CEM CORPORATION

Accent

Accent™ Peptide Cleavage Option

Installation and Operation Instructions

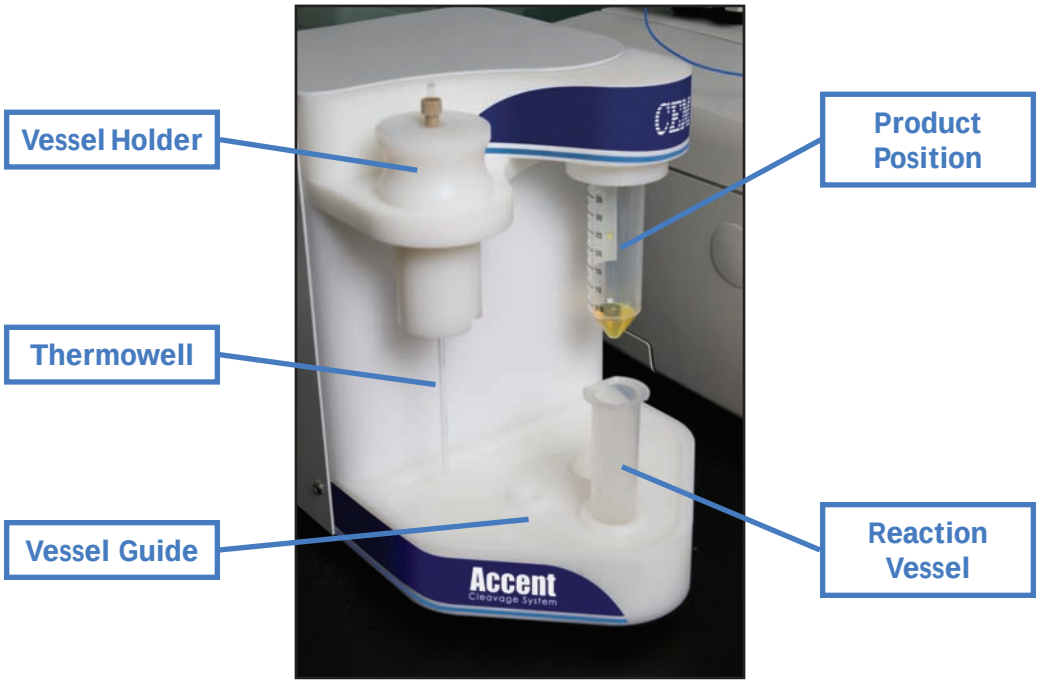
Contents

600125
Rev. 4
Nov. 2014

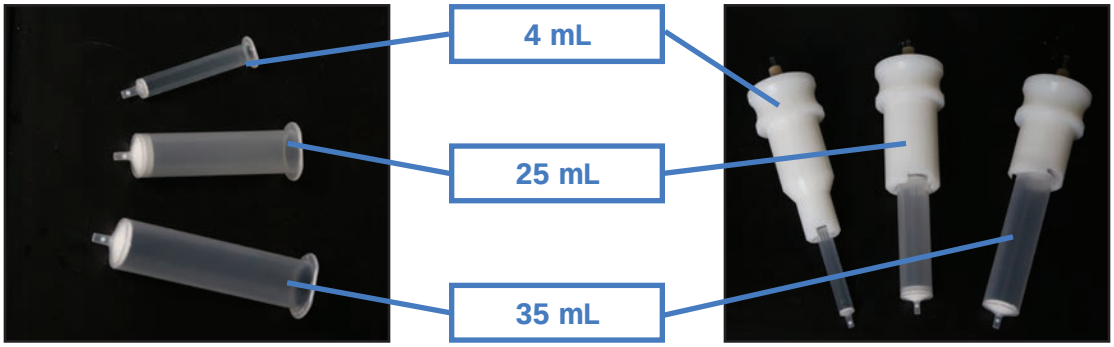
Introduction to the Accent

The Accent Cleavage System is an isolated liquid handling module designed for performing the peptide cleavage step. The isolated module provides the advantage of protecting the Liberty Blue system from the harsh reagents required for the cleavage all while allowing the use of microwave irradiation to provide high quality peptides in excellent yields in a fraction of the time.

Accent Hardware



Reaction Vessels and Holders



System Requirements

Bench and/or Fume Hood Space

The Accent should be positioned on the bench such that access to the electrical outlets for the system is not restricted. The Accent requires the following space for system components:

Discover Microwave Reactor

15" (w) x 20" (d) x 9" (h) [38 cm (w) x 51 cm (d) x 23 cm (h)]
(Depth includes 3" (8 cm) clearance behind instrument for unimpeded airflow at rear fan ducts)

Accent Wash Station

11" (w) x 11" (d) x 9.5" (h) [28 cm (w) x 28 cm (d) x 24 cm (h)]

Environmental Conditions

The Accent is designed for indoor used only.

Temperature Range: 50 °F – 85 °F (10 °C – 29 °C)
Relative Humidity Range: 0 – 85%

Electrical Requirements

The Accent 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 Accent.

Two (2) grounded electrical connections providing a total of 10 A are required for all components:

Discover Microwave Reactor power cord
Accent Wash Station power cord

NOTE

These requirements assume the Accent will be used with an independent Discover Microwave Reactor. The Accent can also be used with a Discover Bio Microwave Reactor installed with a Liberty Blue. Additional requirements for the Liberty Blue can be found in the Liberty Blue Operation Manual (PN 600291).

Hardware Assembly and Setup

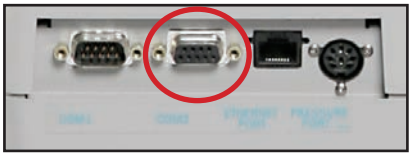
Discover Setup

NOTE

When using the Accent with a Discover Bio Microwave Reactor installed with a Liberty Blue, the Liberty Blue's fiber-optic probe can be used instead of installing an external fiber-optic option. If using the Liberty Blue's fiber-optic probe, proceed to Accent Setup, p. 4.

Temperature Probe Assembly

1. Connect one end of the serial cable to the rear of the FISO temperature control box. Connect the other end of the serial cable to the COM2 port on the rear of the Discover.



2. Connect the blue fiber-optic temperature probe to the FISO control box, taking care not to bend the probe.

Calibration of the Fiber-Optic Probe

1. Connect the power cord to the rear of the Discover. Plug the power cord into an appropriate power source. Locate the power switch on the left side of the Discover and turn the power on.
2. After the Discover has started up, press the EDIT key to open the System Menus.
3. The first menu option will be Temperature. Press ENTER to open the Temperature Menu.
4. Use the right arrow key to scroll to "Select Alternate" and press ENTER.
5. Use the right arrow key to change to "DEVICE = FIBER-OPTIC" and press ENTER.
6. The Discover will return to the Home screen. Press EDIT to open the System Menus, then press ENTER to open the Temperature Menu again.
7. Use the right arrow key to scroll to "Enter Calibration" and press ENTER.
8. Press EDIT, then use the numeric keypad on the right of the Discover to enter the seven digit GF number printed on the white band at the base of the blue fiber-optic probe.
9. Ensure the GF number is correctly entered, and press ENTER to save the calibration value in the software.
10. Press HOME to return to the Home screen.

Setting Discover for Open Vessel Mode

1. From the Home screen, press EDIT to open the System Menus.
2. Use the arrow keys (and) to scroll to Open Vessel and press ENTER.
3. Use the right arrow key to set "RUN OPEN VESSEL: YES" and press ENTER.
4. The Discover will return to the Home screen. Ensure that the top line of the screen says "OPEN VESSEL".
5. Insert the Open Vessel Attenuator so that the two tabs line up with the two slots, and lock into place.

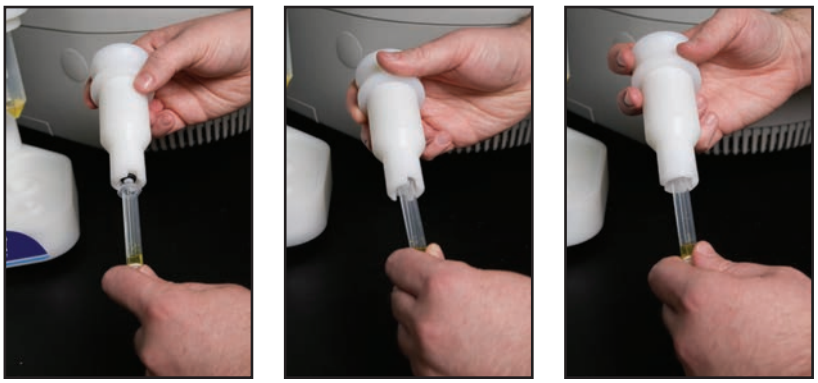
Accent Setup

Hardware Setup

1. Connect the power cord to the rear of the Accent module, then plug the power cord into an appropriate outlet.
2. If using the Accent outside of a fume hood, connect a length of tubing to stainless steel exhaust port on the rear of the Accent using a stainless nut. This tubing should be run to an approved acid-safe fume hood no more than 10' (~3 m) from the Accent.

Vessel Holder Setup

1. Insert the reaction vessel into the appropriate vessel holder, rotating the vessel to lock it in place.



2. Place a yellow ferrule onto the thermowell so that the thinner side of the ferrule points towards the open end of the thermowell.
3. Inset the thermowell, closed end first, through the top of the vessel holder until it touches the bottom of the reaction vessel.
4. Place the PEEK nut onto the thermowell, and tighten it onto the top of the vessel holder.

WARNING
The fitting should be finger-tight. Overtightening the fitting will break the thermowell.

5. Repeat steps 1-4 for the other vessel holder(s).

General Instrument Operation

NOTE
When using the Accent with a Discover Bio Microwave Reactor installed with a Liberty Blue,it will be necessary to remove the spill tray to access the Discover keypad for method programming.

Programming a New Method

1. Press the OPEN key.
2. Use the left arrow key to select “New Method” and press ENTER.
3. The first parameter will be Mode. Use the right arrow key to select SPS mode and press ENTER.
4. Using the numeric keypad, enter the microwave power setting (in W) and press ENTER.
5. Using the numeric keypad, enter the maximum temperature setting (in °C) and press ENTER.
6. Using the numeric keypad, enter the run time (in min:sec) and press ENTER.
7. Using the numeric keypad, set the Delta Temperature value to 5 °C and press ENTER.
8. Using the right arrow key set stirring to “OFF” and press ENTER.

(Cont’d)

General Instrument Operation (cont'd)

Programming a New Method (cont'd)

9. Using the ▶ right arrow key set stirring to "OFF" and press ↵ ENTER.
10. Using the ▶ right arrow key set cooling to "OFF" and press ↵ ENTER.
11. Using the ▶ right arrow key set "SAVE METHOD = (Y)" and press ↵ ENTER.
12. Using the arrow keys (◀ and ▶) to scroll to the desired characters and ↵ ENTER to select, enter a name for the method. When the name has been entered, use the arrow keys (◀ and ▶) to highlight "Exit" and press ↵ ENTER. The method is now saved in the firmware.

Loading an Existing Method

1. Press the OPEN key.
2. Use the arrow keys (◀ and ▶) to scroll to the desired method name, then press ENTER to load the method.
3. The method name should be displayed on the Home screen, along with the programmed parameters.

Recommended Cleavage Parameters

Recommended Microwave Methods

Cleavage can be performed using the Accent either at room temperature or in the microwave. The table below lists CEM's recommended parameters for both microwave and room temperature cleavage.

Method	Power	Maximum Temp.	Run Time	Delta Temp.
Cleavage	10 W	38 °C	30 min	5 °C
Rapid Microcleavage	10 W	38 °C	4 min	5 °C
Room Temperature	0 W	25 °C	3 hours	N/A

NOTE

If the peptide contains multiple arginines the cleavage time may need to be extended.

Recommended Reagents

Various scavenger molecules are added to the TFA to prevent the 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), triisopropyl silane (TIS, scavenges trityl and Pbf cations), ethane dithiol (EDT, scavenges t-butyl cations, reduces oxidation of Cys/Met side chains), dioxa-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).

CEM recommends using a cocktail of **TFA/TIS/H_2O/DODT (92.5/2.5/2.5/2.5)**.

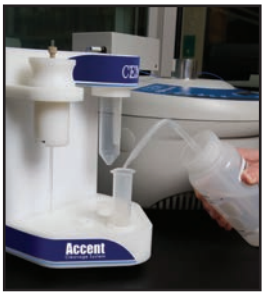
General Cleavage Protocol

1. Rotate the Accent baseplate so that the appropriate size vessel guide is in the front position.

2. Insert a new, clean reaction vessel into the baseplate.

3. Connect a 50 mL centrifuge tube to the product position.

4. Transfer the resin with the completed peptide to the reaction vessel. Turn on the Accent to drain any solvent from the resin.



CAUTION

DMF is a base and will react with the TFA. DCM should be used to rinse the resin prior to cleavage to ensure that no residual DMF is present before adding the cleavage cocktail.

5. Rinse the resin three times with an appropriate volume of dichloromethane.

6. Turn off the Accent. Remove the centrifuge tube and discard the waste.

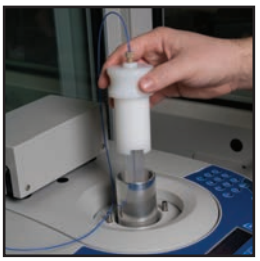
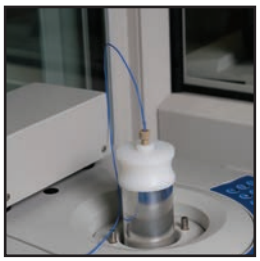
7. Remove the reaction vessel from the baseplate. Place a luer plug on the bottom of the reaction vessel.

8. Add an appropriate volume of cleavage cocktail to the reaction vessel.

9. Insert the reaction vessel into the appropriate vessel holder, rotating the vessel to lock it in place.

10. Insert the fiber optic probe into the thermowell. Ensure that the thermowell reaches the bottom of the vessel and that the fiber-optic probe is completely inserted into the thermowell.


11. Insert the vessel holder into the microwave cavity.

12. Press the Play/Pause key on the Discover to run the loaded method.
13. After the method is finished, connect a clean, labeled centrifuge tube to the product position on the Accent.
14. Remove the reaction vessel from the microwave. Disconnect the reaction vessel from the vessel holder. Place the reaction vessel holder in the rack on the Accent.


15. Turn on the Accent. Remove the luer cap and insert the vessel into the Accent baseplate to drain the cleavage mix containing the peptide into the centrifuge tube.

16. Wash any residual peptide from the resin with additional TFA (up to 1 mL).
17. Turn off the Accent. Remove the reaction vessel with the spent resin from the vacuum manifold and discard.
18. Remove the centrifuge tube containing the cleaved peptide from the Accent. Add an appropriate volume of ice-cold ether (8-10x the volume of cleaved peptide) and allow the peptide to precipitate.

19. Centrifuge the precipitated peptide to isolate the crude product. Carefully decant the ether. Rinse the peptide with additional ether as necessary, centrifuging to isolate the precipitate each time.

20. To clean the Accent, connect a 50 mL centrifuge tube to the product position. Insert an empty reaction vessel into the baseplate. Rinse the system with ~25 mL dichloromethane. Holding the vessel holder over the reaction vessel, rinse the thermowell thoroughly with dichloromethane. Wipe the thermowell with a dry, lint-free paper cloth.

Maintenance Procedures

After Each Use

- Clean the Accent lines.
  - Connect a 50 mL centrifuge tube to the product position.
  - Insert an empty reaction vessel into the baseplate.
  - Rinse the system with ~25 mL dichloromethane.
- Clean the vessel holder thermowell by wiping with a dry, lint-free cloth.

Daily Maintenance

- Clean the Accent baseplate with methanol or another appropriate solvent.

Spare Parts and Accessories

Accent Peptide Cleavage System (Includes Discover Bio)

Product	Part Number
Accent Peptide Cleavage System (Includes the following:)	925514
Discover Bio Microwave Reactor (select one)	
120 V (60 Hz) power supply	909450
240 V (50 Hz) power supply	909455
Power cord	(varies by country)
Accent Wash Station (select one)	
120 V (60 Hz) power supply	908570
240 V (50 Hz) power supply	908575
Power cord	(varies by country)
Fiber optic temperature control	541176
4 mL reaction vessel holder	547125
4 mL reaction vessel (qty 50)	547150-M
25 mL reaction vessel holder	547130
25 mL reaction vessel (qty 50)	576210-M
Luer plug (qty 25)	167999-M
Open vessel attenuator	541130
50 mL centrifuge tube (qty 25)	167150-M
Fuse (1 amp, 5 x 20 mm)	BR198826

Accent Peptide Cleavage Option

Product	Part Number
Accent Peptide Cleavage Upgrade	925516
Accent Wash Station (select one)	
120 V (60 Hz) power supply	908570
240 V (50 Hz) power supply	908575
Power cord	(varies by country)
Fiber-optic temperature control	541176
4 mL reaction vessel holder	547125
4 mL reaction vessel (qty 50)	547150-M
25 mL reaction vessel holder	547130
25 mL reaction vessel (qty 50)	576210-M
Luer plug (qty 25)	167150-M
Open vessel attenuator	541130
50 mL centrifuge tube (qty 25)	167150-M
Fuse (1 amp, 5 x 20 mm)	BR198826

Accessories and Consumables

Product	Part Number
4 mL reaction vessel holder	547125
4 mL reaction vessel (qty 50)	547150-M
25 mL reaction vessel holder	547130
25 mL reaction vessel (qty 50)	576210-M
35 mL reaction vessel holder	547135
35 mL reaction vessel (qty 50)	547175-M
Luer plug (qty 25)	167999-M
50 mL centrifuge tube (qty 50)	167150-M
Replacement thermowell	165016
Fitting for thermowell	167810
Ferrule for thermowell	164315

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CEM has been an ISO-certified facility since 1994.