Symphony & Quartet Quick Start Guide





Symphony & Quartet Quick Start Guide



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WARNING ALL REACTION VESSELS AND/ OR RINSE TUBES MUST BE IN PLACE AT ALL TIMES.



WARNING COLLECTION VIALS MUST BE IN PLACE AT ALL TIMES.



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

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



WARNING THE CLEAVE COLLECT AREA HAS A DRAIN FEATURE SO IF A MECHANICAL FAIL-URE CAUSES THE COLLECT VIALS TO OVER-FLOW, THE FLUID WILL DRAIN INTO THE SOL-VENT CABINET TRAY. SHOULD YOU SEE ANY FLUID IN THE TRAY FROM THIS, YOU NEED TO CONTACT PROTEIN TECHNOLOGIES, INC. OR YOUR DISTRIBUTOR FOR SERVICE.

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www.pti-instruments.com

Introduction

Thank you for purchasing your new Symphony from Protein Technologies, Inc. The Symphony is a fully automated, parallel synthesizer with completely independent reaction vessels, allowing you to start and stop individual syntheses whenever you please. This capability makes the Symphony unmatched in the parallel solid-phase synthesis market.

About This Book

This book helps you get started with the Symphony.

- Chapter 1, **Instrument Layout**, introduces you to the basic layout of the Symphony.
- Chapter 2, **Accessories**, introduces you to the accessories offered for the Symphony.
- Chapter 3, Instrument Setup, introduces you to the basic software and procedures required to set up a synthesis on the Symphony.

About The Company

Protein Technologies, Inc. (PTI) is built on the belief that our products and services must be of the highest possible quality. Our products are supported by a dedicated field service team, and we are proud of our reputation for reliability.

Founded in 1985 by researchers affiliated with the University of Arizona in Tucson, PTI launched its first peptide synthesizer, the PS3, in 1990. Since then, PTI has manufactured and sold the world's finest solid-phase synthesizers. Today we are growing and innovating to serve the needs of the solid-phase synthesis market. We are here to help. If you have any questions or ideas for new instruments, please feel free to contact us:

> Tel: 520-629-9626 Toll Free: 800-477-6834 Fax: 520-629-9806 E-mail: info@pti-instruments.com www.pti-instruments.com

Chapter 1: Instrument Layout

Symphony Front

Collection Rack Reaction 8 \$ 8 8 8 1 1 1 1 Vessel Rack Symphony Protein Amino Acid Bottle Rack Pull-Out . Work Tray Solvent Cabinet



Vacuum/Air Pump Controls:

- Vacuum/Air Hose Outlets
- Circuit Breakers
- Power Plug
- On/Off Switch

1-800-477-6534

Solvent Cabinet



Quick Start Guide

Chapter 1: Instrument Layout

Chapter 2: Accessories

Reaction Vessels & O-Rings





Small Glass 9 mL #SMP-010135

Standard Glass 16 mL #SMP-010092



Large Glass 32 mL #SMP-010134

Reaction Vessel O-Rings: Part #SMP-270054



Disposable Plastic 26 mL #SMP-RV-012 #SMP-RV-100 #SMP-RV-500

Collection Vials

Standard Plastic 15 mL #3100013 (Pack of 50)





Large Glass 45 mL #SMP-LGCLTB (1 each) Instrument modification required for installation

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Amino Acids & Reagents for Peptide Synthesis

Protein Technologies, Inc. supplies high quality, pretested N-Fmoc-protected amino acids preweighed in 5 mmol and 10 mmol quantities in synthesizer-ready bottles (See Appendix A for listings), as well as bulk N-Fmoc-protected amino acids preweighed in 25 g and 100 g quantities (See Appendix B for listings). We recommend using our amino acids for all of your synthesis needs.

Protein Technologies, Inc. supplies the following reagents for peptide synthesis on the Symphony:

CATALOG NO.	DESCRIPTION	QUANTITY
	Reagents:	
PS3-PPR-L	20% Piperidine/DMF (DEP)	0.9 L
PS3-MM-L	0.4 N-Methylmorpholine/DMF (ACT)	0.9 L
B-100-HBTU B-500-HBTU	HBTU	100 g 500 g
B-1K-HBTU	TIBTO	1 kg
SMP-K5	Start-Up Kits: FMOC Amino Acid Start-up Kit for the Symphony and Symphony Quartet. Contains 1 ea. of 20 standard amino acids (5 mmol); 0.9 L Deprotectant; 0.9 L Activator and HBTU (100 g).	1 ea.

Replacement Parts/Accessories

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

Chapter 3: Setup Instructions

Define Amino Acid and Solvent Data Files

Symphony software has standard amino acid ("standard.aa") and solvent ("standard.slv") data files which can be opened and viewed through the SetUp menu under "Reagent (AA) Data" and "Solvent/Activator Data," respectively. These files list the Symphony default settings.

Data files should reflect the solvents and reagents being used in a synthesis. If solvents and/or reagents other than the defaults will be used, new data files must be created.

Create a Reagent (AA) Data File

To create a new Reagent (AA) Data file, select **Reagent (AA) Data** under the **SetUp** menu, and click on **New**:



This will open the Reagent (AA) Data window.

To view default values, click on **Defaults** button in lower left corner. Reagent entries may be made directly or by modifying the default listings.

For each amino acid bottle position (Pos) enter:

- 1. Key Single letter abbreviation
- 2. Abbreviation Three letter abbreviation
- 3. Description Name or description of reagent
- 4. Deprotected Weight Formula weight without protecting group(s)
- Protected Weight Formula weight including protecting group(s)

Pos	Key	Abv.	Description	Deprotected Wt	Protected W A
1	A	Ala	Alanine	89.095	311.380
2	С	Cys	Cys (Trt)	121.159	585.700
3	D	Asp	Asp (But)	133.105	411.500
4	E	Glu	Glu (But)	147.132	425.500
5	F	Phe	Phenylalanine	165.194	387.400
6	G	Gly	Glycine	75.068	297.300
7	Н	His	His (Trt)	155.158	619.730
8	1	Iso	Isoleucine	131.176	353.400
9	K	Lys	Lys (Boc)	146.191	468.600
10	L	Leu	Leucine	131.176	353.400
11	M	Met	Methionine	149.213	371.500
12	N	Asn	Asn (Trt)	132.120	596.700
13	P	Pro	Proline	115.133	337.400
14	Q	Gln	Gln (Trt)	146.147	610.700
15	R	Arg	Arg (Pbf)	174.204	648.770
16	S	Ser	Ser (But)	105.094	383.400
17	T	Thr	Thr (But)	119.121	397.500
18	V	Val	Valine	117.149	339.400
19	W	Trp	Trp (Boc)	204.230	526.600 💌
•					•
		<u>D</u> efau	ilts Clea		se

Save a Data File

To save changes to a Data file, click on the **Save** shortcut shown below:



This will save changes to the currently active window. To save a newly created Data file, click on the **Save As** shortcut shown below:



This will open a new screen. Enter file name and click **Save**.

Create a Solvent/Activator Data File

To create a new Solvent/Activator Data file, select **Solvent/** Activator Data under the **SetUp** menu, and click on **New**:



This will open the Solvent/Activator Data window.

To view default values, click on the **Defaults** button beneath each list. Reagent entries may be made directly or by modifying the default listings.

Under the **Solvents** list, enter the name or description of the solvent under **Description** for each solvent bottle position listed under **Solvent**.

Under the **Activators** list, enter the following for each activator component:

- 1. Description Name or description of activator component
- 2. F.W. Formula weight of activator component
- **3.** Density (g/mL) Density of activator component. If activator component is not a liquid, enter 0.000.

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Solvents	Solvent	Description		
	Solvent 1 Solvent 2 Solvent 3 Solvent 4 Solvent 5 Solvent 6	DMF 20% Piperidin 0.4 NMM/DM Acetic Anhyd Methylene Ch TFA	e/DMF IF ride Ioride	
Activator	<u>D</u> efaults	Clea	1	
Descrip NMM HBTU TBTU PyBop DCC	2	F. W. 101.500 379.300 321.100 520.400 206.300	Density(g/ml) 0.920 0.000 0.000 0.000 0.000	
	<u>D</u> efaults	Cleaj		
	<u>(</u>	Close		

To save Solvent/Activator Data file, follow instructions on page 3-3.

Create a Program

2 programs are required to run a synthesis on the Symphony:

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

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

New Program

To create a new program, click on the New Program shortcut shown below:



Enter Program Values

- 1. Solvent/Activator Data File Click on shortcut to select Solvent/Activator Data file
- 2. Step Number indicates active row that can be modified
- 3. Solvent/Operation Use pull-down menu to select appropriate solvent or operation for step
- Volume Use pull-down menu to select number of 1.25 mL aliquots delivered (1 = 1.25 mL, 9 = 11.25 mL)
- 5. Mix Time Enter mix time in hh:mm:ss
- Drain Use pull-down menu to select "ON" or "OFF" to specify whether reaction vessel will be drained at end of step
- **7. Rep** Use pull-down menu to select number of times step will be performed
- 8. Comment Add comment if desired
- 9. Insert Inserts a new step above selected step
- 10. Delete Deletes selected step
- 11. Close Closes Program Editor

1 DMF			00:00:00			Comment	5-
2		*	00:00:00	-	-		
	-	¥	00:00:00	-	-		
	-	v	00:00:00	Y	*		
	*	¥.	00:00:00	¥	-		
	*	¥	00:00:00	~	*		
	Ŧ	*	00:00:00	×	*		
	*	*	00:00:00	×	Y		
	*	×	00:00:00	*	*		
	*	٣	00:00:00	~	-		
le Description:				So	lvent Data	File standard	
			-	Insert		Delete	

Quick Start Guide

Helpful Hint:

Symphony software includes the basic synthesis program "basic.prg," the basic swelling program "basicsw.prg," and the basic cleavage program "stdclv. clv" You can create new programs by modifying these basic programs and saving them under a new name.

Step	Solvent/Operation		Volu	me	Mix Time	Drain	Rep
1	DMF	•	1	-	00:00:30	ON	3 -
2	20% Piperidine/DMI	•	1	-	00:02:30	ON	2 -
3	DMF	•	1	-	00:00:30	ON	6 -
4	Reagent (AA)	•	1	-	00:00:00	OFF	1 -
5	0.4 NMM/DMF	•	1	-	00:20:00	ON	1 -
6	DMF	•	1	-	00:00:30	ON	3 -



Step	Solvent/Operation	Vol	ume	Mix Time	Drain		Re	P
1	DMF 💌	1	-	00:10:00	ON	-	3	
2	20% Piperidine/DMI	1	-	00:02:30	ON	-	2	•
3	DMF 🔄	1	-	00:00:30	ON	-	6	
4	Reagent (AA)	1	-	00:00:00	OFF	-	1	
5	0.4 NMM/DMF	1	-	00:20:00	ON	-	1	-
6	DMF 💌	1	-	00:00:30	ON	-	3	+

1	DMF	1	•	00:00:30	ON	-	3	•
2	20% Piperidine/DMI	1	-	00:05:00	ON	•	2	•
3	DMF 💌	1	-	00:00:30	ON	•	6	•
4	Methylene Chloride 💌	1	-	00:00:30	ON	•	6	•
5	Dry	1	-	00:10:00	ON	•	1	•
6	TFA	2	•	02:00:00	ON	•	1	•
7	TFA	1	-	00:00:30	ON	•	1	•
8	Methylene Chloride 💌	1	-	00:00:30	ON	-	3	•
9	Dry	11	-	00:02:00	ON	-	1	-

CAUTION Failure to save modifications under a new name will overwrite file.

Open an Existing Program

To open an existing program, click on the **Open program** shortcut shown below:



In the next box, select program type (Synthesis or Cleavage) and click **OK**.

🗃 Open S	ynthesis/Cleav	vage Program 🗙
	Open progr	am
	• Synthesis	C Cleavage
C		<u>C</u> ancel

This will open a new screen. Select a program and click **Open**.

Save a Program

To save changes to an existing program, click on the **Save** shortcut shown below:



This will save the currently active window. To save a new program, click on the **Save As** shortcut shown below:



In the next box, select program type (Synthesis or Cleavage) and click **OK**.



This will open a new screen. Enter file name and click Save.

Create a Peptide

The Symphony allows you to run a different program in each cycle. Use the **Peptide Editor** to enter a peptide sequence and assign a specific program to each amino acid.

New Peptide

To create a new peptide, click on the **New Peptide** shortcut shown below:



Enter a Sequence

- 1. Select Reagent (AA) Data File Click on shortcut button. Select file and click **Open**.
- Select Default Program Click on shortcut button. This will open a new screen. Select program and click Open. This program will automatically be assigned to newly entered amino acids.
- Select Termination Select COOH or CONH2 to specify whether the C-terminus is an acid or an amide, respectively. If COOH is selected, synthesis will begin one amino acid to the left of the C-terminal (right-most) amino acid. If CONH2 is selected, synthesis will begin with the C-terminal amino acid itself.
- 4. Enter Peptide Sequence Use buttons on right of screen or keyboard (using single letter amino acid abbreviations) to enter peptide sequence in the N to C direction.

ptide Sequence Editor Untitled			B	
ynthesis Program Selections	COOH	Amino Acid List Standard	Reagent (Av	
Set basicsw	C CONH2	C Custom	Aria	MMet
			C-Cys	N-Asri
PTT	rogram J*****		D-Asp	P-Pro
			E-Glu	Q-GIn
			F-Phe	R-Arg
			G-Gly	S-Ser
Set Cut Copy Paste Leng	th 3 Molecular Weight	329.40	H-His	T-Thr
escriptio <u>n</u>			l-lso	V-Val
			- au - 1	1

Assign Programs

- 1. Select Set Program Click on shortcut button. This will open a new screen. Select program and click **Open**.
- 2. Highlight Amino Acid(s) Use mouse and/or arrow keys to highlight amino acid(s).
- **3.** Set Program Click Set to assign Set Program to highlighted amino acid(s).

vnthesis Program Selections	Termination	- Amino Acid List	- Reagent (A4	A) Data-
Default basic	соон	Standard	standard	
Set basicsw	CONH2	C Custom	A-Ala	M-M
eguence Current Position 14 Current	Program basicsw		C·Cys	N-A:
	Tragram Inducem	2	D Ann	P.P.
PUTPUTPUTPUTPUT			Divesh	1.11
PTIPTIPTIPTIPTI			E-Glu	Q-G
PTIPTIPTIPTIP	2		E-Glu F-Phe	Q-G R-A
3	2		E-Glu F-Phe G-Gly	Q-G R-Ai S-Se
3 Set Cut Copy Paste Len	2 gth 15 Molecular Weight [1574.94	E-Glu F-Phe G-Gly H-His	Q-G R-A S-Se T-TI
Set Cut Copy Paste Len	gth 15 Molecular Weight	1574.94	E-Glu F-Phe G-Gly H-His Hso	Q-G R-A S-Se T-TI V-V.
Set Cut Copy Paste Len	2 gth 15 Molecular Weight [1574.94	E-Glu F-Phe G-Gly H-His H-so K-Lys	Q-G R-Ar S-Se T-TI V-V.

Set first cycle to run swelling program. If COOH is selected, synthesis will begin one amino acid to the left of the C-terminal (right-most) amino acid. If CONH2 is selected, synthesis will begin with the C-terminal amino acid.

Save a Peptide

To save changes to an existing peptide, click on the **Save** shortcut shown below:



This will save the currently active window. To save a new peptide, click on the **Save As** shortcut shown below:



In the next box, select file type (Peptide or Sequence) and click $\mathbf{OK}.$



This will open a new screen. Enter file name and click Save.

Print a Peptide

To print a peptide, click on the **Print** shortcut shown below:



This will print the currently active window. In the next box, click on **Yes** to print synthesis programs.



NOTE Always print out peptide and program files as a final check of sequences and programs before running a synthesis.

Calculate Reagent Amounts

The **Calculations Screen** can be used to calculate the amounts of resin and reagents you will need to prepare a synthesis on the Symphony. It will also calculate the synthesis time(s) as well as the theoretical peptide yield(s).

Open Calculations Screen

To open the **Calculations Screen**, click on the shortcut shown below:



Calculate Resin Amounts

In the Calculations Screen under the Peptides tab:

- 1. Select Reagent (AA) Data File Click on shortcut to select Reagent (AA) Data File
- 2. Select Solvent/Activator Data File Click on shortcut to select Solvent/Activator Data File
- Load Peptide(s) For each reaction vessel, click on shortcut button to right of Peptide name box. Select peptide and click Open to download peptide to reaction vessel.
- Enter Resin Substitution Use keyboard to enter the resin substitution (in mmol/g) in the "Subs(mmol/g)" column. Press ENTER.
- **5. Enter Scale** Use keyboard to enter the scale (in μmol) in the "Scale (umol)" column. Press **ENTER**.
- 6. Resin Amount The software will calculate the amount of resin (in mg) needed for the reaction and display it in the column marked "Resin (mg)."

lea	ident (AA) Data File	olvent/Activator	∑ields Solveni	t/Activa	tor Data File	standar 1.slv	
	Peptide	Sequence		Term	Subs(n mol/a)	Scale (umol)	Resin (mg)
1	G-LHRH	GHWSYG	LRPG	CONH2	0.40	25	63
2	16 AA 2	VFGTGTKVTVLG	QPKA (соон	U.47	25	53
3	ACP	VQAAID	YING (соон	0.50	25	50
4	ABRF	VKKRCSMWIIPTI	DDEA (соон	0.47	25	53
5	Ę	2	0				
6	P	2	Į.				
7	E	2					
8	P	5	ĺ.				
9	Đ	2	Į.			1	
10	P	F					
11	Ę	8	j.				
12	P	F					
		25 G	200				

Calculate Reagent (AA) Amounts

In the Calculations Screen under the Reagent (AA) Wts tab:

- Enter Concentration Enter amino acid concentration (in mM). Press ENTER.
- Enter Volume By default, the software will display the minimum reagent volumes required for the synthesis. Enter a new value in the "Act Vol(ml)" column if desired. Press ENTER.
- 3. Amino Acid Amount The software will calculate the amino acid weight (in mg) needed for the synthesis based on the Concentration and Actual Volume values and display it in the column marked "Weight (mg)."

	73	5 olvent/Activ	vator <u>T</u> imes	Yields			
	Description	Residues	Min Vol(ml)	Act \ol(ml)	Weig <mark>l</mark> t(m 🔺		
1	A - Alanine	2	12	12	374		
2	C - Cys (Trt)	1	11	1	644		
3	D - Asp (But)	3	14	14	576		
4	E - Glu (But)	1	11	1	468		
5	F - Phenylalanine	1 1	11	1	426		
6	G - Glycine	6	18	18	535		
7	H - His (Trt)	1	11	<u>ा 1</u>	682	Concentration	100.00
8	I - Isoleucine	4	15		510	(mM)	100.00
9	K - Lys (Boc)	4	15	20	937		
10	L Leucine	2	12	42	424		
11	M - Methionine	1	11	11	409		
12	N - Asn (Trt)	1	11	11	656		
13	P - Proline	3	14	14	472		
14	Q - Gln (Trt)	2	12	12	733		
15	R - Arg (Pbf)	2	12	12	779		
16	S - Ser (But)	2	12	12	460		
17	T - Thr (But)	4	15	15	596		
18	V - Valine	5	16	16	543		
19	W - Trp (Boc)	2	12	12	632		
	V T (D)	2	12	12	551 💌		

Calculate Solvent/Activator Amounts

In the **Calculations Screen** under the **Solvent/Activator** tab:

- 1. Minimum Volume The software will calculate and display the minimum solvent volumes required for the synthesis in the "Min Vol (ml)" column.
- 2. Enter Activator Volume Enter activator Actual Volume (in mL) if different from minimum volume. Press ENTER.
- Enter Activator Concentration Enter activator concentration (in mM) if different from default value. Press ENTER.
- Activator Volume The software will calculate the activator volume (in mL) needed for the synthesis based on the Actual Volume and Concentration values and display it in the column marked "Vol (ml)."

	De:	scription	Min Vol (ml	Act Vol (ml)
SOLV 1	DMF		1382	1382
SOLV 2	20% Piperidir	ne/DMF	132	132
SOLV 3	0.4 NMM/DI	MF	71	71
SOLV 4	Acetic Anhy	dride	10	10
SOLV 5	Methylene C	hloride	10	10
SOLV 6	TFA		10	10
NMM		400.00	4	4.060
D	escription	[Conc (mM)]	Vol (ml) W	eight (g)
HBTIL		100.00		3.793
		100,00		3.211
TBTU		100 00		5.204
TBTU PyBop		100,00 1		a (a a
TBTU PyBop DCC		100,00		2.163
TBTU PyBop DCC		100.00		2.163
TBTU PyBop DCC		100 00		2.163
TBTU PyBop DCC		100.00		2163

5. Activator Weight - Activator weights (in g) are displayed in the column marked "Weight (g)."

NOTE Make sure the Amino Acid and Activator concentrations match. The Activators list may also be used to calculate base or additive values.

Calculate Times

In the **Calculations Screen** under the **Times** tab, the estimated synthesis time will be displayed for each peptide.

ILHRH GHWSYGLRPG 9 hours 28 min 30 sec 6AA VFGTGTKVTVLGQPKA 13 hours 58 min 30 sec CP VQAAIDYING 8 hours 34 min 30 sec BRF VKKRCSMWIIPTDDEA 13 hours 58 min 30 sec	Peptide	Sequence	Time
5 AA VFGTGTKVTVLGQPKA 13 hours 58 min 30 sec CP VQAAIDYING 8 hours 34 min 30 sec BRF VKKRCSMWIIPTDDEA 13 hours 58 min 30 sec 	G-LHRH	GHWSYGLRPG	9 hours 28 min 30 sec
VIJAAIDYING 8 nours 34 min 30 sec BRF VKKRCSMWIIPTDDEA 13 hours 58 min 30 sec	16 AA	VFGTGTKVTVLGQPKA	13 hours 58 min 30 sec
			to nours 34 min 30 sec
		9	
Image: second			
· ·			

Calculate Yields

In the **Calculations Screen** under the **Yields** tab, the theoretical yields for the 5, 10, 25, 50, and 100 μ mol scales will be calculated and displayed for each peptide.

				(mg)		
Peptide	Sequence	5 μmol	10 µmol	25 µmol	50 µmol	100 µmol
G-LHRH	GHWSYGLRPG	5.641	11.282	28.206	56.412	112.825
16 AA	VFGTGTKVTVLGQPKA	8.015	16.029	40.073	80.146	160.292
ACP	VQAAIDYING	5.316	10.632	26.580	53.159	106.319
ABRF	VKKRCSMWIIPTDDEA	9.461	18.922	47.306	94.612	189.225
			,			

Create a Good Laboratory Practice File

After calculations are complete, create a Good Laboratory Practice or GLP file by clicking on the **Create GLP** button at the bottom of the **Calculations Screen**. This will open a new window. Enter a file name and click **Save**.

Instrument Setup

Prior to setting up the instrument in this section, you will need to prepare resins, amino acid solutions, and solvent solutions according to Symphony software calculations. In this section, you will learn to install:

- 1. reaction vessels
- 2. amino acid bottles
- 3. collection vials
- 4. solvent containers
- 5. waste container(s)

Reaction Vessel Installation

- 1. Position lever vertically to release clamp. Place vessel in lower seat first, then straighten it into upper seat.
- 2. Position lever horizontally to secure vessel in place. Twist vessel to ensure it is seated properly.
- 3. To remove vessel, lift lever and remove.



2





Quick Start Guide

Amino Acid Bottle Installation

- 1. Pull back metal tab. Push bottle up into place.
- 2. Push metal tab in to secure but DO NOT FORCE.
- 3. To remove bottle, pull back metal tab and remove.







Collection Vial Installation

- 1. Insert vial, top first. Make sure the tubing is inserted IN-SIDE the vial to avoid spills and loss of product.
- 2. Secure in bottom seat.
- 3. To remove, lift vial up to release from bottom seat.
- 4. Remove vial.









<u>CAUTION</u> Be careful when removing vials containing cleavage solution. Follow MSDS handling guidelines provided with the individual reagents.

Quick Start Guide

Solvent Container Installation

- 1. Fill 20 L (or 12 L) tank with solvent 1. Make sure inlet and outlet lines are connected (see below).
- 2. Place solvents 2-6 in appropriate bottles.



Waste Container Installation

- 1. Empty container. Replace lid.
- 2. Make sure waste level sensor cable is connected.



Bottle Preparation

Once reagent and solvent bottles have been filled and placed on the Symphony, the bottles should be pressurized and primed using the **Bottle Preparation Screen**. After a synthesis, the bottles should be vented and flushed with nitrogen prior to removal from the instrument.

Open Bottle Preparation Screen

To open the **Bottle Preparation Screen**, click on the shortcut shown below:



Pressurize and Prime Bottles

In the **Bottle Preparation Screen** under the **Solvent Bottles** tab:

- 1. Select Active Good Laboratory Practice File Click on shortcut to select file. Refer to page 3-22.
- 2. Select Solvent Check box to left of solvent or use Select All button at lower left to select all solvents.
- 3. Pressurize Selected Solvents Click Pressurize. Pressurized solvents will display "On" in the Pressure column
- 4. Prime Selected Solvents Repeat Step 1 to select solvents. Click Prime. Primed solvents will display "Fluid" in the Sensor column.

ttle Freparation			
olver Bottles R	eagent (AA) Bottles	لر	
Active Good La	aboratory Practice File stand	dard.glp	Ð
Solvent	Description	Pressure	Sensor
OLV1	DMF	Off	No Fluid
SOLV2	20% Piperidine/DMF	Off	No Fluid
E SOLV3	0.4 NMM/DMF	Off	No Fluid
SOLV4	Acetic Anhydride	Off	No Fluid
E SOLV5	Methylene Chloride	Off	No Fluid
SOLV6	TFA	Off	No Fluid
Select <u>A</u> ll	Prime	Pressurize	Flush <u>N</u> 2
	⊻ n t	<u><u>St</u>ip</u>	Flus <u>h</u> Solv
	Close	1	

In the **Bottle Preparation Screen** under the **Reagent (AA) Bottles** tab:

- 1. Select Bottles(s) Check box to left of reagent or use Select All button at lower left to select all reagents.
- 2. Pressurize Selected Reagents Click Pressurize. The status bar beneath the reagent list will display "PRESSUR-IZED" when the reagents are pressurized.
- 3. Prime Selected Reagents Repeat Step 1 to select reagents. Click Prime. Primed reagents will display "Yes" in the Fluid column.

Iver t Bottl	es Heagent (AA)	Bottles	IN MAR			
Active G Bottle	ood Laboratory Pra Reagent (AA)	ctice File star Fluid	idard.glp Bottle	🗾 Reagent (A	A)	Fluid
	A-Ala 💌	No	🗆 11 🕅	1-Met	•	No
 2	C-Cys 💌	No	T 12	l-Asn	-	No
Г 3	D-Asp	No	□ 13 F	-Pro	-	No
Γ4	E-Glu 💌	No	14 0	l-Gin	-	No
□ 5	F-Phe	No	T 15 F	I-Arg	-	No
F 6	G-Gly 💌	No	T 16 S	-Ser	•	No
Γ7	H-His 💌	No	T 17 T	-Thr	•	No
□ 8	I-lso 💌	No	T 18 V	-Val	-	No
9	K-Lys 💌	No	T 19 🗸	V∙Trp	•	No
line 10	L-Leu 💌	No	C 20 Y	'-Tyr	•	No
_		NOT PRES	SURIZED			
Sele	ect <u>A</u> ll	Prime	Press	urize	Flus	h <u>N</u> 2
		⊻ent	<u></u>	p	Flus	n Solv
		Close	1			

Synthesis Setup

Once all resins, reagents, solvents, collection vials and waste container(s) have been placed on the Symphony and the reagent and solvent bottles have been pressurized and primed, you are ready to begin a synthesis.

Open Reaction Vessel Operations Screen

To open the **Reaction Vessel Operations Screen**, click on the shortcut shown below:



Start a Synthesis

In the **Reaction Vessel Operations Screen** under the **Automated Operations** tab:

1. Load Peptide - Click on shortcut button. This will open a new screen. Select peptide and click **Open**.

utomate	c Oper	ation	IS Manual (Dper <mark>t</mark> io	ons					-				1	
łun		RV	Peptide	I	.engtł	AA#	Program	Step	Reagent	Mix Time	Rep	Action	Cleavage	1	CI
	Start	1	G-LHRH		10	10				00:00:00			stdclv		
s	Start	2	16 AA		16	15				00:00:00			stdclv		(
b _ s	Start	3	ACP	1	10	9	<u>.</u>			00:00:00			stdclv		(
b _s	Start	4	ABRF	- 📂	16	15				00:00:00			stdclv	6	4
s s	Start	5		1						00:00:00					
 s	Start	6		- 📂	i i					00:00:00				•	-1
S	Start	7		-						00:00:00					
) _ s	Start	8		-						00:00:00					1
 s	Start	9	-	-						00:00:00					1
) _ s	Start	10		-						00:00:00					1
s s	Start	11		-						00:00:00					
	Stret	12		-	1 0					00:00:00					

Quick Start Guide

- Load Cleavage Program Click on shortcut button. This will open the Set Cleave Time and Date for RV _ screen.
 - a. Select Cleavage Program Click on shortcut button. This will open a new screen. Select program and click Open.
 - **b. Select Cleave Time** Click circle to left of desired cleave time.
 - c. Set Cleave Date and Time If "Cleave by Date and Time" selected, enter in the date and time you want to begin cleavage. Otherwise, proceed to d.
 - d. Click OK.



3. Start Synthesis - Click on the Start button.

Helpful Hint:

If you are planning to run the same peptide or cleavage conditions on multiple reaction vessels, use the **Set** button.

Hun		RV	Peptide	Length	AA# Program	Step	Reagent	Mix Time	Rep	Action	Cleavage		C
•	Start	1	G-LHRH	10	10			00:00:00			stdclv		(
	Start	2	16 AA	16	15			00:00:00			stdclv		1
•	Start	3	ACP	10	9			00:00:00			stdclv	•	
•	Start	4	ABRF	16	15			00:00:00			stdclv	6	-1
	Start	5	[5				00:00:00				•	
•	Start	6	[5				00:00:00				•	-1
•	Start	7		5				00:00:00					E.
•	Start	8	[]	- <u>-</u>				00:00:00					21
	Start	9		5				00:00:00				•	
•	Start	10		- <u>-</u>				00:00:00					21
•	Start	111	-	5				00:00:00				•	
•	Start	12		-				00:00:00					

This will open the **Automated Operations Global Edit** screen.

continued on next page...



Chapter 4: Post-Synthesis Procedures

The following steps are required after every synthesis to clean the Symphony and prepare it for the next synthesis.

Collect Clean

Collect Clean is only necessary if automated cleavage was performed on the instrument. After cleavage collection is complete, the word **Rinse** will be displayed on the **Start** button in the **Reaction Vessel Operations Screen** under the **Automated Operations** tab.

- Remove collection vials containing cleaved peptide and replace with empty collection vials. Peptides can be precipitated in the removed collection vials with cold (< 0°C) ether.
- 2. Click on Rinse button.
- 3. This will open a warning window. Click **OK**. The collection lines will be rinsed with solvent 5.
- 4. When rinse is complete, remove collection vials, discard rinse solution and place clean, empty collection vials on the instrument for the next collection.

Clear Reaction Vessels

Following the Collect Clean operation, clear reaction vessels as follows. In the **Reaction Vessel Operations Screen** under the **Automated Operations** tab:

un	R١	/ Peptide	Len	gth AA#	Program	Step	Reagent	Mix Time	Rep	Action	Cleavage		CLV
Sta	t 1	G-LHRH	1	10				00:00:00			stdclv		0
Sta	t 2	16 AA	1	3 15	<u>[</u>			00:00:00			stdclv		C
Sta	t 3	ACP	1	9	<u> </u>			00:00:00			stdclv		C
Sta	t 4	ABRF	1	5 15	<u> </u>			00:00:00			stdclv	6	0
🔰 Sta	t 5	[-					00:00:00					C
) Sta	t 6	[-					00:00:00				•	C
Sta	t 7	[5					00:00:00					C
Sta	t 8		5					00:00:00					C
Sta	t 9		5					00:00:00				•	C
Sta	t 10		5					00:00:00					C
Sta	t 11	-	5					00:00:00				•	C
Sta	t 12		-					00:00:00					C

1. Click on Clear button.

This will open the Clear Reaction Vessels Settings window.

- Select RV(s) Check box to left of RV(s) or use Select All button to select all RVs
- 2. Clear Click on Clear to clear loaded peptide(s) from Automated Operations screen.
- 3. Close Click on Close to exit window.



RV Clean

NOTE RV clean cannot be run on a reaction vessel channel while other reaction vessels are running.

After a synthesis has been performed in a reaction vessel(s), that channel(s) should be cleaned prior to running a new synthesis. Replace reaction vessel(s) with rinse tube(s). Install rinse tubes the same way as a reaction vessel with the skinny end at the top. Refer to section on **Reaction Vessel instal-lation**, page 3-23. (Rinse tubes are also pictured alongside the reaction vessel.) This operation will rinse the channel

twice each with solvent 2, solvent 1 and solvent 5. Make sure these solvent bottles contain solvent and are pressurized and primed. In the **Reaction Vessel Operations** screen under the **Manual Operations** tab:

- Select RV Clean Use the pull down menu to select RV Clean from the Operation/Solvent column. Do not specify mix times, repetitions (Rep) or volumes (Vol). Repeat for each desired reaction vessel.
- 2. Start Click Start button for desired reaction vessel(s).
- Clear After cleaning is complete, click on Clear button. This will open the Clear Reaction Vessels Settings window. Refer to page 3-33 for remaining instructions.

utomat	ed open	adons	mandal operation		2		-				1212121	4.145	
Run	Church	RV	Operation/Solv	/ent	Heagent (AA)	Mix Lime	Drain		He	P	Vol	Action	1
~	Staft	1	INV Llean	_ _	N N	00.00.00		-	1	-]
	Start	2	DME			00.00.00	ON	-	1	-			1
	Start		DME			00.00.00	ION	-	1	-			1
	Start	15	DME	-		00:00:00	ON	-	1	-	1 1		1
	Start	6	DMF	-	×	00:00:00	ON	-	1	1	1 -		1
. 1	Start	17	DMF	-	*	00:00:00	ON	-	1	-	1 -		
•	Start	8	DMF	-		00:00:00	ON	-	1	-	1 -		,]
	Start	9	DMF	-	*	00:00:00	ON	•	1	•	1 -	-	1
•	Start	10	DMF	-	Ŧ	00:00:00	ON	•	1	-	1 💌		1
•	Start	11	DMF	•	×	00:00:00	ON	-	1	-	1 💌	8]
•	Start	12	DMF	-		00:00:00	ON	•	1	•	1 💌]
				Set	Pause All	Cjea		5					
			-			-							

NOTE We recommend that RV clean be run on ALL channels even if only a few reaction vessels were used.

System N2 Flush

Prior to this operation, make sure to:

- 1. Place reaction vessels or rinse tubes in all reaction vessel positions.
- 2. Place collection vials in all collect positions.
- 3. Connect waste tank(s).
- 4. Place bottles in all bottle positions.

Under the **Tools** menu, under **Cleaning**, select **System N2 Flush**.



This will open a new window. Click **Start** to begin. This process should take approximately 20 minutes. Click **Close** to exit window upon completion.

Appendix

Appendix A

Symphony and Quartet Bottles Containing N-FMOC-Protected Amino Acids, Preweighed

CAT. NO.	DESCRIPTION	QUANTITY
SMP-05-A SMP-10-A	FMOC-L-Ala	5 mmol 10 mmol
SMP-05-RP SMP-10-RP	FMOC-L-Arg(Pmc)	5 mmol 10 mmol
SMP-05-RBF SMP-10-RBF	FMOC-L-Arg(Pbf)	5 mmol 10 mmol
SMP-05-NT SMP-10-NT	FMOC-L-Asn(Trt)	5 mmol 10 mmol
SMP-05-DB SMP-10-DB	FMOC-L-Asp(OtBu)	5 mmol 10 mmol
SMP-05-CA SMP-10-CA	FMOC-L-Cys(Acm)	5 mmol 10 mmol
SMP-05-CT SMP-10-CT	FMOC-L-Cys(Trt)	5 mmol 10 mmol
SMP-05-EB SMP-10-EB	FMOC-L-Glu(OtBu)	5 mmol 10 mmol
SMP-05-QT SMP-10-QT	FMOC-L-GIn(Trt)	5 mmol 10 mmol
SMP-05-G SMP-10-G	FMOC-L-Gly	5 mmol 10 mmol
SMP-05-HT SMP-10-HT	FMOC-L-His(Trt)	5 mmol 10 mmol
SMP-05-I SMP-10-I	FMOC-L-Ile	5 mmol 10 mmol
SMP-05-L SMP-10-L	FMOC-L-Leu	5 mmol 10 mmol
SMP-05-KBC SMP-10-KBC	FMOC-L-Lys(Boc)	5 mmol 10 mmol

Appendix

CAT. NO.	DESCRIPTION	QUANTITY
SMP-05-M SMP-10-M	FMOC-L-Met	5 mmol 10 mmol
SMP-05-F SMP-10-F	FMOC-L-Phe	5 mmol 10 mmol
SMP-05-P SMP-10-P	FMOC-L-Pro	5 mmol 10 mmol
SMP-05-SB SMP-10-SB	FMOC-L-Ser(tBu)	5 mmol 10 mmol
SMP-05-TB SMP-10-TB	FMOC-L-Thr(tBu)	5 mmol 10 mmol
SMP-05-W SMP-10-W	FMOC-L-Trp	5 mmol 10 mmol
SMP-05-WBC SMP-10-WBC	FMOC-L-Trp(Boc)	5 mmol 10 mmol
SMP-05-YB SMP-10-YB	FMOC-L-Tyr(tBu)	5 mmol 10 mmol
SMP-05-V SMP-10-V	FMOC-L-Val	5 mmol 10 mmol

Appendix B

Bulk N-FMOC-Protected Amino Acids, Preweighed

CAT. NO.	DESCRIPTION	QUANTITY
B-25-A B-100-A	FMOC-L-Ala	25 g 100 g
B-25-RP B-100-RP	FMOC-L-Arg(Pmc)	25 g 100 g
B-25-RBF	FMOC-L-Arg(Pbf)	25 g
B-25-NT B-100-NT	FMOC-L-Asn(Trt)	25 g 100 g
B-25-DB B-100-DB	FMOC-L-Asp(OtBu)	25 g 100 g
B-25-CA	FMOC-L-Cys(Acm)	25 g
B-25-CT B-100-CT	FMOC-L-Cys(Trt)	25 g 100 g
B-25-EB B-100-EB	FMOC-L-Glu(OtBu)	25 g 100 g
B-25-QT B-100-QT	FMOC-L-GIn(Trt)	25 g 100 g
B-25-G B-100-G	FMOC-L-Gly	25 g 100 g
B-25-HT B-100-HT	FMOC-L-His(Trt)	25 g 100 g
B-25-I B-100-I	FMOC-L-Ile	25 g 100 g
B-25-L B-100-L	FMOC-L-Leu	25 g 100 g
B-25-KBC B-100-KBC	FMOC-L-Lys(Boc)	25 g 100 g
B-25-M B-100-M	FMOC-L-Met	25 g 100 g
B-25-F B-100-F	FMOC-L-Phe	25 g 100 g

Appendix

CAT. NO.	DESCRIPTION	QUANTITY
B-25-P B-100-P	FMOC-L-Pro	25 g 100 g
B-25-SB B-100-SB	FMOC-L-Ser(tBu)	25 g 100 g
B-25-TB B-100-TB	FMOC-L-Thr(tBu)	25 g 100 g
B-25-W B-100-W	FMOC-L-Trp	25 g 100 g
B-25-WBC	FMOC-L-Trp(Boc)	25 g
B-25-YB B-100-YB	FMOC-L-Tyr(tBu)	25 g 100 g
B-25-V B-100-V	FMOC-L-Val	25 g 100 g

Larger quantities available upon request.

Appendix C

Symphony Accessories/Replacement Parts

CAT. NO.	DESCRIPTION	QUANTITY
SMP-010135 SMP-010092 SMP-010134	Reaction Vessels: Small Glass Reaction Vessel (9 mL) Standard Glass Reaction Vessel (16 mL) Large Glass Reaction Vessel (32 mL)	1 ea. 1 ea. 1 ea.
SMP-220056	Cap for Glass Reaction Vessel	1 ea.
SMP-RV-012 SMP-RV-100 SMP-RV-500	Plastic Reaction Vessel (26 mL)	Pkg. of 12 Pkg. of 100 Pkg. of 500
SMP-270054	O-ring, Kalrez, for all Reaction Vessels	1 ea.
SMP-010139	RV Rinse Tube (new style)	1 ea.
SMP-270096	Rinse Tube O-ring	1 ea.
SMP-010141	Safety Shield, Plastic	1 ea.
SMP-VX-020 SMP-VX-100	Replacement Parts: Empty AA Reservoirs & Septa	Pkg. of 20 Pkg. of 100
SMP-270044	Seal, Reagent Reservoir	Pkg. of 20
3100013	Collection Vial (Plastic, Standard)	Pkg. of 50
SMP-LGCLTB	Collection Vial (Glass, Large)	1 ea.
SMP-RF-100	Bottle Filter	Pkg. of 100
SMP-260187	Bottle Filter Housing	1 ea.
SMP-300007 SMP-300008 SMP-300002 SMP-300004	Bottle amber, 0.5 L Bottle amber, 1 L Bottle amber, 2.5 L Bottle amber, 4 L	1 ea. 1 ea. 1 ea. 1 ea.
SMP-260205	Cap for 0.5, 1, 2.5 and 4 L Bottles	1 ea.
PS3-270042	O-ring for 0.5, 1, 2.5, 4 and 20 L Bottles	1 ea.
SMP-350019	Quick-connect, male	1 ea.
SMP-690015	SMC valve	1 ea.
SMP-010105 SMP-010106	Vessel Assembly, 20 L Vessel Assembly, 12 L	1 ea. 1 ea.

Appendix

CAT. NO.	DESCRIPTION	QUANTITY
SMP-010096	Waste Tank Assembly	1 ea.
SMP-010103	Waste Spigot Assembly	1 ea.
SMP-260264	Cabinet Floor Tray	1 ea.
SMP-260265	Cabinet Shelf Tray	1 ea.
	Tools:	
SMP-010129	Nitrogen Flow Meter	1 ea.
SMP-680018	Vacuum Test Jig	1 ea.
SMP-680019	Pressure Test Jig	1 ea.
SMP-680010	0.125 Lee Tool	1 ea.

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