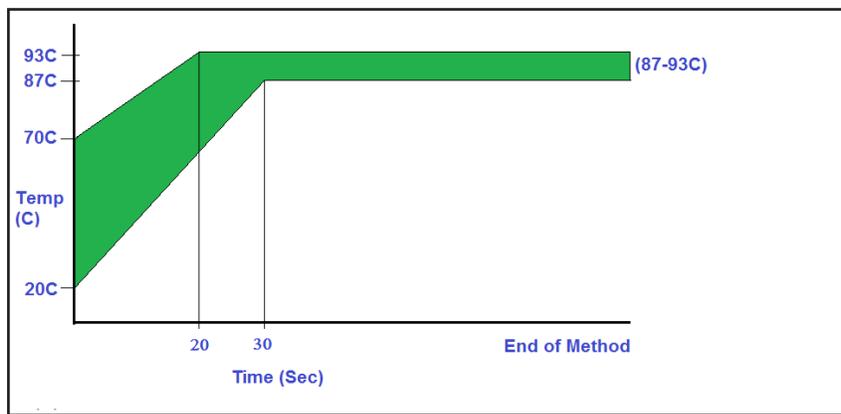
Duplicating and Modifying an Existing Microwave Method

Often, it is easier to copy and modify an existing microwave method rather than creating an entirely new method. Methods can easily be duplicated and modified within the  Microwave Editor, and then be moved to different scale folders as needed. To copy an existing method:

1. Click on the filter buttons at the top of the  Microwave Editor to open the filter list.
2. Select a scale and method type (the currently selected parameters will be highlighted). Click out of the filter list to open the selected cycle type.
3. Click on a  Microwave Method to select it and click the  Duplicate button.
4. The new  Microwave Method will appear named “Copy of (method name)”. Type a new name and press Enter.
5. Make any changes to the method, then click Save to save the modified method.

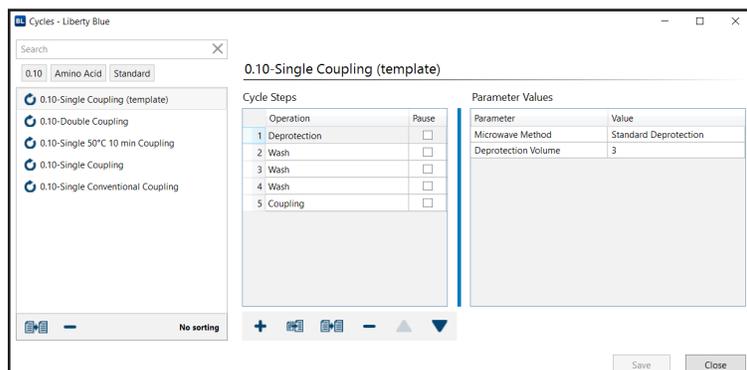
Calibrating Rapid Liberty Blue Microwave Methods

The rapid microwave methods used by the Liberty Blue employ rapid heating rates. For both the deprotection and coupling methods the set temperature of 90 °C should be reached within 20-30 seconds and maintained for at least 45 seconds and 100 seconds respectively. The starting temperature for each reaction will be significantly warmer than room temperature as residual heat will remain from the previous reaction. The graph below shows the desired heating profile for Liberty Blue rapid deprotection and coupling microwave methods. The green area represents the acceptable temperature range.



Cycle Editor

The Cycle Editor allows for the full control and customization of any step of a synthesis. This allows for optimization of each step of a given peptide synthesis. The Cycle Editor can be accessed from the Edit menu. The Cycle Editor contains separate cycles for each scale. Within each scale, cycles are sorted by type and then by resin type, allowing for the development of optimized cycles for each scale. A cycle will only appear in the Method Editor when creating a method of the same scale.



The Liberty Blue application software comes with optimized default cycles for resin swelling, amino acid addition, and final deprotection for each of the available synthetic scales for both standard and high swelling resins. The default cycle for each scale is listed first in the Cycle Editor.

Cycle Types

Resin: Resin cycles control the swelling of the resin before synthesis.

Amino Acid: Amino acid cycles control how the deprotection and coupling steps of any one amino acid in the sequence occur.

Final Deprotection: Final deprotection cycles control the removal of the N-terminal protecting group, and also allow for N-terminal acetylation of the peptide before cleavage.

Operations in the Cycle Editor

The Cycle Editor allows for full customization of cycles using a number of modular, preset operations. An operation is selected for a given step, and then the parameters for that operation are selected.

Addition Operations

Add Deprotection: This will add deprotection solution (in mL) from the deprotection bottle to the reaction vessel through the bottom, then purge the line (in seconds) to the reaction vessel.

Add Reagent: This will add solution (in mL) from any of the 27 amino acid positions or from the activator or activator base position through the Reagent line. Reagent: Any of the of the 27 amino acid positions, activator, or activator base can be selected. Selecting “(from method)” will add from the amino acid the cycle is assigned to in the method.

Add Main Solvent (MS2): This will add main solvent (in mL) to the reaction vessel through the Wash line.

Wash Operations

Wash: This will wash the vessel by adding main solvent (in mL) through the Wash line, bubbling the vessel, then draining the vessel (in seconds).

Wash Thru Manifold: This will wash the manifolds and the vessel by adding main solvent (in mL) through the Reagent line, purging the manifold to the vessel, bubbling, then draining the vessel (in seconds).

Microwave Operations

Microwave Deprotection: This will add deprotection solution (in mL) from the deprotection bottle to the reaction vessel through the bottom, then purge the line to the reaction vessel. The microwave will then microwave the vessel using the selected method, then drain the vessel.

Microwave Coupling: This will add amino acid, activator, and activator base (in mL) from the selected bottles to the reaction vessel. The microwave will then microwave the vessel using the selected method, then drain the vessel.

- **Amino Acid:** This is the amino acid that will be added. Any of the of the 27 amino acid positions, activator, or activator base can be selected. Selecting “(from method)” will add from the amino acid the cycle is assigned to in the method.
- **Delayed Reagent Time (After Purge):** This is the time (in seconds) after the 30 second purge that the fourth reagent (in mL) will be added to the reaction vessel. This should not exceed the total microwave time of the selected microwave method.
- **Delayed Reagent Bottle Position:** Any of the of the 27 amino acid positions, activator, or activator base can be selected. Selecting “(from method)” will add from the amino acid the cycle is assigned to in the method.

Microwave Coupling (Chloride Loading): This is the operation used for loading the first amino acid on CEM ProTide™ Chloride resin. The vessel is drained afterwards.

Microwave Method: This will run the selected microwave method and then drain the vessel.

Microwave Method NO DRAIN: This will run the selected microwave method, but will not drain the contents of the reaction vessel after the method has finished.

Miscellaneous Operations

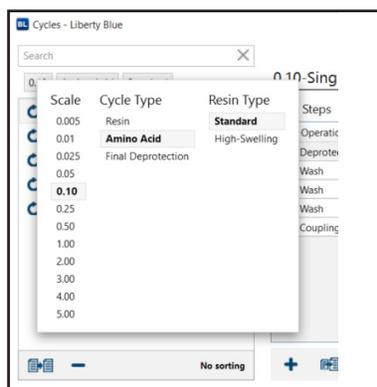
Swell Resin: This will add main solvent (in mL) to the reaction vessel through the Wash line, wait a specified time (in seconds) to allow the resin to swell, then drain the reaction vessel.

Drain: This will drain the reaction vessel for the specified time (in seconds).

Creating a Cycle

 Cycles allow for control of each action taken in a given step of a run. The Liberty Blue application software comes with default cycles for each synthetic scale; however, cycles can be customized to accomplish a number of

non-standard chemistries.



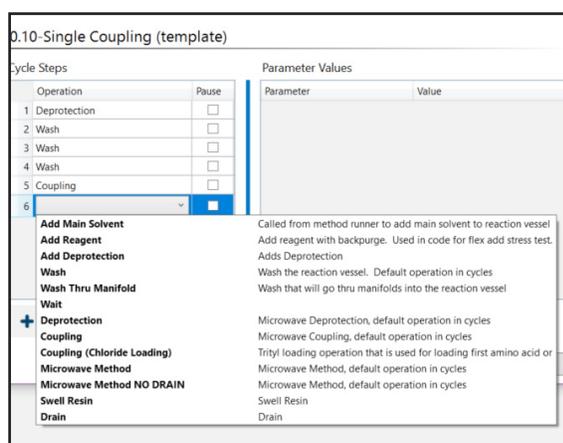
To create a new cycle:

1. Click on the filter buttons at the top of the Cycle Editor screen to open the filter list.
2. Select a scale, cycle type, and resin type. The currently selected parameter for each will be displayed in bold. Click out of the filter list to open the selected cycle type.
3. Select a default cycle to use as a template and click the Duplicate button.

i NOTE

A cycle will only be available for use in the Method Editor for methods created at that scale and with that resin type.

4. The new cycle will appear named "(cycle name) 2". Type a new name and press Enter.
5. Make any desired changes to the cycle.
 - 5.1. To modify an existing step:
 - 5.1.1 Click on the step to be modified to highlight it.
 - 5.1.1.1 To change the operation, double-click the Operation box to open the operations drop-down and select the operation to be performed.



- 5.1.1.2 To change the parameters, double-click on the parameter value in the Parameter Values table. A pop-up will appear to configure the parameter. Make any changes, then click OK to close the pop-up.

5.1.1.3 To add a pause after the operation is carried out, check the Pause box.

	Operation	Pause
1	Deprotection	<input type="checkbox"/>
2	Wash	<input type="checkbox"/>
3	Wash	<input type="checkbox"/>
4	Wash	<input checked="" type="checkbox"/>
5	Coupling	<input type="checkbox"/>

NOTE

The method will not resume until the user presses the Start button on the main screen of Liberty Blue Software.

5.2. To add a new step:

5.2.1 Click the **+** Add Step button. The new step will appear at the end of the cycle.

5.2.2 Double-click the Operation box to open the operations drop-down and select the operation to be performed.

Operation	Pause
1 Deprotection	<input type="checkbox"/>
2 Wash	<input type="checkbox"/>
3 Wash	<input type="checkbox"/>
4 Wash	<input type="checkbox"/>
5 Coupling	<input type="checkbox"/>
6	<input checked="" type="checkbox"/>

Parameter	Value
-----------	-------

+ Add Main Solvent	Called from method runner to add main solvent to reaction vessel
Add Reagent	Add reagent with backpurge. Used in code for flex add stress test.
Add Deprotection	Adds Deprotection
Wash	Wash the reaction vessel. Default operation in cycles
Wash Thru Manifold	Wash that will go thru manifolds into the reaction vessel
Wait	
+ Deprotection	Microwave Deprotection, default operation in cycles
+ Coupling	Microwave Coupling, default operation in cycles
Coupling (Chloride Loading)	Trityl loading operation that is used for loading first amino acid or
Microwave Method	Microwave Method, default operation in cycles
Microwave Method NO DRAIN	Microwave Method, default operation in cycles
Swell Resin	Swell Resin
Drain	Drain

5.2.3 The default parameters for the chosen operation will appear in the Parameter Values table. Double-click on a Parameter Value to change the value.

5.2.4 To add a pause after the operation is carried out, check the Pause box.

5.3. To delete a step, click the step to highlight it, then click the **-** Delete Step button.

5.4. To insert a new step between existing steps, click a step to highlight it, then click the **+** Insert Step button. The new step will be inserted above the selected step.

5.5. To copy a step, click a step to highlight it, then click the **+** Duplicate Step button. The copy will be inserted after the highlighted step.

5.6. To move a step up or down, click the step to highlight it, then click the **▲** Move Up or **▼** Move Down buttons.

6. Click Save to save the cycle.

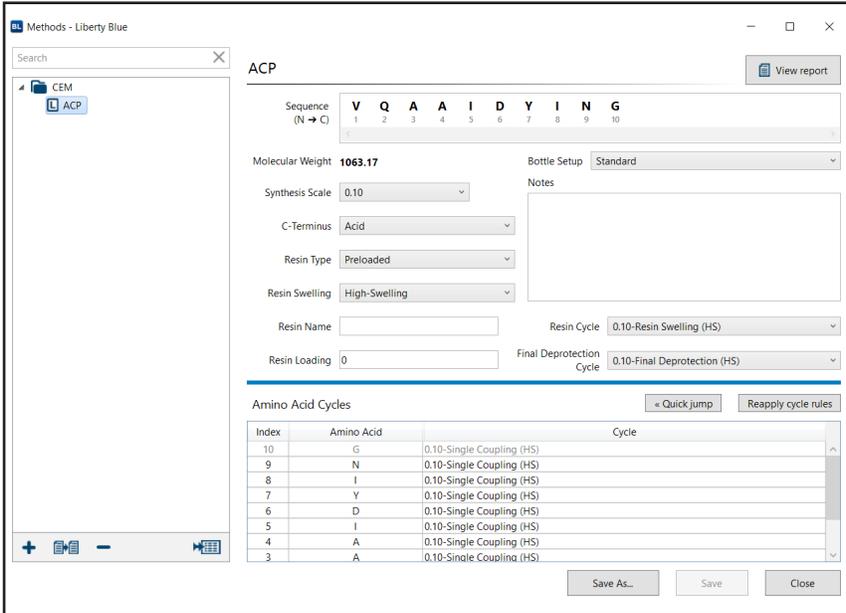
Editing an Existing Cycle

The Cycle Editor allows for settings on existing cycles to be modified and saved as needed. To modify an existing cycle:

1. Click on the filter buttons at the top of the  Cycle Editor screen to open the filter list.
2. Select a scale, cycle type, and resin type. The currently selected parameter for each will be displayed in bold. Click out of the filter list to open the selected cycle type.
3. Click on the  cycle to be modified.
4. Make any changes as needed.
5. Click Save to save the changes to the cycle.
6. Click the  Add Step button. The new step will appear at the end of the cycle.
 - 6.1. Double-click the Operation box to open the operations drop-down and select the operation to be performed.
 - 6.2. The default parameters for the chosen operation will appear in the Parameter Values table. Double-click on a Parameter Value to change the value.
 - 6.3. To add a pause after the operation is carried out, check the Pause box.
7. To delete a step, click the step to highlight it, then click the  Delete Step button.
8. To insert a new step between existing steps, click a step to highlight it, then click the  Insert Step button. The new step will be inserted above the selected step.
9. To copy a step, click a step to highlight it, then click the  Duplicate Step button. The copy will be inserted after the highlighted step.
10. To move a step up or down, click the step to highlight it, then click the  Move Up or  Move Down buttons.
11. Click Save to save the cycle.

Liberty Method Editor

The  Liberty Method Editor is used to program the specific conditions to be used for a given synthesis. Once the sequence is entered, several options for the run can be configured that will determine the specific steps to be used in the synthesis. The Liberty Method Editor can be accessed from the Edit menu.

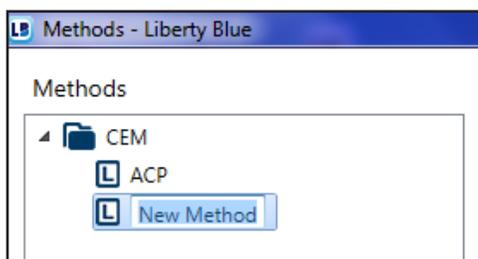


The screenshot shows the Liberty Method Editor interface. The sequence is V Q A A I D Y I N G. The amino acid cycles table is as follows:

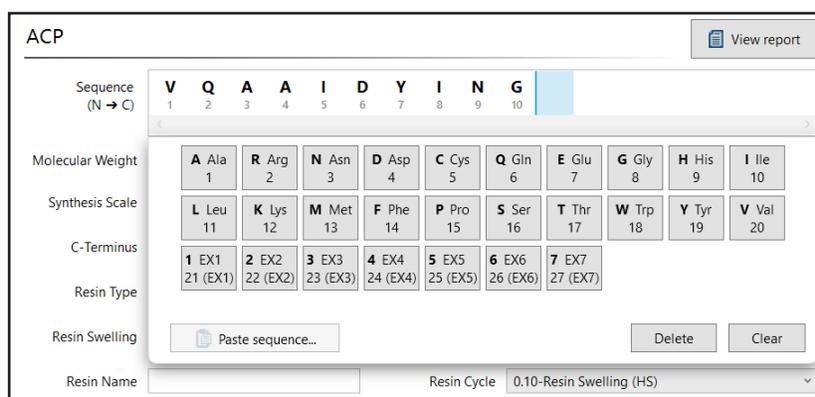
Index	Amino Acid	Cycle
10	G	0.10-Single Coupling (HS)
9	N	0.10-Single Coupling (HS)
8	I	0.10-Single Coupling (HS)
7	Y	0.10-Single Coupling (HS)
6	D	0.10-Single Coupling (HS)
5	I	0.10-Single Coupling (HS)
4	A	0.10-Single Coupling (HS)
3	A	0.10-Single Coupling (HS)

Creating a New Liberty Method

1. Open the appropriate folder or sub folder in the Methods box for the new method.
2. Click the **+** New Method button.



3. The **L** method will appear in the appropriate folder. Enter a name for the method and press Enter. Click into the Sequence box. A list of the amino acids in the current bottle setup will drop down. To enter the sequence, either click the appropriate amino acid positions in the drop-down, or type the one-letter abbreviation for the desired amino acids.

A screenshot of the "ACP" method configuration window. At the top, there is a "View report" button. Below it is a "Sequence (N → C)" field containing the sequence "V Q A A I D Y I N G" with positions 1 through 10. Below the sequence is a grid of amino acid options. The grid is organized into rows: "Molecular Weight" (A Ala 1, R Arg 2, N Asn 3, D Asp 4, C Cys 5, Q Gln 6, E Glu 7, G Gly 8, H His 9, I Ile 10), "Synthesis Scale" (L Leu 11, K Lys 12, M Met 13, F Phe 14, P Pro 15, S Ser 16, T Thr 17, W Trp 18, Y Tyr 19, V Val 20), "C-Terminus" (1 EX1, 2 EX2, 3 EX3, 4 EX4, 5 EX5, 6 EX6, 7 EX7), and "Resin Type" (21 (EX1), 22 (EX2), 23 (EX3), 24 (EX4), 25 (EX5), 26 (EX6), 27 (EX7)). At the bottom, there is a "Resin Swelling" section with a "Paste sequence..." button, "Delete" and "Clear" buttons, and a "Resin Name" field. The "Resin Cycle" is set to "0.10-Resin Swelling (HS)".

i NOTE

To delete an amino acid from the sequence, press the backspace key (to delete the residue before the cursor) or click the delete button on the drop-down (to delete the highlighted residue).

To insert an amino acid into the sequence, click an amino acid in the sequence to highlight it, then either click the desired amino acid in the drop-down, or type the one-letter abbreviation for the desired amino acid. The new residue will be inserted before the highlighted amino acid.

4. Select the scale (0.005 mmol to 5.0 mmol) from the Synthesis Scale box, then select the resin swelling type (Standard or High Swelling) from the Resin Swelling box.

i NOTE

The scale and resin type must be selected before changing any other parameters, as each scale and resin type has specific default options that will be loaded upon selection.

5. The remaining options can now be selected.
 - 5.1. C-Terminus: The C-terminus (acid or amide) is only used to calculate the molecular weight, and does

not affect the synthesis conditions.

- 5.2. Resin Type: The resin type will determine the first coupling step of the reaction. If Preloaded is checked, the software assumes the C-terminal amino acid is already loaded on the resin and therefore skips that coupling. If Not Preloaded is selected, the first coupling will proceed as normal. If Chloride Loading is selected, a chloride coupling step will be used for the C-terminal amino acid coupling.
- 5.3. Resin Swelling: Choosing the correct resin swelling condition ensures the resin is completely submerged during a run. Select (HS) for high swelling resins such as PEG-based resins, and Standard for polystyrene resins.
- 5.4. Resin Name: The resin name entered in this box will appear in the Method Report, but will not affect the synthesis conditions.
- 5.5. Resin Loading: The substitution value entered in this box (in meq/g) will appear in the Method Report, but will not affect the synthesis conditions.
- 5.6. Resin Cycle: The resin cycle determines the swelling conditions used to prepare the resin at the beginning of the synthesis. Make sure the amino acid cycles match the Resin Swelling Cycle (HS vs. Standard).
- 5.7. Final Deprotection Cycle: By default, the N-terminal protecting group is removed at the end of the synthesis. Selecting an alternate Final Deprotection cycle allows control of this step.
- 5.8. Bottle Setup: If using a bottle setup other than the standard default, it must be selected prior to entering the sequence.

i NOTE

The Chloride Loading resin type is not available for Liberty Lite.

6. Assign cycles for each residue that will not use the default cycle.

i NOTE

By default, the Double Coupling cycle is used for arginine. The default standard bottle setup uses His(Boc). For other His derivatives, use Single 50°C 10 min Coupling cycle. The Single Coupling cycle is used for all other amino acids. To restore all amino acids in a method to the default cycles, click on Reapply Cycle Rules.

i NOTE

Clicking the Quick Jump button will open a shortcut to all amino acids in the sequence.

- 6.1. From the Amino Acid Cycles table, click on the amino acid to highlight it, then double-click the cycle to open the drop-down of available amino acid cycles.

i NOTE

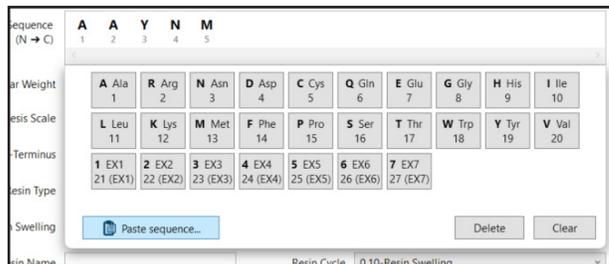
To select multiple amino acids, hold Ctrl and left-click the desired amino acids. This will highlight multiple rows in the Amino Acid Cycles table. When all the desired amino acids are highlighted, click the Mark Rows button at the top of the Amino Acid Cycles table. The total number of selected rows will be listed next to the  flag icon. When multiple rows are marked (indicated by a blue highlight), any change made to one row will be made to all selected rows. When all changes are made, click the Clear button to deselect all rows.

- 6.2. From the drop-down, select the desired cycle. This will change the cycle for that highlighted amino acid only. To change the cycle for all amino acids to the selected cycle, right-click the cycle and select "Apply this cycle to all".
7. When the method is complete, click Save to save the method.

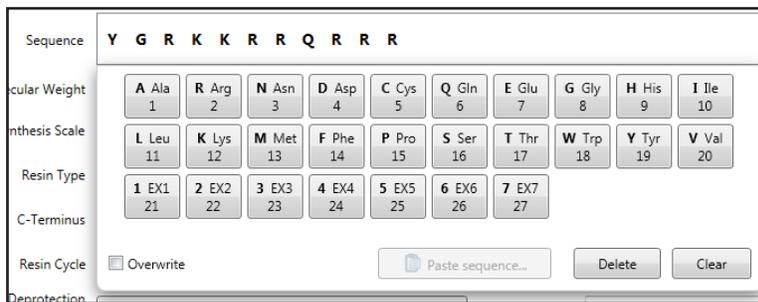
Importing a Sequence to the Method Editor

When creating a new **L** method, the sequence can be copied and pasted from outside the Liberty Blue application software, allowing for the importation of sequences from documents, presentations, websites, etc. To paste a sequence:

1. Highlight the sequence in the source document and press CTRL + C to copy to the system clipboard.
2. In the sequence editor, click the Paste Sequence button. (If the object on the clipboard is not able to be imported, the Paste Sequence button will be inactive.)

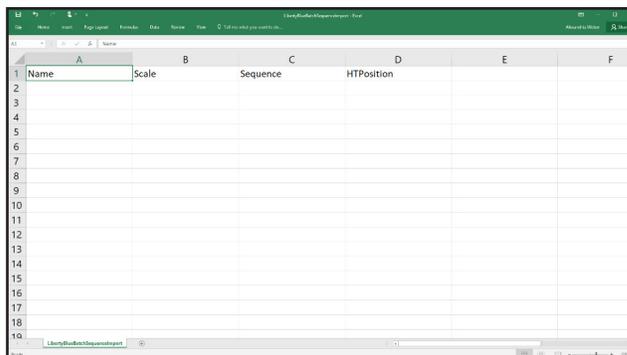


3. If there are any amino acids in the sequence that are not a part of the current bottle setup, the Sequence Importer will open and display the sequence. The amino acids shown in red will not be imported.
4. The sequence should be in N-terminal to C-terminal order.
5. If the sequence appears correct, click Import Sequence. The sequence will be loaded into the method.



Importing a Batch of Sequences to the Method Editor

1. From the Liberty Blue Software, save the Batch Sequence Import Template (File -> Import -> Save Batch Sequence Import Template).
2. Open the Batch Sequence Import Template.



- Input the Name, Scale, and Sequence of each Liberty Method to be created, and save/rename the file.

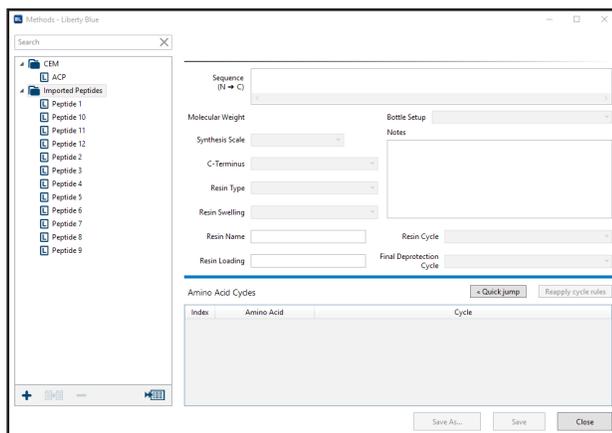
Name	Scale	Sequence	HTPosition
Peptide 1	0.005	ACDEFGHIKLMNPQRST	
Peptide 2	0.01	ACDEFGHIKLMNPQRST	
Peptide 3	0.025	ACDEFGHIKLMNPQRST	
Peptide 4	0.05	ACDEFGHIKLMNPQRST	
Peptide 5	0.1	ACDEFGHIKLMNPQRST	
Peptide 6	0.25	ACDEFGHIKLMNPQRST	
Peptide 7	0.5	ACDEFGHIKLMNPQRST	
Peptide 8	1	ACDEFGHIKLMNPQRST	
Peptide 9	2	ACDEFGHIKLMNPQRST	
Peptide 10	3	ACDEFGHIKLMNPQRST	
Peptide 11	4	ACDEFGHIKLMNPQRST	
Peptide 12	5	ACDEFGHIKLMNPQRST	

NOTE

To load the Liberty Methods into the HT Grid immediately upon import, input the desired HT position for each Liberty Method.

Name	Scale	Sequence	HTPosition
Peptide 1	0.005	ACDEFGHIKLMNPQRST	1
Peptide 2	0.01	ACDEFGHIKLMNPQRST	2
Peptide 3	0.025	ACDEFGHIKLMNPQRST	3
Peptide 4	0.05	ACDEFGHIKLMNPQRST	4
Peptide 5	0.1	ACDEFGHIKLMNPQRST	5
Peptide 6	0.25	ACDEFGHIKLMNPQRST	6
Peptide 7	0.5	ACDEFGHIKLMNPQRST	7
Peptide 8	1	ACDEFGHIKLMNPQRST	8
Peptide 9	2	ACDEFGHIKLMNPQRST	9
Peptide 10	3	ACDEFGHIKLMNPQRST	10
Peptide 11	4	ACDEFGHIKLMNPQRST	11
Peptide 12	5	ACDEFGHIKLMNPQRST	12

- Import the Batch Sequence Import Template file into the Liberty Blue Software (File -> Import -> Sequences). The Liberty Method Editor window will open, revealing the recently imported sequences.



Editing an Existing Liberty Method

- Open the Liberty Method Editor.
- Open the appropriate folder or sub folder in the Methods box for the new method.

3. Click on the method to be modified.
4. Make any changes to the method as needed.
5. Click Save to save the changes to the method, or click Save As to save the changes as a new Liberty method.

Duplicating and Modifying an Existing Liberty Method

Liberty methods can easily be duplicated and modified within the Liberty Method Editor, and then they can be moved to different folders as needed. To copy an existing Liberty method:

1. Open the  Liberty Method Editor.
2. Open the appropriate folder or sub folder in the Methods box for the new method.
3. Click on a  Liberty method to select it and click the  Duplicate button.
4. The new Liberty method will appear named "Copy of [method name]". Type a new name and press Enter.
5. Make any changes to the method as needed, then click Save to save the changes to the method.

Generating a Method Report

A Liberty Method Report records all settings selected in the Method Editor when creating the method. Liberty Method Reports are created as PDF files which can be saved and printed to allow for easy recording of experimental parameters.

The level of detail included in a Liberty Method Report can be configured during report generation. To generate a Liberty Method Report:

1. Open the  Liberty Method Editor.
2. Open the appropriate folder or sub folder in the Methods box for the new method, then click on the method to be reported.
3. Click the View Report button in the upper right corner of the Liberty Method Editor screen to open the Liberty Method Report Generator.
4. Select the options to be included in the Liberty Method Report.
 - 4.1. To include a header image, check the Image box under Header. To select a custom image, click the image on the right, then open the desired image.
 - 4.2. To include the calculated molecular weight in the header, check the Molecular Weight box.
 - 4.3. Select the details to be included in the report by checking the appropriate boxes.
 - 4.3.1 Selecting Sequence will include the sequence.
 - 4.3.2 Selecting Method Details will include the synthesis scale, resin type, preloaded or not preloaded status, C-terminus, resin cycle, final deprotection cycle, resin name (if entered), and resin loading (if entered).
 - 4.3.3 Selecting Method Notes will include any text entered in the Notes field.
 - 4.3.4 Selecting Amino Acid Cycles will include a list of the cycles assigned to each amino acid in the sequence. This list only includes the names of the cycles.
 - 4.3.5 Selecting Cycle Details will include the details for each cycle used in the Liberty Method (including all operations and parameters in the cycle).
 - 4.4. To include a footer image, check the Image box under footer. To select a custom image, click the image on the right, then open the desired image.

Exporting Microwave Methods and Operations

The Liberty Blue application software allows operations, microwave methods, and associated microwave cycles to be transferred from one Liberty Blue Software database to another.

To export existing operations:

1. Open the  Export Menu (File → Export).
2. Click on the Operations tab and select the operations and categories to export. Click Select All to select all operations.

3. Click  Export. The selected operations will be saved as an XML file (.xml).

To export existing Liberty methods:

1. In the Export menu, click on the Methods tab and select the methods to export. Click Select All to select all methods.
2. Click  Export. The selected operations will be saved as a JSON file (.json).

CAUTION

All operations must be exported separately from the Liberty methods. Attempting to export methods and operations at the same time will not work as two different file types cannot be created at the same time.

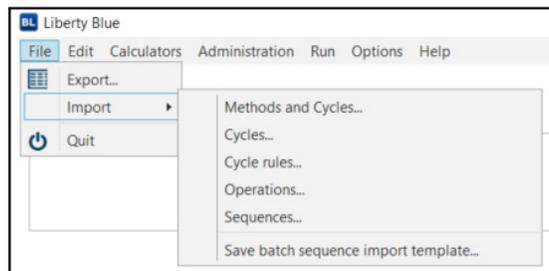
NOTE

When exporting Liberty methods, all associated cycles are also exported with the method. These include default and custom resin, amino acid, and final deprotection cycles.

Importing Microwave Methods, Operations, and Settings

The Liberty Blue application software allows settings, operations, Liberty methods, cycles, and associated microwave methods to be transferred from one Liberty Blue Software database to another. To import existing operations, settings, or Liberty methods:

1. To import files, select File then Import.



2. To import methods and cycles, select “Methods and Cycles...”. Select the appropriate JSON file from the computer then click Open. The Liberty methods will automatically start importing to the current database.
3. To import operations, select “Operations...”. Select the appropriate XML file from the computer then click Open. The operations will automatically start importing to the current database.
4. To import settings, select “Settings...”. Select the appropriate JSON file from the computer then click Open. The settings will automatically start importing to the current database.

NOTE

When importing Liberty methods into a new database, all associated cycles are also imported. These cycles include default and custom resin, amino acid, and final deprotection cycles.

Calculators

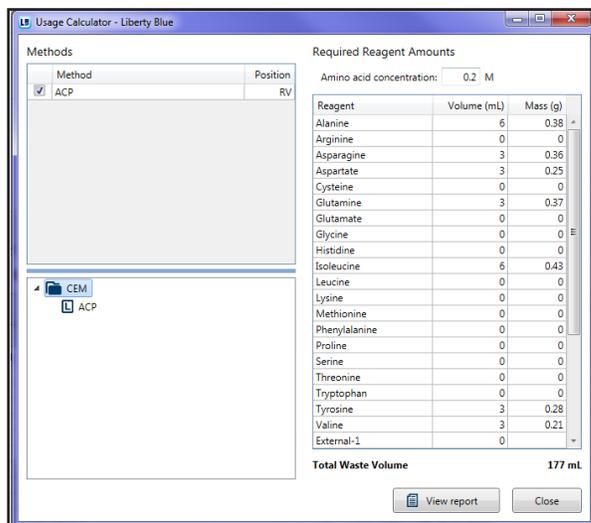
The Liberty Blue application software contains two calculators that allow for easy determination of reagent needs and reagent preparation: the  Usage Calculator and the  Reagent Calculator. These calculators are accessed from the Calculators menu.

Usage Calculator

The  Usage Calculator can be used to determine the amount of each reagent needed for a given method. Multiple methods can be loaded into the calculator, so that sufficient reagents can be loaded onto the Liberty Blue for multiple peptides.

To use the Usage Calculator:

1. From the Calculators menu, select  Usage Calculator.



2. From the Methods box at the bottom left of the Usage Calculator, right-click the  method to be included in the calculation and select Add to Calculation. The method will then appear in the table at the top left of the Usage Calculator.
3. The calculator will automatically determine the total usage for each reagent, as well as the mass of each amino acid required, and estimate the total waste produced.
4. To prepare enough reagents for multiple runs, repeat step 2 for each method to be included in the calculation. The calculator will automatically update the total usage for each reagent. To remove a selected method from the calculation, uncheck the box next to the method name. If different amino acid concentrations are required across the selected runs, a warning will appear to notify that amino acid concentration from the largest scale will be used in the calculation.
5. To run using a different concentration of amino acid, enter the desired concentration in the box at the top of the Required Reagent Amounts table. The calculator will automatically update to reflect the new concentration.
6. To print a Usage Report, click the View Report button at the bottom of the Usage Calculator screen. This will generate a report as a PDF which can be printed or saved for future reference.

Reagent Calculator

The  Reagent Calculator can be used to determine how to prepare all reagents to be loaded on the system at the correct concentrations. There are five tabs: Resin, Deprotection, Activators, Activator Bases, and Amino Acids.

Resin Calculator Tab

1. Enter the scale for the synthesis.
2. Enter the resin substitution value in the Resin Substitution box. This value is provided by the supplier of the resin, and is usually expressed in mmol/g or meq/g.
3. The mass required will automatically be calculated (in grams).
4. To include this reagent in the Reagent Report, check the Include In Report box.

Deprotection Calculator Tab

1. Click on the desired deprotection reagent from the list.
2. Enter the desired concentration (in percent volume).
3. Enter the total volume of deprotection solution required.
4. The required amount of deprotection reagent in milliliters (for liquids) and grams (for solids) will automatically be calculated.
5. To include the desired reagent in the Reagent Report, check the Include In Report box.

Activators and Activator Bases Calculator Tabs

1. Click on the desired reagent from the list.
2. Enter the desired concentration (in M).
3. Enter the total volume of reagent solution needed.
4. The required mass or volume (in grams or mL respectively) will automatically be calculated.
5. To include the desired reagent in the Reagent Report, check the Include In Report box.

Amino Acids Calculator Tab

1. Click on an amino acid to select it, then double-click the volume and enter the desired volume (in mL).
2. Double-click the concentration and enter the desired concentration (in M).
3. The required mass (in grams) will automatically be calculated for the selected amino acid.
4. To calculate for all amino acids, right-click and select "Apply volume to all" and/or "Apply concentration to all". The required mass (in g) will automatically be recalculated for all amino acids using the selected parameter.
5. To include amino acids in the Reagent Report, check the Include In Report box for each amino acid to be reported.

Reagent Calculator Report

A Reagent Calculator Report includes the required masses and volumes for all selected reagents for a given synthesis. The report will be generated as a PDF which can be printed or saved for future reference.

To Generate A Reagent Calculator Report:

1. For each reagent to be included in the report, check the Include in Report box for that specific reagent.
2. Click View Report to render the report.

Edit Users and User Group

New users and user authority is determined by the default administrator. User group privileges can also be edited by the administrator. All changes are tracked in the audit trail. To enable users/user groups, click on the Options tab and select Settings. In the Application folder, select User/Password Security. In the drop-down next to Enable users - authentication type, select Local and click Save.

Creating a new user

1. Log in as administrator.
2. In the Administration menu, select Edit Users.
3. To edit a current user (Name, Group, or add picture), select the user name.
4. To create a new user, select the "+" icon.
5. Create user name, group type, and change password.
6. Follow the prompts. Password must be at least 8 character(s) and cannot contain any part of user name.
7. Select the green Save icon.

Removing a user

1. Log in as administrator.
2. In the Administration menu, select Edit Users.
3. Once a user is created, they cannot be removed. They can be deactivated.
4. Select the user.
5. Select the “-“ icon and select “Deactivate” to deactivate the account or “Cancel” to ignore.
6. To reactivate, select the “reactivate” icon (curved circular arrow).
7. Re-enter new password credentials.

Edit a User Group

1. Log in as administrator.
2. In the Administration menu, select Edit User Groups.
3. Select the user group and make edits as needed. The following is the comprehensive list of user groups and default privileges.

Permission	Administrator	Power User	User	Guest
Admin				
Edit users	Permitted	Denied	Denied	Denied
Edit system settings	Permitted	Permitted	Denied	Denied
Edit calibrations	Permitted	Permitted	Permitted	Denied
Approve changes	Permitted	Permitted	Denied	Denied
Make changes without approval	Permitted	Denied	Denied	Denied
View audit trail	Permitted	Permitted	Denied	Denied
View hardware configuration	Permitted	Permitted	Denied	Denied
View current operation step	Permitted	Denied	Denied	Denied
View components status	Permitted	Denied	Denied	Denied
View application state via OPC	Permitted	Permitted	Permitted	Denied
Call OPC methods	Permitted	Permitted	Denied	Denied
Methods				
View methods (all)	Permitted	Permitted	Denied	Denied
View methods (group)	Permitted	Permitted	Permitted	Denied
View methods (lesser groups)	Permitted	Permitted	Permitted	Denied
Edit methods (all)	Permitted	Denied	Denied	Denied
Edit methods (group)	Permitted	Permitted	Permitted	Denied
Edit methods (lesser groups)	Permitted	Permitted	Permitted	Denied
Create methods	Permitted	Permitted	Permitted	Denied
Edit template cycles and microwave methods	Permitted	Denied	Denied	Denied
Import/Export methods	Permitted	Permitted	Permitted	Denied
Results				
View results (all)	Permitted	Permitted	Permitted	Permitted
View results (group)	Permitted	Permitted	Permitted	Permitted
View results (lesser groups)	Permitted	Permitted	Permitted	Permitted
Miscellaneous				
View camera	Permitted	Permitted	Permitted	Permitted

Data Integrity

The Liberty Blue provides a software solution for automated microwave peptide synthesis users to aid in achieving compliance with the FDA's guidance on electronic records and electronic signatures: 21 CFR Part 11.

- 1.** Software allows viewing of records for validity. Records include a date of last modification and a revision number which is incremented upon modification.
- 2.** Software has the ability to authenticate individuals via local accounts within the application software. User accounts can be created, disabled and re-enabled by the administrator.
- 3.** Software authenticates users based on both a unique user name and password security. Each user is a member of a particular user group. Each user group specifies a list of privileges allowed and disallowed to users of that group.
- 4.** Software stores records in encrypted databases to ensure easy retrieval and prevents records being read or altered outside of the application.
- 5.** Software requires user to sign records and reports as well as changes in methods with a unique user name and password. User name, the date and time the signature was executed, and the meaning of the signing are recorded.
- 6.** Software records the signature(s) in the record audit trail which may also be viewed and printed.

Setting up a Synthesis

Loading a Method

Once a  method has been created and saved in the  Liberty Method Editor, it is available from the Methods tab on the main Liberty Blue Software screen. To load a method:

1. Open the appropriate  folder in the Methods tab.
2. Click on the desired  method, and drag the method to the resin position. Alternatively, right-click on the desired  method and select “Queue for run”.
3. The method name will appear in the Loaded Method indicator. The indicator will turn blue, meaning the method is loaded and ready to run.

Standard Reagents and Concentrations

The Liberty Blue uses stock solutions of all reagents. The table below details the concentrations used on the Liberty Blue and/or CEM's preferred reagents.

Reagents	0.005 mmol	0.01 mmol	0.025 mmol	0.05 mmol	0.1 - 0.5 mmol	1.0 - 5.0 mmol
Wash Solvent*	DMF	DMF	DMF	DMF	DMF	DMF
Deprotection Cocktail	4% Piperidine in DMF or 2% Piperazine (w/v) in EtOH:NMP (10:90)	4% Piperidine in DMF or 2% Piperazine (w/v) in EtOH:NMP (10:90)	20% Piperidine in DMF or 10% Piperazine (w/v) in EtOH:NMP (10:90)	20% Piperidine in DMF or 10% Piperazine (w/v) in EtOH:NMP (10:90)	20% Piperidine in DMF or 10% Piperazine (w/v) in EtOH:NMP (10:90)	20% Piperidine in DMF or 10% Piperazine (w/v) in EtOH:NMP (10:90)
Amino Acids	0.04 M in DMF	0.08 M in DMF	0.2 M in DMF	0.2 M in DMF	0.2 M in DMF	0.4 M in DMF
Activator**	0.05 M DIC in DMF	0.10 M DIC in DMF	0.25 M DIC in DMF	0.50 M DIC in DMF	1.0 M DIC in DMF	1.0 M DIC in DMF
Base	0.05 M Oxyma in DMF	0.1 M Oxyma in DMF	0.25 M Oxyma in DMF	0.5 M Oxyma in DMF	1.0 M Oxyma in DMF	1.0 M Oxyma in DMF

*NMP is suitable as a wash solvent or for dissolution/dilution of reagents, but requires 75°C coupling cycles to reduce the risk of insertions.

**View the CarboMAX application note, ap0124, at www.cem.com for further details.

NOTE

The Liberty Blue is not equipped to perform peptide cleavages. Cleavages should be carried out at room temperature or heated in the Razor™.

Recommended Reagents and Strategies for Microwave SPPS on the Liberty Blue

This section is intended to provide general guidance for reagent selection when synthesizing peptides on the Liberty Blue. For each case CEM's preferences are ranked in order with #1 being the preferred choice. The corresponding MW methods recommended are subsequently described in the Microwave Methods Selection.

! WARNING

Handle all chemicals under a fume hood, and wear suitable protective clothing such as safety glasses, chemical resistant gloves, and a laboratory coat. Dispose of all waste in accordance with all applicable local, state, and federal health and safety recommendations. For detailed information on the safety requirements for the chemicals used on the Liberty Blue, refer to the appropriate SDS documents.

Resin Selection

Default cycles are available for both standard resins (such as polystyrene) and high-swelling resins (such as PEG). It is important to use the appropriate cycles for the resin being used in the synthesis. When the resin type is selected in the [Liberty Method Editor](#), the appropriate default cycles for that type will automatically be loaded. Although most resins are compatible with microwave SPPS on the Liberty Blue, CEM recommends the resins shown in the table below for optimal results.

Peptide Length	C-Terminal Acid	C-Terminal Amide
Standard (<25 residues)	Cl-TCP(Cl) ProTide	Fmoc Rink Amide ProTide
Long (>25 residues)	Cl-MPA ProTide (LL)	Fmoc Rink Amide ProTide (LL)

Mesh Size

Only 100-200 mesh (or lower) resin (>75 micron) should be used with the Liberty Blue. The use of higher mesh resins will result in clogging of the reaction vessel frit and damage to the system.

Deprotection Solution Selection**General Strategy**

Historically, piperidine has been the most common deprotection reagent. Piperazine, however, is a great alternative and is growing in popularity; it is a non-controlled substance, relatively inexpensive, and less malodorous than piperidine. Although piperazine has limited solubility in DMF and NMP, it shows improved solubility and deprotection activity in a solution of 10% ethanol in NMP.

CEM Preference	Deprotection Cocktail	Microwave Method
1	20% Piperidine (v/v) in DMF or NMP	Standard
2	10% (w/v) Piperazine in EtOH:NMP (10:90)	Standard

For sequences where aspartimide formation is a concern, 0.1 M HOBt or 0.1 M Oxyma should be added to the deprotection cocktail to suppress the side reaction.

i NOTE

Allow up to 1 hour for piperazine to completely dissolve with stirring. If 0.1 M Oxyma is added then 30 minutes is enough to completely dissolve with stirring. Gentle heating on a hotplate can help. Calibration of the deprotect timed addition should be performed for the particular reagent used (piperazine or piperidine).

Special Case: Sequences Containing Asp-Gly (DG)

The Asp-Gly (DG) segment is particularly sensitive to base-catalyzed aspartimide formation. Simple addition of HOBt or Oxyma to the standard deprotection solutions may not be sufficient to prevent this side reaction. Therefore, less aggressive deprotection conditions should be used or a Dmb protected dipeptide that prevents this side reaction. Below are a few additional approaches for combating aspartimide formation.

Suppression Strategy	Microwave Method
5% (w/v) Piperazine w/ 0.1 M Oxyma Pure in EtOH:NMP (10:90)	Two step room temperature deprotection: 1. Conventional initial deprotection 2. Conventional deprotection
Use Fmoc-Asp(OtBu)-(Dmb)Gly-OH dipeptide and any deprotection cocktail applicable for standard peptides	Standard
Use Fmoc-Asp(OMpe)-OH and any deprotection cocktail applicable for standard peptides.	Standard

Activation Strategy Selection

General Strategy

The two most popular activation strategies for solid phase peptide synthesis are carbodiimide promoted coupling (using a carbodiimide such as DIC with an additive such as HOBt or Oxyma to quickly convert the O-acylisourea intermediate to a reactive ester) and onium salt promoted coupling (using a base such as DIEA to initiate conversion of the amino acid to the activated ester through reaction with an onium salt such as HBTU). The table below details CEM's preferred strategies and recommended microwave conditions for each strategy.

CEM Preference	Reagents	Cycle	Exceptions
1	AA/DIC/Oxyma ¹ in DMF	Single Coupling ²	Arg: Double Coupling
2	AA/HBTU ³ /DIEA ⁴ in DMF	90°C 1 min Deprotection Wash (x4) 75°C 5 min Single Coupling	Cys: Single 50°C 10 min Coupling His: Single 50°C 10 min Coupling Arg: 1 st Coupling: 25 min room temp, 75°C 2 min 2 nd Coupling: 75°C 5 min
3	AA/DIC/Oxyma ¹ in NMP	90°C 1 min Deprotection Wash (x4) 75°C 10 min Coupling	Cys: Double 50°C 10 min Coupling His: Double 50°C 10 min Coupling Arg: 1 st Coupling: 25 min room temp, 75°C 2 min 2 nd Coupling: 75°C 5 min
4	AA/HBTU ³ /DIEA ⁴ in NMP	90°C 1 min Deprotection Wash (x4) 75°C 5 min Single Coupling	Cys: Single 50°C 10 min Coupling His: Single 50°C 10 min Coupling Arg: 1 st Coupling: 25 min room temp, 75°C 2 min 2 nd Coupling: 75°C 5 min

¹HOBt, 6-Cl-HOBt, or HOAt can be used in place of Oxyma.

²Recommended cycles assume employment of His(Boc). For other His derivatives, use Single 50°C 10 min Coupling cycle.

³These conditions should be used for all onium activators (HBTU, 6-Cl-HBTU, HATU) and for the phosphonium activators (PyBOP, PyAOP).

⁴DIEA should be prepared in NMP.

i NOTE

For assistance with cycle and microwave method programming, please contact CEM Applications Support at peptide.support@cem.com.

Special Case: Hyper Acid Sensitive Resins (e.g. 2-chlorotrityl)

Acidic additives (HOBt, HOAt, Oxyma) can lead to premature cleavage of the peptide from the resin (low yields). Activation methods containing DIEA are more basic and thus preferred to DIC/Oxyma for these resins at high temperatures. Since lower temperatures should be used, the more aggressive HATU activator is recommended.

CEM Corporation offers Cl-TCP(Cl) ProTide which shows excellent stability to high temperatures and acidic additives such as Oxyma Pure, yet still exhibits hyper-acid sensitivity. The Cl-TCP(Cl) ProTide resin can be cleaved under 1% TFA/DCM conditions.

CEM Preference	Reagents	Resin/Linker	Coupling Microwave Method ¹	Exceptions
1	AA/DIC/Oxyma ² in DMF	Cl-TCP(Cl) ProTide Resin	Standard Coupling (90°C 2 min)	N/A ³
		2-Chlorotrityl resin	60°C 5 min Coupling ⁴	N/A ³
2	AA/HBTU/DIEA ⁵ in DMF	Cl-TCP(Cl) ProTide Resin	75°C 5 min Coupling	Cys/His: 50°C 10 min Coupling
		2-Chlorotrityl resin	50°C 10 min Coupling	N/A

¹Standard deprotection (90°C 1 min) can be performed in all cases.

²0.1 equivalents DIEA should be added to Oxyma solution

³Recommended Coupling Microwave Method assumes employment of His(Boc). For other His derivatives, use 50 °C 10 min Coupling Microwave Method.

⁴Coupling time can be extended to 10 min.

⁵DIEA should be prepared in NMP.

i NOTE

The rapid microwave methods used by the Liberty Blue employ rapid heating rates. For both the deprotection and coupling methods the set temperature of 90 °C should be reached within 20-30 seconds, and maintained for at least 45 seconds and 100 seconds respectively. The starting temperature for each reaction will be significantly warmer than room temperature as residual heat will remain from the previous reaction.

Preparing Reagents

! WARNING

Handle all chemicals under a fume hood, and wear suitable protective clothing such as safety glasses, chemical resistant gloves, and a laboratory coat. Dispose of all waste in accordance with all applicable local, state, and federal health and safety recommendations. For detailed information on the safety requirements for the chemicals used on the Liberty Blue, refer to the appropriate SDS documents.

Preparing Activator Solution

1. Weigh out the appropriate amount of activator (as calculated using the  Usage and Reagent Calculators) and transfer to a 250 mL glass bottle.
2. Add the appropriate volume of solvent.
3. Put a cap on the bottle. Gently swirl the bottle to ensure that all of the activator has gone into solution.

Preparing Activator Base Solution

1. Measure out the appropriate amount of activator base (as calculated using the  Usage and  Reagent Calculators) and transfer to a 250 mL glass bottle.

CAUTION

When using DIEA, NMP must be used as the solvent because DIEA is not miscible in DMF at the standard concentration of 2 M. The final concentration of DIEA in the reaction vessel is low enough that miscibility is not an issue.

2. Add the appropriate volume of solvent to give the correct final volume.
3. Put a cap on the bottle. Gently swirl the bottle to ensure all of the activator base has gone into solution.

Preparing Amino Acids

1. Weigh out the appropriate amount of each amino acid (as calculated using the  Usage and  Reagent Calculators).
2. Transfer each amino acid to a clean 50 mL centrifuge tube.
3. Add the appropriate volume of DMF to each centrifuge tube.
4. Place a cap on each centrifuge tube and shake vigorously to ensure the amino acid has fully gone into solution.

CAUTION

All centrifuge tubes used on the system must be ultra-high performance grade, and must NOT use CentriStar closures.

Preparing Deprotection Solution

1. Measure out the appropriate amount of deprotection reagent (as calculated using the  Usage and  Reagent Calculators).
2. Transfer the deprotection reagent to a 250 mL glass bottle.
3. Add the appropriate volume of DMF to give the correct final volume as calculated in the Reagent Calculator.
4. Add HOBT or Oxyma if necessary.
5. Place a cap on the bottle. Swirl the bottle gently to ensure the deprotection reagent (and HOBT/Oxyma, if used) has fully gone into solution.

NOTE

Piperazine may take up to an hour to go into solution. It is helpful to add a magnetic stir bar and stir the solution to speed dissolution.

Setting up the Liberty Blue

Loading Reagents onto the Liberty Blue

1. Connect amino acid centrifuge tubes to the appropriate positions on the system. Connect empty centrifuge tubes to all unused positions.

i NOTE

All reagent positions must have a centrifuge tube in place. If a given reagent is not required for a synthesis, connect an empty centrifuge tube to that position.

2. Connect activator base and activator bottles to the appropriate positions on the system.

i NOTE

It is important that the reaction vessel be empty before running the Change Bottle Deprotection operation. The Change Bottle Deprotection operation will add deprotection solution to the reaction vessel as part of the priming procedure, and then wash the reaction vessel with main solvent.

3. Check the level of deprotection solution remaining in the deprotection bottle. If more deprotection is required, use the Change Bottle Deprotection operation to replace the bottle (see Change Bottle Deprotection in Section 4).
 - 3.1. From the Options menu, select  Maintenance.
 - 3.2. From the Change Bottle folder, select the Change Bottle Deprotection operation and click Run Operation.
 - 3.3. The Change Bottle window will appear. Follow the on screen instructions to depressurize and remove the existing bottle.
 - 3.4. Add the required volume of deprotection solution to the bottle. Ensure the reagent line has a main solvent filter in place (PN 551000), then connect the bottle to the cap.
 - 3.5. Click OK to continue with the Change Bottle procedure. The Liberty Blue will automatically pressurize the bottle and click OK to prime the lines.
4. Check the level of solvent remaining in the solvent bottle. If more solvent is required, use the Change Bottle Main Solvent operation to replace the bottle. (see Change Bottle Main Solvent).
 - 4.1. From the Options menu, select  Maintenance.
 - 4.2. From the Change Bottle folder, select the Change Bottle Main Solvent operation and click Run Operation.
 - 4.3. The Change Bottle window will appear. Follow the on screen instructions to depressurize and remove the existing bottle.
 - 4.4. Ensure the dip tube has a main solvent filter (PN 551000) in place, then screw the cap onto the solvent bottle.
 - 4.5. Click Next to continue with the Change Bottle procedure. The Liberty Blue will automatically pressurize the bottle and prime the lines.
5. Add the resin to the reaction vessel.

! WARNING

It is vital to use the appropriate reaction vessel for the scale of the synthesis. The standard (30 mL) vessel should be used for syntheses between 0.005 and 0.25 mmol scale (up to 1 g of resin maximum). The large (125 mL) vessel should be used for syntheses at 0.5 mmol scale and higher (up to 8 g of resin maximum).

To replace the vessel:

1. Remove the reaction vessel from the microwave cavity.
2. Disconnect the bottom fitting of the brown union connector.
3. Disconnect the vessel body from the attenuator.
4. Disconnect the filtered drain line from the vessel body at the brown fitting.
5. Connect the bottom portion of the union to the new vessel body.

6. Connect the new vessel body to the attenuator.
7. Connect the filtered drain line to the new vessel body using the union, being careful not to cross thread or over tighten the fitting.
8. Place the vessel back into the microwave cavity, securing the attenuator.

- 5.1. Use  Reagent Calculator to determine the amount of resin required for the synthesis.
- 5.2. Remove the reaction vessel from the microwave cavity.
- 5.3. Disconnect the filtered drain line from the vessel body using the union.

WARNING

The Liberty Blue reaction vessel is designed to work with 100-200 mesh resins only. The use of 200-400 mesh resins will clog the frit on the bottom of the reaction vessel, resulting in failure to drain the vessel and a possible reaction vessel overflow event.

- 5.4. Disconnect the vessel body from the attenuator.
- 5.5. Weigh the appropriate amount of dry resin (100-200) mesh into the vessel body.
- 5.6. Connect the vessel body to the attenuator.
- 5.7. Connect the filtered drain to the vessel body using the brown union.
- 5.8. Place the vessel back into the microwave cavity, securing the attenuator.

Running the Liberty Blue

Preparing the Liberty Blue to Run

1. Verify the level of the waste container to ensure there is sufficient room before starting another synthesis.
2. Verify the nitrogen supply to ensure there is sufficient nitrogen to complete the synthesis.
3. Verify that the fiber optic probe is fully inserted into the thermowell.

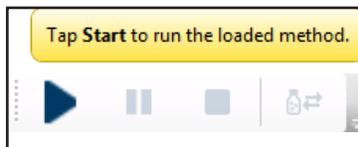
WARNING

If the probe is not inserted all the way to the bottom of the vessel the Liberty Blue will not accurately measure the temperature, and overheating of the vessel can occur. This will result in poor synthesis quality and/or serious damage to the vessel.

4. Verify that the method is correct by looking at the steps outlined in the Method box. If there are any errors, make corrections and reload the method.
 - 4.1. Clear the method by clicking the small X at the top of the resin indicator.
 - 4.2. To correct the method:
 - 4.2.1 Open the  Liberty Method Editor.
 - 4.2.2 Open the  folder where the method was saved, then click on the method to load it.
 - 4.2.3 Make any  corrections as needed and then click Save to save the method. Then, close the Liberty Method Editor.
 - 4.2.4 Load the corrected method as described above.

Running the Liberty Blue

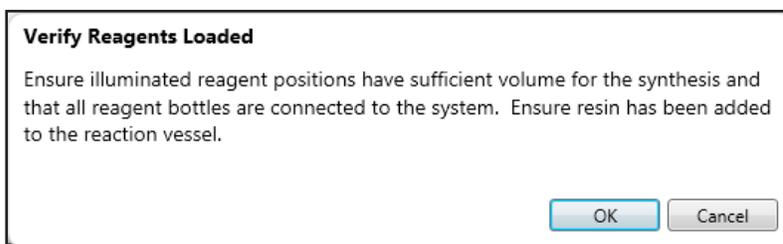
1. Click the Start button at the top of the Liberty Blue Software main screen.



2. The Loaded Method indicator will turn yellow to indicate the method is running.



3. The Liberty Blue will illuminate all required amino acids positions for the method. Ensure that all reagents are connected and have sufficient volume, empty centrifuge tubes are connected to any unused reagent positions, and that there is sufficient solvent and deprotection solution, then click OK to continue.



4. The Liberty Blue will go through a series of initialization steps and leak checks.
5. During the run, the current step of the method is highlighted in yellow in the Cycle Steps table of the Current Run tab. A status bar in the lower right of the main screen indicates relative progress of the run.
6. When the run is complete, the Loaded Method indicator will turn green, indicating the method was successfully completed.



Generating A Run History Report

The  Run History Report records each command executed by Liberty Blue Software during a run with a date/time stamp. In addition, the  Run History Report records any system errors that occur, allowing for easy diagnosis and troubleshooting of failed syntheses.

Creating a Run History Report

From the Run History tab:

1. Open the  folder for the date on which the method was run.
2. Click the desired run. The run history information will load into the table on the right.
3. Click the  Generate Report button.
4. The Report Viewer window will open, and the method report will be rendered as a PDF.
5. Click Save to save the report.

Cleavage

Various scavenger molecules are added to the TFA to prevent cleaved protecting groups from reattaching to the peptide. The particular scavengers used depend on the specific peptide sequence. Common scavengers include water (scavenges t-butyl cations), triisopropylsilane (TIS, scavenges trityl and Pbf cations), ethanedithiol (EDT, scavenges t-butyl cations, reduces oxidation of Cys/Met side chains), dioxo-1,8-octane-dithiol (DODT, scavenges t-butyl cations, suppresses oxidation of Cys/Met side chains), phenol (protects Tyr and Trp side chains from oxidation), and thioanisole (aids in removal of Pbf protecting groups from Arg(Pbf), suppresses oxidation of Cys/Met side chains).

Cleavage can be performed heated, or at room temperature. The table below lists CEM's recommended parameters for both heated and room temperature cleavages.

CEM Preference	Cleavage Cocktail	Temperature	Time
1	TFA/TIS/H ₂ O/DODT (92.5/2.5/2.5/2.5)	40-42°C	30 min
2	TFA/TIS/H ₂ O/DODT (92.5/2.5/2.5/2.5)	Room Temperature	3 h

i NOTE

If the peptide contains multiple arginines, cleavage time may need to be extended to ensure complete Pbf removal.

Maintenance of the Liberty Blue

Recommended Routine Maintenance Schedule

A routine maintenance protocol is vital for the long-term operation of the Liberty Blue.

Daily Maintenance

- Ensure the fiber optic probe is fully inserted into the thermowell of the reaction vessel.
- Empty waste reservoir and fill main wash position.

Biweekly Maintenance

- Backflush all positions except Main Wash (see Backflush Reagent Positions & Backflush Deprotection).
- Replace amino acid, activator, and activator base solutions.

Monthly Maintenance

- Perform a timed addition calibration for all timed additions (see Timed Addition Calibration).
- Verify the performance of the filtered drain (see Reaction Vessel Drain Test).
- Review recent run history reports to ensure that the Liberty Blue is heating properly.

Semiannual Maintenance

- Replace all amino acid diptube filters.
- Replace the Main Solvent and Deprotection filters.
- Replace the reaction vessel (or as needed by software Maintenance Tracker).
- Verify performance of waste sensor (see Verification of Waste Sensor Operation).

Annual Maintenance

- Replace all amino acid and reagent manifold o-rings. If the HT resin loader option is included, replace these manifold o-rings also.
- Tighten the diptube nuts in the manifold ports for each reagent position. If the HT resin loader option is included, tighten these diptube nuts also.

The following chart is provided for a record of routine maintenance procedures. As each procedure is completed as indicated in the record, either a check, a date, or the user's initials should be entered in the appropriate box to record the maintenance procedure as completed.

Biweekly

Week	2	4	6	8	10	12	14	16	18	20	22	24	26
System Backflush													

Monthly

Month	1	2	3	4	5	6	7	8	9	10	11	12
Timed Addition Calibration												
Reaction Vessel Drain Test												
View run history reports to ensure proper heating*												

Semiannual

Month	6	12	18	24	30	36	42	48	54	60	66	72
Replace AA Dip Tube Filters												
Replace Main Solvent and Deprotection Filters												
Verify proper operation of the waste sensor												

Annual

Year	1	2	3	4	5	6	7	8	9	10	11	12
Replace all amino acid and reagent manifold o-rings.												
Tighten the diptube nuts in the manifold ports for each reagent position.												

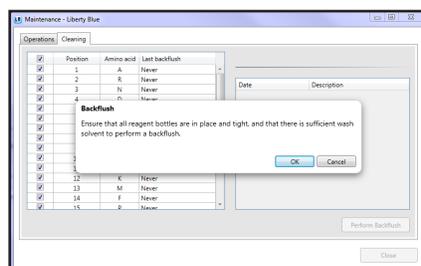
i NOTE

A complete listing of spare and replacement parts with part numbers and images can be found on the CEM website (www.cem.com).

Maintenance Procedures

Backflush Reagent Positions

1. Select "Maintenance" from the "Options" menu.
2. Select the Cleaning tab.
3. Replace the centrifuge tubes or bottles for positions to be backflushed with empty tubes or bottles.
4. Ensure that all reagent positions have a bottle connected.
5. From the Cleaning Tab, check the box next to each position to be backflushed. To backflush all reagents, check the box at the top of the table to select all positions.
6. Click the Perform Backflush button. A warning box will pop up. Verify that empty bottles have been connected and tightened for all positions to be backflushed, and that there is sufficient solvent for the operation. Click OK to continue.



7. A progress window will pop up showing the progress of the backflush procedure for all amino acids.

<input checked="" type="checkbox"/>	Position	Amino acid	Last backflush
<input checked="" type="checkbox"/>	1	A	10/25/2013 4:29:20 PM
<input checked="" type="checkbox"/>	2	R	10/25/2013 4:29:22 PM
<input checked="" type="checkbox"/>	3	N	10/25/2013 4:29:24 PM
<input checked="" type="checkbox"/>	4	D	10/25/2013 4:29:27 PM

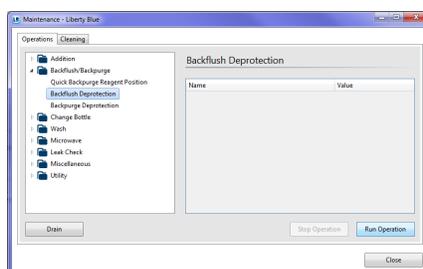
i NOTE

As each position is backflushed, the Last Backflush date will be updated to the current date and time.

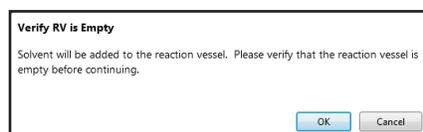
- Once all reagent positions have been backflushed, the manifold will be cleared, and the system will perform a backpurge of selected positions.
- After backflushing, observe the volume in the amino acid bottles. If the bottles have less than 2 mL of Main Wash added, replace the dip tube filter in the bottle(s) that are low and perform a backflush on those specific positions again. If volume is still below 2 mL, contact CEM.
- Once all positions have been backpurged, remove the bottles from those positions and dispose of the liquid.

Backflush Deprotection

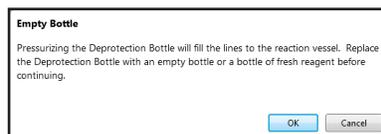
- Select “Maintenance” from the “Options” menu.
- Select the  Backflush/Backpurge folder and then select the Backflush Deprotection operation
- Click Run Operation.



- The software will prompt to verify the reaction vessel is empty. Ensure the reaction vessel is empty, then click OK to continue.



- Solvent will be added to the reaction vessel, then backpurged into the deprotection bottle. To avoid filling the lines with the solvent, the software will prompt to replace the bottle. Replace the bottle, then click OK to complete the backflush operation.



Timed Addition Calibration

There are four timed additions that must be calibrated for optimal functionality of the Liberty Blue: Wash, Wash Thru Manifold, Deprotection, and Wash Thru RV Bottom. To calibrate a timed addition:

- Place the reaction vessel into the volume calibration stand.
 - Remove the reaction vessel assembly from the cavity of the Discover.
 - Insert the reaction vessel into the calibration stand so that the drain line from the bottom of the vessel fits into the slot on the right of the stand.
- From the Calibration screen, under the Timed Addition  folder, select the  addition to be calibrated. The table on the right will update with the calibration history for the selected addition.
- Click the Calibrate button to begin the calibration operation.

4. Enter the volume to be used for calibration, then click OK. Typically, 5 mL of reagent are used for calibration.

Calibrate Wash(MS2) Volume

Remove the reaction vessel from the microwave cavity and place it in the calibration stand.

Enter the desired volume into the text box below and hit OK to add desired volume into the reaction vessel.

Desired Volume:

5. The selected reagent will be added to the reaction vessel. Once the addition is finished, measure the volume delivered to the reaction vessel using the graduations on the calibration stand.
6. Enter the volume into the Delivered Volume box.
7. Click OK to drain the reaction vessel. The software will then calculate the new flow rate. Click OK to save the new calibration values.
8. Once the new rate has been saved, click OK to complete the operation.
9. If the new timed addition flow rate is less than 1.0 mL/s, replace the position's filter with PN 551000 and recalibrate. If the flow rate is still below 1.0 mL/s, contact CEM.

Reaction Vessel Drain Test

1. In the Liberty Blue application software, open the  Maintenance screen.
2. Under  Addition, select the Add Main Wash (MS2) operation.
3. On the Parameters tab, double click the value for Volume, enter 10 in the pop-up, then click OK.
4. Click the Run Operation button to execute the operation. This will add 10 mL of solvent to the vessel.
5. Click the Drain button at the bottom of the Maintenance screen.
6. The liquid should drain within 10 seconds. If the liquid requires more than 10 seconds to fully drain, replace the entire vessel (PN 167260).

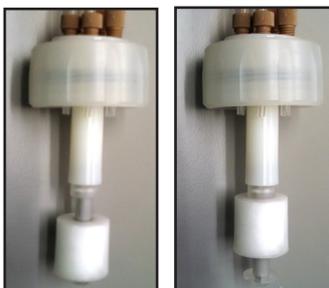
Verification of Waste Sensor Operation

The Waste Sensor is a float sensor located on the top of the waste bottle. It is connected to the Liberty Blue through a 9-pin connector cable plugged into the left side of the Liberty Blue module. Periodically, operation of the Waste Sensor should be verified.

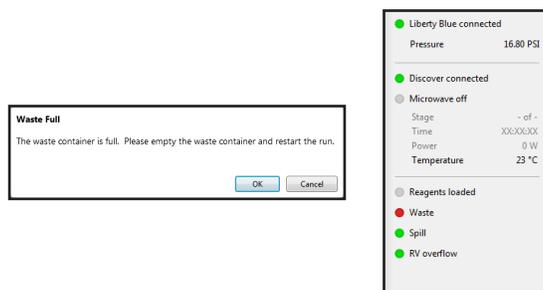
WARNING

Handle all waste under a fume hood, and wear suitable protective clothing. Dispose of all waste in accordance with all applicable local, state, and federal health and safety recommendations.

1. Ensure the Liberty Blue is idle.
2. Remove the cap from the waste container.
3. Lift the float sensor.



- The software should prompt a Waste Full error. In addition, the Waste indicator will turn red.



- If the Waste Full error does not prompt, ensure the 9-pin connector is securely plugged into the Liberty Module. If the plug is secure and the error does not prompt, replace the waste sensor assembly.
- Release the float sensor, connect the cap to the waste container, then click OK to clear the Waste Full error.

Standby Procedure

If the Liberty Blue will be idle for a period of two weeks or more:

- Remove all reagents (amino acids, activator, activator base) from the system.
- Select “Maintenance” from the “Options” menu.
- Use the Change Bottle Deprotection operation to replace the deprotection bottle with an empty bottle and properly clear the lines.

i NOTE

During the priming step, the system will get an Empty Deprotection Bottle Error. This is normal. Click Cancel to clear the error and end the Change Bottle operation.

- Backflush all positions, including deprotection.
- Use the Change Bottle Main Solvent operation to replace the main wash solvent bottle with an empty bottle and properly clear the lines.

i NOTE

During the priming step, the system will get an Empty Solvent Bottle Error. This is normal. Click Cancel to clear the error and end the Change Bottle operation.

- Use the Depressurize System operation to depressurize the system.

Prior to using the Liberty Blue after it has been in standby:

- Connect the main wash solvent using the Change Bottle Main Solvent operation to properly prime the lines and pressurize the bottles.
- Connect the deprotect bottle using the Change Bottle Deprotection operation to properly prime the lines and pressurize the bottle.
- Perform a backflush on all positions.
- Perform a Timed Addition Calibration for all timed additions.

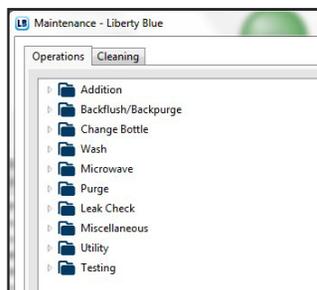
✖ Maintenance Screen

The ✖ Maintenance screen is accessed from the Options menu. From the Maintenance screen, important maintenance procedures and manual system commands can be accessed.

Operations Tab

From the Operations tab, various manual operations and cleaning procedures can be performed.

To perform an operation:



1. Open the  folder for the operation type, and select the operation to be performed.
2. Select any parameters for the operation in the table to the right.
3. Click the Run Operation button to execute the operation.

Operations Folders and Specific Operations

Change Bottle Main Solvent: The Change Bottle operation will depressurize the main solvent bottle and prime the lines for both the wash to the reaction vessel and the manifold wash.

Change Bottle Deprotection: The Change Bottle operation will depressurize the deprotection bottle, clear the lines to the bottle, and prime the lines to the reaction vessel.

NOTE

Other Operations Folders/Specific Operations in the Operations Tab should only be performed under the guidance of a CEM representative.

Using the Change Bottle Operations

The Change Bottle operations will depressurize the main solvent bottle and prime the lines for both the wash to the reaction vessel and the manifold wash.

Change Bottle Main Solvent

1. From the Operations tab, open the  Change Bottle folder and click on the Change Bottle Main Solvent operation.
2. Click the Run Operation button.
3. The Liberty Blue will vent the pressure from the system.
4. The software will prompt to replace the Main Solvent bottle. Remove the solvent bottle from the system and either refill the bottle or connect a new bottle to the main solvent line.
5. Click OK to continue. The Liberty Blue will pressurize the bottle and prompt to begin priming the lines. Click OK to begin the priming procedure, or click Cancel to skip priming the lines.

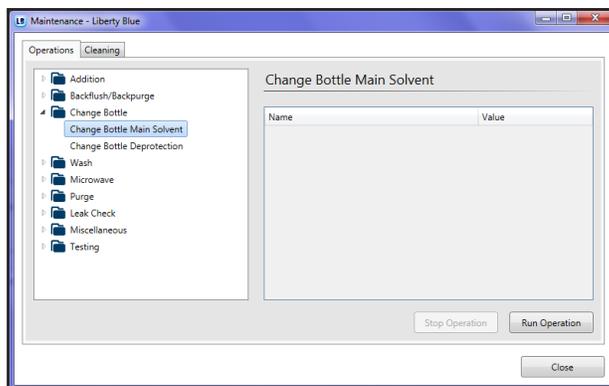
NOTE

Priming the lines will add solvent to the reaction vessel and drain the reaction vessel. The lines must be primed if the main solvent bottle was empty or when changing to a different solvent (for example, when changing from DMF to NMP). If the bottle was not empty, priming can be skipped.

- The Liberty Blue will prime the line to the reagent manifold (through valve MS1), purge the liquid to the reaction vessel, then drain the vessel. Next, the Liberty Blue will prime the direct wash line to the reaction vessel (through valve MS2), then drain the vessel.

Change Bottle Deprotection

- From the Operations tab, open the  Change Bottle folder and click on the Change Bottle Deprotection operation.



- Click the Run Operation button.
- The Liberty Blue will vent the pressure from the deprotection bottle, then clear the lines to the deprotection bottle.
- The software will prompt to replace the deprotection bottle. Replace the bottle, then click OK to pressurize the bottle.
- The software will prompt to begin priming the lines. Click OK to begin the priming procedure, or click Cancel to skip priming.

NOTE

The deprotection line must be primed or the first deprotect addition will not be accurate.

- The Liberty blue will prime the lines from the deprotection bottle to the reaction vessel, then drain the reaction vessel.
- Following the priming, the reaction vessel will be washed and drained.

Calibration Screen

The  Calibration screen allows for the calibration of the timed delivery of reagents to the reaction vessel, as well as all pressure and liquid sensors. The Calibration screen can be accessed from the Options menu.

Timed Addition Calibration

There are four timed additions that must be calibrated for optimal functionality of the Liberty Blue: Wash, Wash Thru Manifold, Deprotection, and Wash Thru RV Bottom. Timed Addition Calibration should be performed monthly as part of routine maintenance.

NOTE

Refer to the Timed Addition Protocol. All other calibrations should be performed under guidance of a CEM representative.

Main Pressure Calibration

Main Pressure calibration is not necessary for routine maintenance.

! WARNING

Main Pressure calibration should only be done on installation of the Liberty Blue or as part of a troubleshooting protocol as advised by CEM service or applications support.

Liquid Sensor Calibration

The Liberty Blue uses four liquid sensors for various error monitoring functions: LS1 (Wash Solvent sensor), LS2 (Deprotection/Drain sensor), LS3 (Reaction Vessel Overflow sensor), LS4, and LS7 (Spill sensor).

! WARNING

Liquid sensor calibration of LS3, LS4, and LS7 should only be done on installation of the Liberty Blue or as part of a Preventative Maintenance visit performed by a CEM-trained technician.

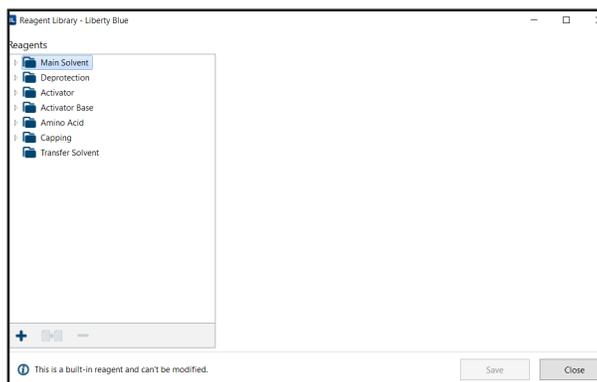
Advanced Features

Cycle Rules

Default cycles for resin swelling, amino acids, and final deprotection are located in Cycle Rules. Please contact CEM Applications Support at peptide.support@cem.com for assistance assigning/changing default cycles.

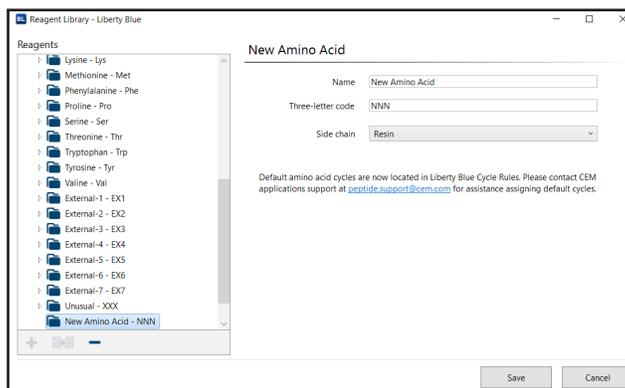
Reagent Library

The Reagent Library is a list of common reagents for peptide synthesis, including solvents, deprotection reagents, amino acids, activators, and activator bases. The Reagent Library is fully customizable; reagents may be added to or deleted from the library through the Reagent Library panel. Any reagent in the Library will appear in the appropriate tab of the Reagent Calculator. The Reagent Library can be opened by selecting Reagents from the Edit menu.



Adding a New Reagent to the Library

1. Open the folder for the appropriate reagent type.
2. Click the + New Reagent button.



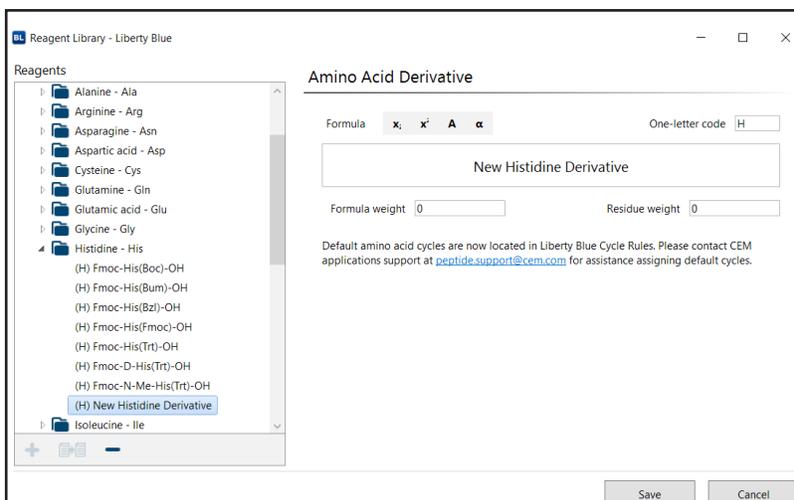
3. Enter the name and other properties into the blanks on the right side of the Reagent Library window. The name in the reagent list will automatically update when the new name is entered in Reagent Properties.
4. Click the Save button to save the new reagent.

Creating a New Specific Derivative

The Reagent Library allows the user to select the specific derivative of an amino acid (including derivatives with various side chain protection strategies) used in a particular Bottle Setup. Specific derivatives do not need to have the same 1-letter code as their parent.

To create a new specific derivative:

1. Open the Amino Acid folder.
 - 1.1. If the new derivative is a derivative of an existing amino acid, open the folder for the parent amino acid, then proceed to step 2.
 - 1.2. If the new derivative is not a derivative of an existing amino acid, select one of the External (corresponding to external amino acid positions on the instrument) or Unusual - XXX folders in which to add your new derivative.



2. With the folder for the parent amino acid type selected, click the **+** New Specific Derivative button. A new specific derivative will appear in the parent amino acid folder.
3. Enter the name, one-letter code, and other properties for the specific derivative; then click Save. By default, the Single Coupling cycle will be assigned as the default for all scales and resin types. To assign a different default cycle, a new cycle rule must be created.

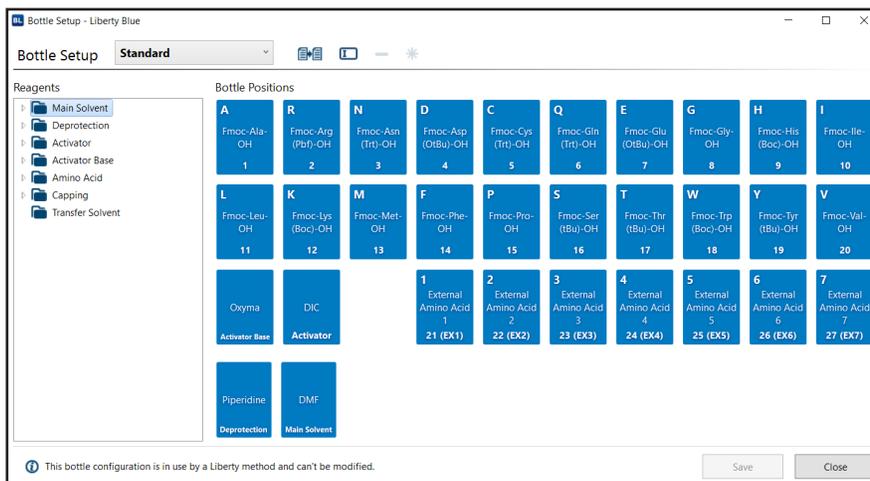
Copying and Deleting Reagents

A new reagent can be created as a copy of an existing reagent by selecting a reagent from the list and clicking the Duplicate Reagent button. When copying an existing specific derivative of an amino acid, the copy will be created in the same parent amino acid folder.

To delete a reagent from the Library, select the reagent and click the Delete button. Select Yes to confirm the deletion.

Bottle Setup

The  Bottle Setup screen allows the user to assign any reagent to any position on the Liberty Blue from within the Liberty Blue software. Individual instrument setups can be saved to allow for quick and easy configuration of the instrument for a variety of syntheses. The  Bottle Setup screen can be accessed from the Edit menu.



Creating a New Bottle Setup

1. Click the Copy Bottle Setup button.
2. A duplicated setup will appear in the Bottle Setup List. Name the new setup and press Enter.
3. To assign different amino acids and reagents, delete the appropriate reagent from the setup and replace it with the desired reagent from the list to the left.
4. Once all amino acids and reagents have been assigned, click Save to save the current layout.

NOTE

Every position must have a reagent assigned to use the Bottle Setup.

Modifying an Existing Bottle Setup

1. From the Bottle Setup screen, select the desired setup from the Bottle Setup List.
2. Make any changes to the setup.
3. Click Save.

Deleting an Existing Bottle Setup

1. From the Bottle Setup screen, select the setup to be deleted from the Bottle Setup List.
2. Click the  Delete Setup button.

NOTE

If the setup is used in a saved method, the software will not allow the setup to be deleted.

Selecting and Using a Bottle Setup

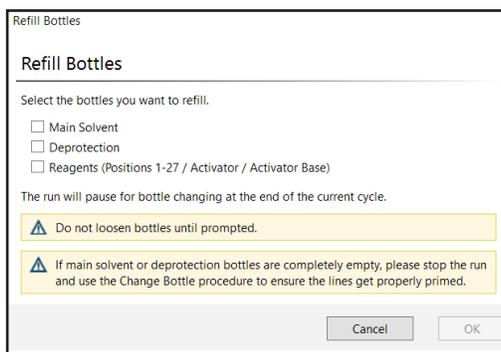
When creating a new Liberty Method, the bottle setup can be selected in the Method Editor. Select the new bottle setup before typing in the peptide sequence. The Method Editor will use the reagents assigned in the selected bottle setup to calculate the total molecular weight. When loading a method to run, the Usage Calculator will use the formula weights from the selected bottle setup to calculate the mass of each amino acid required.

Refill Reagents Button

The Refill Reagents button allows for the replacement of insufficiently filled reagent bottles while a method is running without the need to stop and restart the method manually.

To use the Refill Reagents button:

1. While the run is in progress, press the  Refill Reagents button to open the Refill Reagents window.



2. Check the box for each reagent to be refilled.
3. The run will continue until it reaches a safe stopping point (at the end of a cycle). Once the run has reached a stopping point, the system will depressurize the reagent bottles.
4. The system will prompt to refill the selected reagents. Remove the bottles, refill, replace the bottles, empty the waste container (if needed), then click OK to resume the method.

i NOTE

Only the selected reagent bottles will be depressurized.

i NOTE

The Refill Reagents button re-pressurizes bottles but does not prime reagent lines. Therefore, use of the Refill Reagents button is not recommended if deprotect or Main Wash bottles are empty.

5. The system will re-pressurize the reagent bottles, then automatically resume the run.

Diagnostics Screen

The Diagnostics Screen allows a number of manual controls and operations that are not intended for normal use. The Diagnostics screen should only be accessed under the guidance of a CEM representative.

Settings Screen

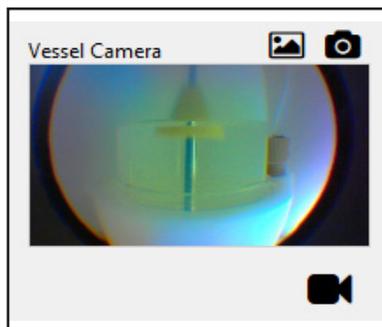
The Settings Screen can be accessed from the Options menu. The Settings screen allows configuration of a number of important system parameters.

i CAUTION

The default values for each setting have been developed for optimal performance. These values should only be changed under direction of CEM service or applications support.

Reaction Vessel Camera Option

The lightweight, easy-to-use reaction vessel camera captures full-color images which can be added to presentations and publications, or for real time method optimization and troubleshooting.



Camera Hardware Installation

Liberty Blue instruments ordered with the camera option will have the camera hardware installed in the Discover microwave prior to shipment. To connect the camera, plug the black USB camera cable into a USB port on the computer.

For field installation of the camera option, please contact your local CEM representative to order the Discover Bio Camera Retrofit Kit (PN 730125).

Camera Software

The Liberty Blue with camera option includes a USB drive with the necessary software driver for operating the camera option. To download this driver, connect the USB drive to a computer and open the Dell Webcam folder. Double click Setup to begin installation.

Enabling the Camera

1. Open the Settings screen in the Liberty Blue application software (Options -> Settings).
2. In the Application folder, select Vessel Camera.
3. Check the box next to Vessel Camera Enabled and save.
4. The vessel camera icon  will appear in the bottom right corner of the application software.

Capturing Images During a Run

1. Install the camera spill cup (PN 169560).
2. Click on the vessel camera icon to open the camera window and view the vessel in real time. To capture an image, click the  snapshot icon. The camera window will flash briefly, indicating a snapshot has been taken.
3. To view all captured images, click the  gallery icon. Alternatively, select File then Show Vessel Camera Snapshots. All images are saved to the computer hard drive (C:\ProgramData\CEM Corporation\Liberty Blue\Snapshots).

Warranty

Limited Warranty Information

What Is Covered:

CEM Corporation warrants that the instrument will be free of any defect in parts or workmanship and will, at its option, replace or repair any defective part (excluding consumables) or instrument.

For How Long:

This warranty remains in effect for 365 days from date of delivery to the original purchaser.

What Is Not Covered:

This warranty does not cover parts or workmanship damaged due to:

- Neglect, abuse or misuse,
- Damage caused by or to test samples,
- Damage incurred during instrument relocation,
- Damage caused by or to any attached equipment,
- Use of incorrect line voltages or fuses,
- Fire, flood, “acts of God” or other contingencies beyond the control of CEM Corporation,
- Improper or unauthorized repair, or
- Any other damage caused by purchaser or its agents.

Responsibilities of Purchaser:

To ensure warranty coverage, the purchaser must:

- Use the instrument according to directions,
- Connect the instrument properly to a power supply of proper voltage,
- Replace blown fuses,
- Replace consumables and
- Clean the instrument as required.

How to Get Service:

Purchaser should contact the Service Department of CEM Corporation or the nearest CEM subsidiary or distributor for return authorization and for proper crating and shipping instructions to return instrument, freight prepaid, for service. On-site repairs by an authorized service technician are available through the CEM Service Department. Travel costs will be charged to the purchaser for on-site repairs.

Within the U.S.

CEM Corporation
3100 Smith Farm Rd.
Matthews, NC 28105-5044
(800) 726-5551
Fax: (704) 821-4368

Outside the U.S.

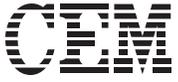
CEM Corporation
3100 Smith Farm Rd.
Matthews, NC 28105-5044
(704) 821-7015
Fax: (704) 821-4368

Warranty Disclaimer:

CEM Corporation hereby excludes and disclaims any warranty of merchantability or fitness for any particular purpose. No warranty, express or implied, extends beyond the face hereof. CEM Corporation shall not be liable for loss of use of instrument or other incidental or consequential costs, expenses or damages incurred by the purchaser or any other user. This warranty is not transferable.

Purchaser's Rights under State Law:

This warranty gives the purchaser specific legal rights, and the purchaser may also have other rights that vary from state to state.



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November 12, 2018 11:53 AM
Rev. 6
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