# Synergy™

## Personal Peptide Synthesizer

Installation Guide

Applied Biosystems

**For Research Use Only. Not for use in diagnostic procedures.**

# 1 Synergy Installation Guide

This Installation Guide describes the steps necessary to prepare the Applied Biosystems Synergy Personal Peptide Synthesizer for its first run. When you have completed all the procedures in this section, Synergy should be operating and have synthesized a 4-residue, test peptide. The entire installation procedure usually takes from four to six hours. You may walk away from the instrument for a break at any step of the installation.

| | |
|---|---|
| *Note* | *Before proceeding with the installation procedure, review the Synergy Pre-Installation Manual. The Pre-Installation Manual contains the MSDSs and Synergy Waste Profile, and describes the recommended ventilation system, space requirements, and gas tank regulator for Synergy.* |

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# Preparation for Installation

***WARNING*** **PHYSICAL INJURY HAZARD*. Use of improper lifting techniques can cause painful and sometimes permanent back injury. Use proper and safe lifting techniques to move the instrument***

1. The Synergy Peptide Synthesizer weighs about 35 lb (16 kg); moving the instrument may require two people. Remove Synergy from the shipping container and place it on its work surface.

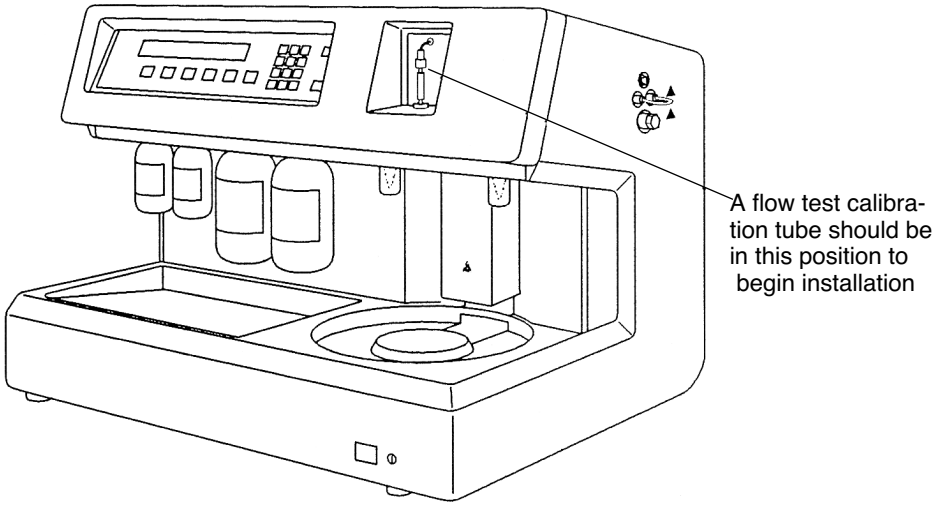



**Figure 1. The Synergy Peptide Synthesizer before installation**

2. **Check that you have the following tools** to complete installation:
   - a flat-head screwdriver
   - open wrenches, (1/4 in., 1/2 in., 5/8 in., 9/16 in.), or an adjustable wrench
   - an indelible ink marking pen or wax marker

   You also **must** have access to a scale that is accurate to ± 1 mg.

3. **Remove all the additional parts** that have been shipped with Synergy and place them near the synthesizer. You will need the following parts to install the Synergy Peptide Synthesizer:
   - one amino acid column wheel
   - two calibration test fixtures: PSC and a wheel test fixture (Figure 2). A package with two more should be in the shipping container.

- a one-gallon, plastic waste bottle with cap, tubing, and ferrule
- reagent bottle seals: 2 each for 40 mL and 8 oz. bottles
- one RS 232 electrical cable
- an AC (alternating current) power cord
- a printer with power supply
- Parflex tubing (1/4 in. O.D.)
- Teflon™ tape (1/4 in.)

**Reagents and Amino Acids**

A package with six bottles of chemical reagents for Synergy and amino acids for the installation procedure are shipped separately. This package contains:

- DMF (N, N-dimethylformamide), 4L
- THF (tetrahydrofuran), 200 mL
- 0.4 M DIEA/DMSO/NMP (N,N-Diisopropylethylamine/Dimethyl sulfoxide/N-Methylpyrrolidone), 40 mL
- HBTU [2-(1 H-Benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate], 8 mmol
- 0.2 M HOBt/DMSO/NMP (1-Hydroxybenzotriazole/Dimethyl sulfoxide/N-Methylpyrrolidone), 40 mL
- Piperidine, 200 mL
- six amino acid columns (AAC) [Figure 2]
- two peptide synthesis columns (PSC) [Figure 2]

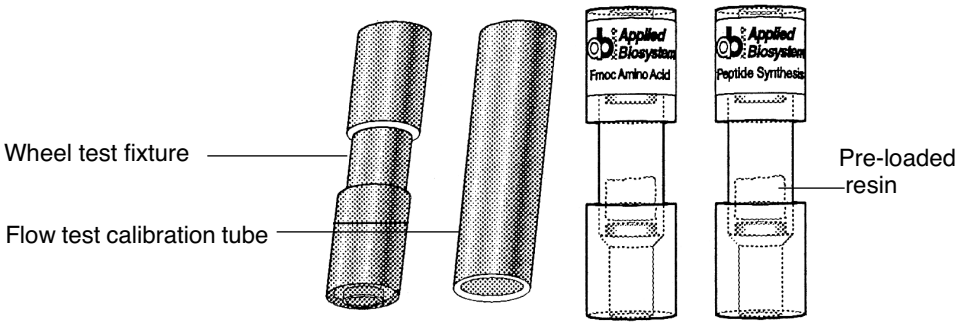



**Figure 2. Calibration Test Fixtures, Amino Acid, and Peptide Synthesis columns**

Synergy peptide synthesis columns (PSCs) each contain 25 µmol of either pre-loaded resin or amide resin. Synergy amino acid columns (AACs) each contain 75 µmol amino acid, enough for a one-time use only. Calibration test fixtures contain two tubes and one wheel. These are reusable and should be kept.

## Printer Assembly

1. **Remove the printer from its packaging.** Follow the instructions in the printer manual for attaching the power supply cord and the printhead cartridge. The power supply cord should plug into a grounded wall socket.
2. **Mode Select Switches**: The Mode Select Switches on the rear of the printer have been pre-set by Applied Biosystems.
3. **Connect the RS 232 electrical cable (Figure 3).** Plug the DB-25 terminal into the RS 232 port on the printer. By hand, tighten the two screws on the DB-25 terminal.

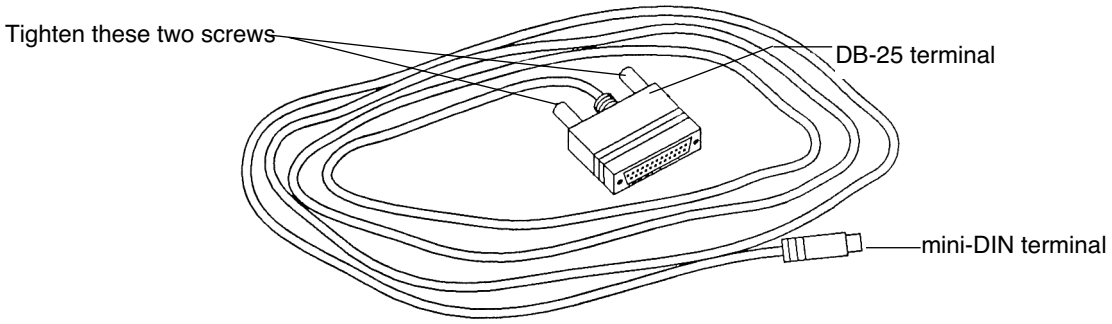



**Figure 3. RS 232 cable terminals**

Plug the mini-DIN terminal of the cable into the printer port on Synergy (Figure 5 on page 6).

| *Note* | *The mini-DIN terminal may be either round or D-shaped. The printer port on Synergy is D-shaped.* |
|---|---|

If the mini-DIN terminal is round, line up the arrow on the terminal with the flat side of the D-shaped port. See Figure 4.

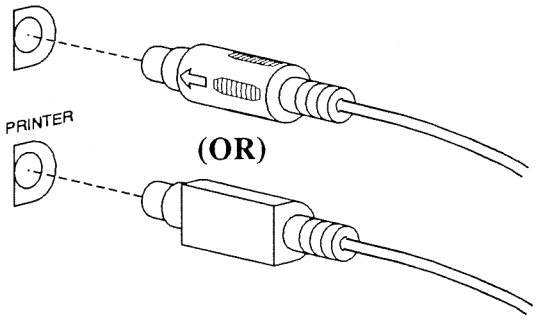



**Figure 4. Mini-DIN terminal connection to D-shaped port**

4. **Load Printer Paper.** Follow the instructions in the printer manual for feeding the paper through the printer.

5. **Attach the Printer.** To conserve workspace, you may mount the printer on top of Synergy. An optional printer mounting rack (shown in Figure 5) may be purchased from Applied Biosystems.

   If you prefer to place the printer directly on the adjacent work surface, place it on the left side of Synergy. Reserve space on the right side of the instrument for two external bottles in polyethylene safety carriers.

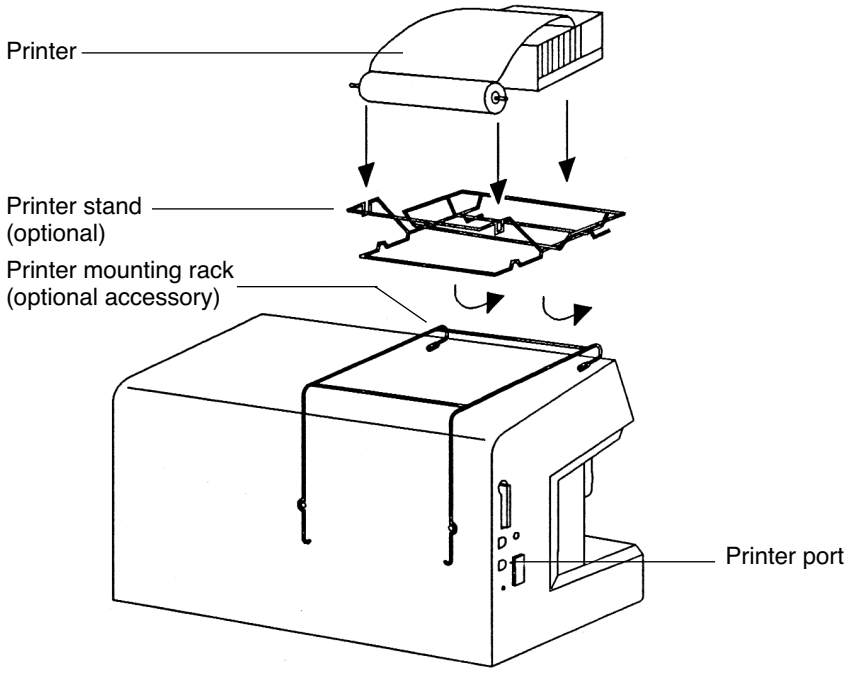



**Figure 5. Printer installation on the Synergy Peptide Synthesizer**

6. Press the power switch on the rear of the printer. The red light labeled "PWR" on top of the printer appears when the printer is on.

| | |
|---|---|
| *Note* | *The "Computer" port above the Printer port currently has no user-related functions. It may be used occasionally by trained Applied Biosystems Service personnel.* |

## Prepare the Synergy Peptide Synthesizer for Installation

Three fittings, labelled “P” (Pressure), “D” (Delivery), and “Hazardous Waste” on the right side of Synergy allow fluids to move in and out of two external bottles (Figure 6). Before shipping, a plug is placed in the waste fitting, and a polyethylene loop is attached to the Pressure and Delivery fittings.

1. Verify that a plug is in the Waste port and that a loop connects the “P” and “D” fittings.

| | |
|---|---|
| ***WARNING*** | **ELECTRICAL FIRE HAZARD** ***Improper voltage supply can damage the instrument wiring system and cause a fire. Before proceeding with these instructions, verify that the instrument voltage specified on the back of the instrument matches the voltage supply in your laboratory*** |

2. Plug one end of the AC power cord into the rear of Synergy. Plug the other end into a grounded wall socket.

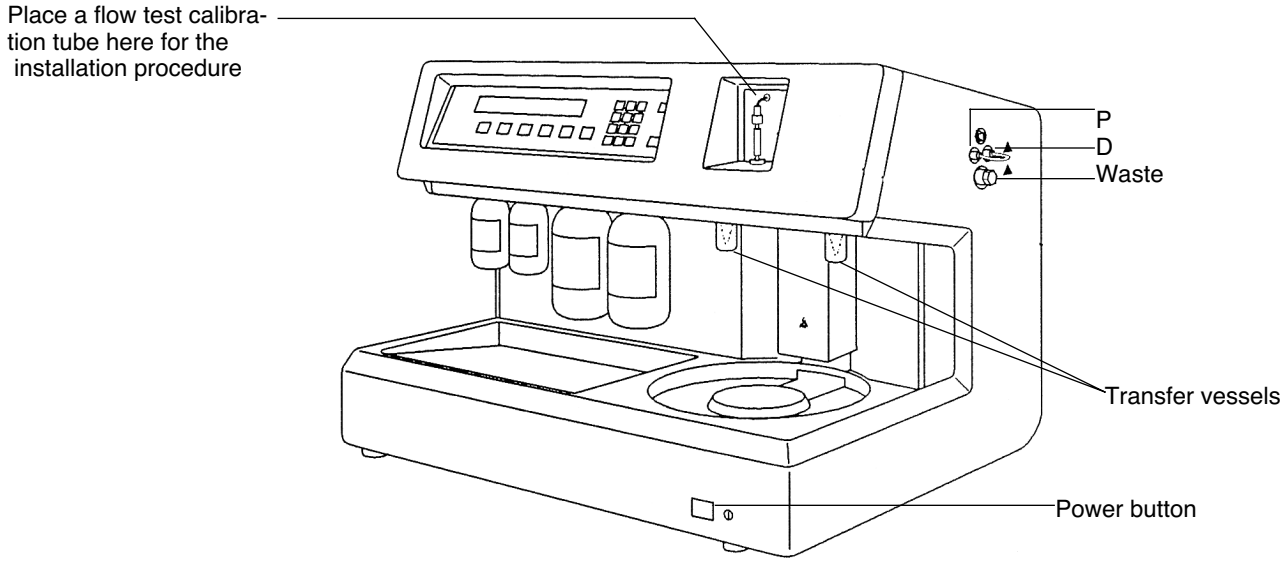



**Figure 6. The Synergy Peptide Synthesizer before installation**

## The LCD and Keyboard

Read this page for a brief description of the LCD (liquid crystal display) and keyboard on the front of Synergy.

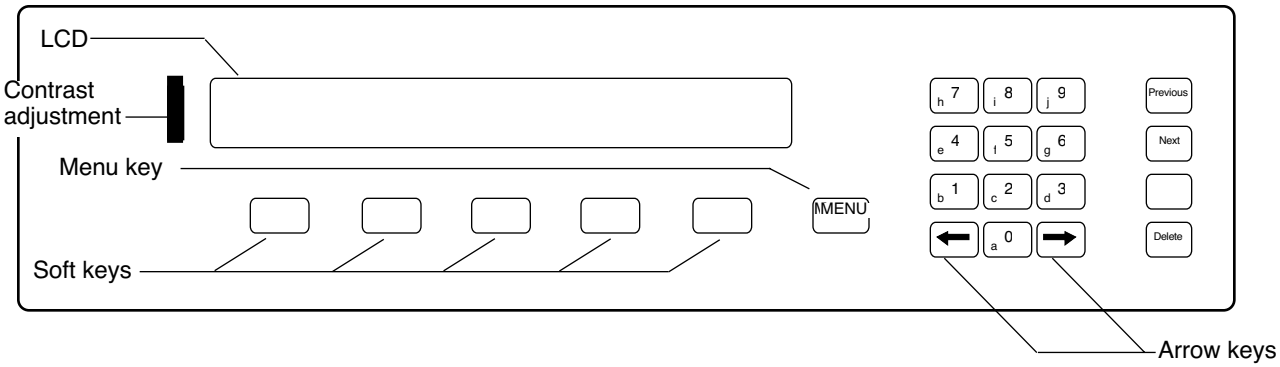



**Figure 7. Synergy LCD and keyboard**

**The LCD** on the front of Synergy displays messages on two lines of 40 characters each. Usually, the top line displays information or directions. The bottom line assigns names to the soft keys located directly under the LCD.

A **cursor**, represented by a blinking, horizontal line (_) on the LCD, appears under text that you can modify.

**Five soft keys under the LCD** correspond to choices displayed on the bottom line of the LCD. The ***HOLD*** and ***PAUSE*** soft keys are like toggle switches. When you press the ***HOLD*** or ***PAUSE*** soft key once, an asterisk (*****) appears next to the key to indicate the key is operating or "on." Press the key again, and the asterisk goes away to indicate the key is "off."

**Sixteen labeled keys** to the right of the LCD let you interact with the Synergy software controller by way of the LCD.

- Use the **alphanumeric keys** to enter numbers on the LCD. In some menus, these keys can represent lower case letters (**a, b, c,** etc.) or upper case letters (**A, B, C,** etc.).
- Use the **arrow** keys (←→) to move the cursor on the LCD to the left or to the right.
- Use the **Previous** and **Next** keys to increase or decrease the value currently selected by the cursor.
- Use the **Delete** key to erase the entry that is currently selected by the cursor.
- Press the **Menu** key to see the preceding LCD display and to return to the Main Menu.

# Technical Support

## Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

## To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

| Product Area | E-mail address |
|---|---|
| Genetic Analysis (DNA Sequencing) | galab@appliedbiosystems.com |
| Sequence Detection Systems and PCR | pcrlab@appliedbiosystems.com |
| Protein Sequencing,<br>Peptide and DNA Synthesis | corelab@appliedbiosystems.com |
| Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers | tsupport@appliedbiosystems.com |
| LC/MS<br>(Applied Biosystems/MDS Sciex) | apisupport@sciex.com<br>or<br>api3-support@sciex.com |
| Chemiluminescence (Tropix) | tropix@appliedbiosystems.com |

## Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

| Product | Hours |
|---|---|
| Chemiluminescence | 8:30 a.m. to 5:30 p.m. Eastern Time |
| Framingham support | 8:00 a.m. to 6:00 p.m. Eastern Time |
| All Other Products | 5:30 a.m. to 5:00 p.m. Pacific Time |

## To Contact Technical Support by Telephone or Fax

### In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

| Product or Product Area | Telephone Dial... | Fax Dial... |
|---|---|---|
| ABI PRISM® 3700 DNA Analyzer | 1-800-831-6844, then press 8 | 1-650-638-5981 |
| DNA Synthesis | 1-800-831-6844, then press 21 | 1-650-638-5981 |
| Fluorescent DNA Sequencing | 1-800-831-6844, then press 22 | 1-650-638-5981 |
| Fluorescent Fragment Analysis (includes GeneScan® applications) | 1-800-831-6844, then press 23 | 1-650-638-5981 |
| Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments) | 1-800-831-6844, then press 24 | 1-650-638-5981 |
| ABI PRISM® 3100 Genetic Analyzer | 1-800-831-6844, then press 26 | 1-650-638-5981 |
| BioInformatics (includes BioLIMS®, BioMerge™, and SQL GT™ applications) | 1-800-831-6844, then press 25 | 1-505-982-7690 |
| Peptide Synthesis (433 and 43X Systems) | 1-800-831-6844, then press 31 | 1-650-638-5981 |
| Protein Sequencing (Procise® Protein Sequencing Systems) | 1-800-831-6844, then press 32 | 1-650-638-5981 |
| PCR and Sequence Detection | 1-800-762-4001, then press 1 for PCR, 2 for the 7700 or 5700, 6 for the 6700 or dial 1-800-831-6844, then press 5 | 1-240-453-4613 |
| Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations | 1-800-899-5858, then press 13 | 1-508-383-7855 |
| Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products) | 1-800-899-5858, then press 14 | 1-508-383-7855 |
| Expedite™ Nucleic acid Synthesis Systems | 1-800-899-5858, then press 15 | 1-508-383-7855 |

| Product or Product Area | Telephone Dial... | Fax Dial... |
|---|---|---|
| Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers) | 1-800-899-5858, then press 15 | 1-508-383-7855 |
| PNA Custom and Synthesis | 1-800-899-5858, then press 15 | 1-508-383-7855 |
| FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader | 1-800-899-5858, then press 16 | 1-508-383-7855 |
| Chemiluminescence (Tropix) | 1-800-542-2369 (U.S. only), or 1-781-271-0045 | 1-781-275-8581 |
| Applied Biosystems/MDS Sciex | 1-800-952-4716 | 1-650-638-6223 |

## Outside North America

| Region | Telephone Dial... | Fax Dial... |
|---|---|---|
| Africa and the Middle East | | |
| Africa (English Speaking) and West Asia (Fairlands, South Africa) | 27 11 478 0411 | 27 11 478 0349 |
| South Africa (Johannesburg) | 27 11 478 0411 | 27 11 478 0349 |
| Middle Eastern Countries and North Africa (Monza, Italia) | 39 (0)39 8389 481 | 39 (0)39 8389 493 |
| Eastern Asia, China, Oceania | | |
| Australia (Scoresby, Victoria) | 61 3 9730 8600 | 61 3 9730 8799 |
| China (Beijing) | 86 10 64106608 | 86 10 64106617 |
| Hong Kong | 852 2756 6928 | 852 2756 6968 |
| Korea (Seoul) | 82 2 593 6470/6471 | 82 2 593 6472 |
| Malaysia (Petaling Jaya) | 60 3 758 8268 | 60 3 754 9043 |
| Singapore | 65 896 2168 | 65 896 2147 |
| Taiwan (Taipei Hsien) | 886 2 22358 2838 | 886 2 2358 2839 |
| Thailand (Bangkok) | 66 2 719 6405 | 66 2 319 9788 |
| Europe | | |
| Austria (Wien) | 43 (0)1 867 35 75 0 | 43 (0)1 867 35 75 11 |
| Belgium | 32 (0)2 712 5555 | 32 (0)2 712 5516 |
| Czech Republic and Slovakia (Praha) | 420 2 61 222 164 | 420 2 61 222 168 |
| Denmark (Naerum) | 45 45 58 60 00 | 45 45 58 60 01 |
| Finland (Espoo) | 358 (0)9 251 24 250 | 358 (0)9 251 24 243 |
| France (Paris) | 33 (0)1 69 59 85 85 | 33 (0)1 69 59 85 00 |
| Germany (Weiterstadt) | 49 (0) 6150 101 0 | 49 (0) 6150 101 101 |
| Hungary (Budapest) | 36 (0)1 270 8398 | 36 (0)1 270 8288 |
| Italy (Milano) | 39 (0)39 83891 | 39 (0)39 838 9492 |

| Region | Telephone<br>Dial... | Fax<br>Dial... |
|---|---|---|
| Norway (Oslo) | 47 23 12 06 05 | 47 23 12 05 75 |
| Poland, Lithuania, Latvia, and Estonia (Warszawa) | 48 (22) 866 40 10 | 48 (22) 866 40 20 |
| Portugal (Lisboa) | 351 (0)22 605 33 14 | 351 (0)22 605 33 15 |
| Russia (Moskva) | 7 095 935 8888 | 7 095 564 8787 |
| South East Europe (Zagreb, Croatia) | 385 1 34 91 927 | 385 1 34 91 840 |
| Spain (Tres Cantos) | 34 (0)91 806 1210 | 34 (0)91 806 1206 |
| Sweden (Stockholm) | 46 (0)8 619 4400 | 46 (0)8 619 4401 |
| Switzerland (Rotkreuz) | 41 (0)41 799 7777 | 41 (0)41 790 0676 |
| The Netherlands (Nieuwerkerk a/d IJssel) | 31 (0)180 331400 | 31 (0)180 331409 |
| United Kingdom (Warrington, Cheshire) | 44 (0)1925 825650 | 44 (0)1925 282502 |
| All other countries not listed (Warrington, UK) | 44 (0)1925 282481 | 44 (0)1925 282509 |
| Japan | | |
| Japan (Hacchobori, Chuo-Ku, Tokyo) | 81 3 5566 6230 | 81 3 5566 6507 |
| Latin America | | |
| Del.A. Obregon, Mexico | 305-670-4350 | 305-670-4349 |

## To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

**http://www.appliedbiosystems.com/techsupp**

To submit technical questions from North America or Europe:

| Step | Action |
|---|---|
| **1** | Access the Applied Biosystems Technical Support Web site. |
| **2** | Under the Troubleshooting heading, click Support Request Forms, then select the relevant support region for the product area of interest. |
| **3** | Enter the requested information and your question in the displayed form, then click Ask Us RIGHT NOW (blue button with yellow text). |
| **4** | Enter the required information in the next form (if you have not already done so), then click Ask Us RIGHT NOW.<br><br>You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours. |

## To Obtain Documents on Demand

Free, 24-hour access to Applied Biosystems technical documents, including MSDSs, is available by fax or e-mail or by download from our Web site.

| To order documents... | Then... |
|---|---|
| by index number | a. Access the Applied Biosystems Technical Support Web site at http://www.appliedbiosystems.com/techsupp<br>b. Click the Index link for the document type you want, then find the document you want and record the index number.<br>c. Use the index number when requesting documents following the procedures below. |
| by phone for fax delivery | a. From the U.S. or Canada, call 1-800-487-6809**,** or from outside the U.S. and Canada, call 1-858-712-0317.<br>b. Follow the voice instructions to order the documents you want.<br>**Note** There is a limit of five documents per request. |
| through the Internet for fax or e-mail delivery | a. Access the Applied Biosystems Technical Support Web site at http://www.appliedbiosystems.com/techsupp<br>b. Under Resource Libraries, click the type of document you want.<br>c. Enter or select the requested information in the displayed form, then click Search.<br>d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click Deliver Selected Documents Now (or click the PDF icon for the document to download it immediately).<br>e. Fill in the information form (if you have not previously done so), then click Deliver Selected Documents Now to submit your order.<br>**Note** There is a limit of five documents per request for fax delivery but no limit on the number of documents you can order for e-mail delivery. |

# Synergy Installation Program

The first time you turn on the power to the Synergy Peptide Synthesizer, an installation software program automatically begins. This installation program displays instructions on the LCD. As you follow these instructions, you prepare the synthesizer for the first test peptide synthesis.

1. Turn on the power on the Synergy Peptide Synthesizer by pressing the power button on the front of the instrument (Figure 7 on page 8).

   As the software program begins to operate, you should hear a series of beeps and see a message on the LCD. If you hear a beep, but cannot see a message, move the contrast adjustment knob (Figure 7 on page 8) at the left of the LCD until the letters become visible.

   **Initializing Synergy**

   **Please wait...**

   **Circuit testing...**

   These messages always appear when you turn on Synergy. If the Circuit Test fails, the LCD displays one of the following messages:

   - ***ROM memory error***
   - ***Valve continuity failure XXXXXXXXXXXXXXXXXXX***

     The zeroes and ones that appear, in place of the X's shown here, are a code that indicates which valve connections have caused the test failure.

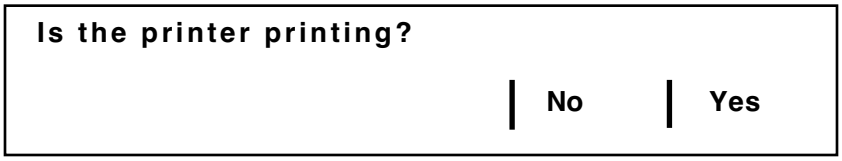
     If the Circuit Test fails, make a note of the number code and call Applied Biosystems Technical Support.

   In the installation program, if the electrical circuits are properly connected, the following display appears after circuit testing.

   **Synergy Instrument Install.**

   **Press [OK] key to continue.** | **OK**

2. Press the soft key under the word ***OK.***




**Printer Test**. If the printer was connected correctly and turned on before you pressed the Synergy power button, it should now be printing the words "The printer is working!"

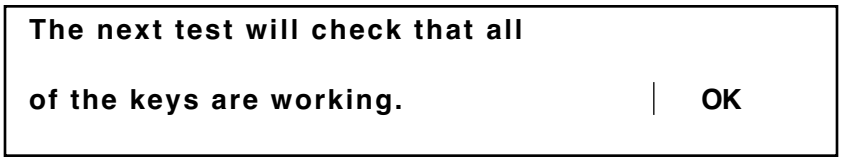
3. **If the printer is printing**, press the ***Yes*** soft key.

   As you go through this installation procedure, the printer records each step of the installation in a report. Do not discard this report; if you call Applied Biosystems Technical Support, they may need information contained in this report in order to assist you.

   **If the printer is not working**, perform the following operations:

   - Check that the power supply and the RS 232 cable have been properly connected.
   - Check that the printer paper is properly loaded. If the white light on the printer blinks, set the top of the paper form.
   - If necessary, prime the ink cartridge again.
   - Check that the printer power switch is ON.

   After you press the ***Yes*** soft key, the following display appears:




4. Press the ***OK*** soft key.

## Keyboard Self Test

The Keyboard Self Test verifies that all keys are working.

```
Press all keys:                    (MENU) to exit

S1S2S3S4S5  <>0123456789 | PREV|NEXT|DEL
```

1. Press each of the 5 soft keys under the LCD.

   As you press each key, the program emits a beep and the name of the key (***S1***, ***S2***, etc.) disappears from the LCD.

2. Press the left and right arrow keys (←→), and each of the alphanumeric keys, ***0-9***.

3. Press the keys labelled *Previous*, *Next*, and *Delete*.

4. When all the key names have disappeared, press the Menu key.

   If the keyboard is working properly, the next display should read:

```
Test passed.

                                  | OK
```

5. Press the ***OK*** soft key.

```
Use the next menu to enter the date

and time.                         | OK
```

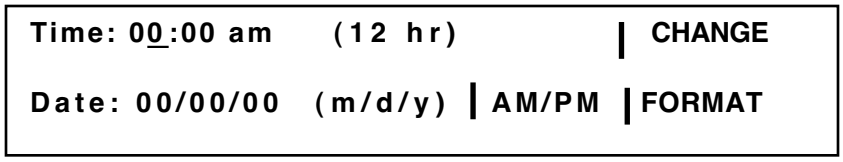
6. Press the ***OK*** soft key to continue the installation.

## Time and Date Entries

Now you can enter the current time and date. At the end of each peptide synthesis, the printer prints the time and date the synthesis began and ended. For this particular menu, use both lines on the LCD to enter numbers.

Use the top line of the LCD to enter the **Time**. When the cursor is in the top line, the soft key under the words ***Change Format*** toggles between 12-hour and 24-hour time displays.

Use the bottom line to enter the **Date**. When the cursor is in the bottom line, the soft key under the words ***Change Format*** toggles between two different date displays, ***m/d/y*** (month/day/year) and ***d/m/y*** (day/month/year).




1. **Enter the time on the top line.** Press the appropriate alphanumeric keys to enter the correct time. Use the arrow keys (←→) to move the cursor to the left or the right in the **Set Time** entry line. To enter a two-digit number, place the cursor in the right-most digit position.

   Press the ***Change Format*** soft key to choose either a 12-hour or 24-hour time display. If you choose a 12-hour time display, press the ***AM/PM*** soft key to choose either of these time suffixes.

2. **Enter the date on the bottom line.** Press the appropriate alphanumeric keys to enter the correct date. Use the arrow keys (←→) to move the cursor from the top line to the bottom line.

   Press the ***Change Format*** soft key to choose a date display format.

3. After the time and date have been entered, press the Menu key to continue the installation.

   The printer should now print an Installation Report heading, with the date and time values you just entered.

## External Gas Connections

| | |
|---|---|
| *Note* | *Refer to the Synergy Pre-Installation Manual for a description of the recommended external gas regulator configuration.* |

The first set of instructions in this section review how to attach the Parflex tubing to the gas regulator on the nitrogen gas tank. These instructions presume that the low pressure port of the gas regulator has female threads. If you have already completed this connection, skip this page and continue at the top of page 19.

***To attach tubing to the external gas tank regulator***

1. First, wrap a short piece of 1/4 in. Teflon tape **counter-clockwise** around the threads of the male pipe fitting (MPF). The tape should not extend beyond the outside lip of the pipe (Figure 8).

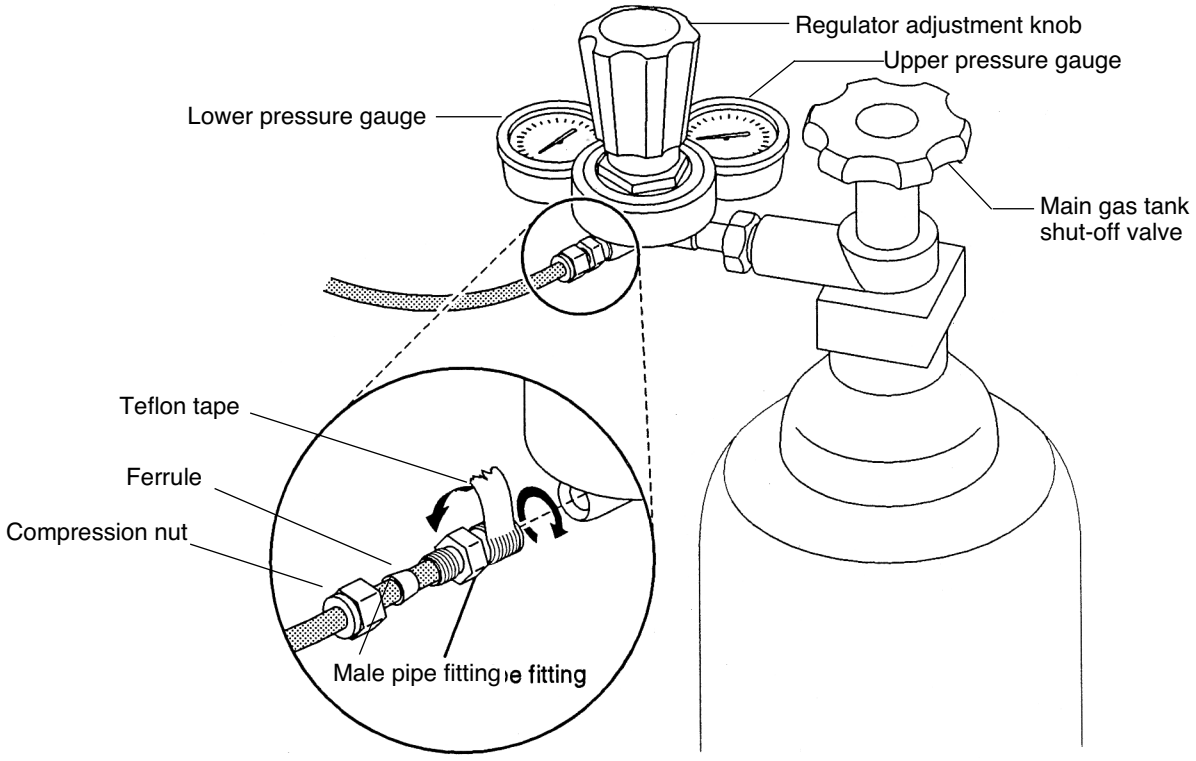



**Figure 8. Attaching Parflex tubing to the gas regulator**

2. Screw the taped fitting **clockwise** into the low pressure port of the gas regulator.
3. Slide a brass ferrule and a compression nut on to the end of the tubing (Figure 8). Insert the tubing into the MPF, push the ferrule into the

MPF, and tighten the compression nut finger-tight. Use a 9/16 in. wrench to turn the fitting 1/4 turn more.

***WARNING*** **GAS TANK EXPLOSION HAZARD** ***Pressurized gas cylinders are explosive. Attach pressurized gas cylinders to a wall or bench by means of approved brackets or chains. Always cap the gas cylinder when not in use.***

If you have followed the instructions in the Pre-Installation Manual, you have already connected Parflex tubing to the gas regulator on the nitrogen gas tank. Start with the following procedure.

***To connect gas tubing to the Synergy Peptide Synthesizer***

| **Make sure all bottles are on tight** | |
|---|---|
| **and connect the gas to the 432A.** | **OK** |

1. Synergy is shipped with four empty reagent bottles and two transfer vessels attached. Check that these bottles and the two transfer vessels (Figure 6 on page 7) are tightly screwed in place.

***Caution*** ***Do not overtighten the bottles! If you turn the bottle past the first point of resistance, you may crack the bottle seal or damage the ratchet cap assembly.***

2. Remove the compression nut and ferrule at the gas entry port. They are covered by a protective plastic cap. Slide them on to the end of the tubing opposite the gas tank regulator (Figure 9).

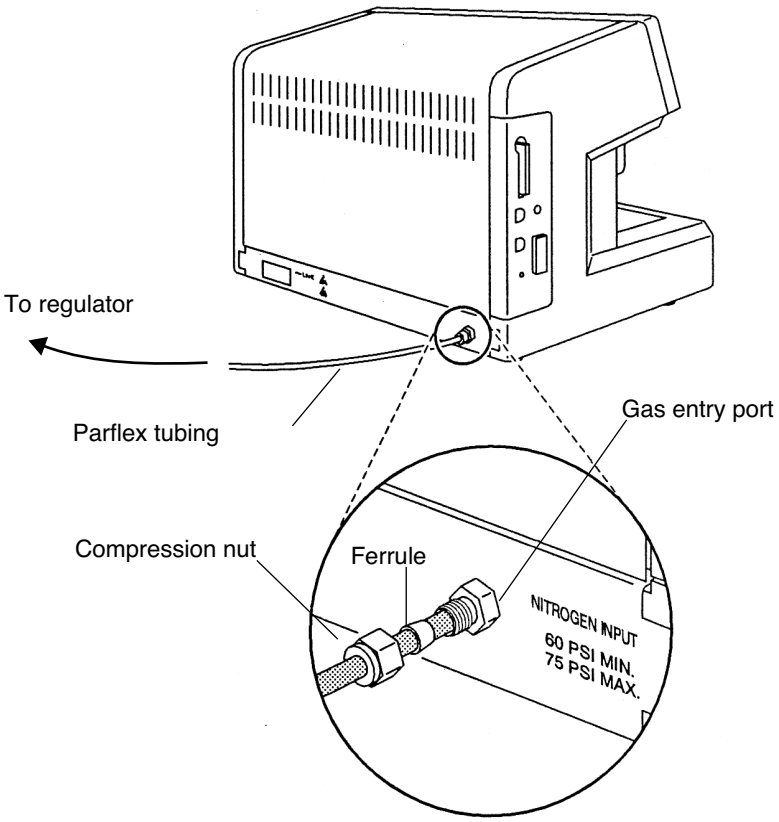



**Figure 9. Attaching the Parflex tubing to the Synergy gas entry port**

3. Insert the tubing into the gas entry port on the back of Synergy, labeled "NITROGEN INPUT."
4. Slide the ferrule and compression nut onto the protruding gas entry port. Turn the fitting clockwise to tighten finger-tight, then use a 9/16 in. wrench to turn the fitting 1/4 turn more. Avoid over-tightening this connection.

| | |
|---|---|
| ***Caution*** | ***The application of excessive force to tighten the compression fitting connection to the Synergy gas entry port can strip the fitting threads! This connection is tested for leaks during the installation process.*** |

5. Press the ***OK*** soft key.

| | |
|---|---|
| **Switch the Pressure/Vent to Vent.** | |
| **Turn on the gas and set to 55 psi.** | **OK** |

6. Set the Pressure/Vent switch to "Vent." (Figure 10).

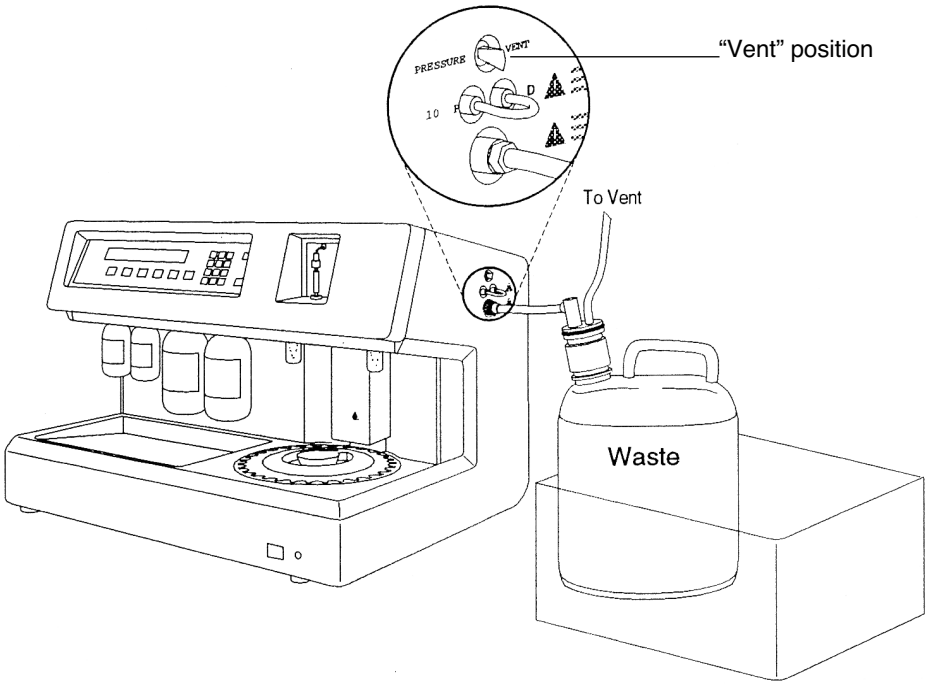



**Figure 10. The pressure/vent switch on the right side panel of Synergy**

7. Open the main supply valve on the gas tank and the regulator needle valve, if one exists. Turn on the external gas regulator. Adjust the external regulator pressure to 55 psi.

| | |
|---|---|
| **Turn off the regulator** | |
| **and wait 5 minutes.** | **OK** |

8. Perform an external gas delivery leak test by turning the regulator knob 3-5 complete turns counterclockwise. As you turn the knob, the lower pressure gauge needle immediately drops 1-5 psi and then stabilizes.

Note the reading on the lower gauge when it stops dropping. Wait five minutes. Press the ***OK*** soft key.

| **Is the gas still at about 55 psi?** | | |
|---|---|---|
| | **NO** | **YES** |

9. **If the lower gauge reading has remained stable after 5 minutes,** there are no leaks in the external gas delivery system. Press the ***YES*** soft key and continue with step 10.

   **If the regulator needle moves down more than 1 psi in 5 minutes**, there is a leak in the external gas delivery line. Press the ***NO*** soft key.

| **Check connections for leaks.** | |
|---|---|
| | **OK** |

   a. Fill a large syringe with soapy water and squirt the solution around the connections between the regulator, Synergy, and the gas delivery line to locate leaks.

   b. Tighten any leaky connections.

   c. Press the ***OK*** soft key and repeat the external gas delivery leak test that starts at step 8 of this procedure.

   **If you cannot eliminate the leak**, call Applied Biosystems Technical Support. **Do not continue with the installation procedure.**

| **Set the gas to 65 psi.** | |
|---|---|
| | **OK** |

10. When the external gas delivery lines are free of leaks and the nitrogen tank regulator pressure, set the external gas pressure to 60 - 75 psi. Press the ***OK*** soft key to continue the installation procedure.

## Adjust Internal Pressure

Reagent delivery and flow throughout a synthesis depends on adequate internal gas pressure. During this stage of the installation, you adjust the internal gas pressure to 5.0 psi. This internal pressure is fairly stable and should require little adjustment during normal operation.

| **Switch the Pressure/Vent to Pressure.** | **OK** |
|---|---|

1. Set the Pressure/Vent switch to “Pressure.”

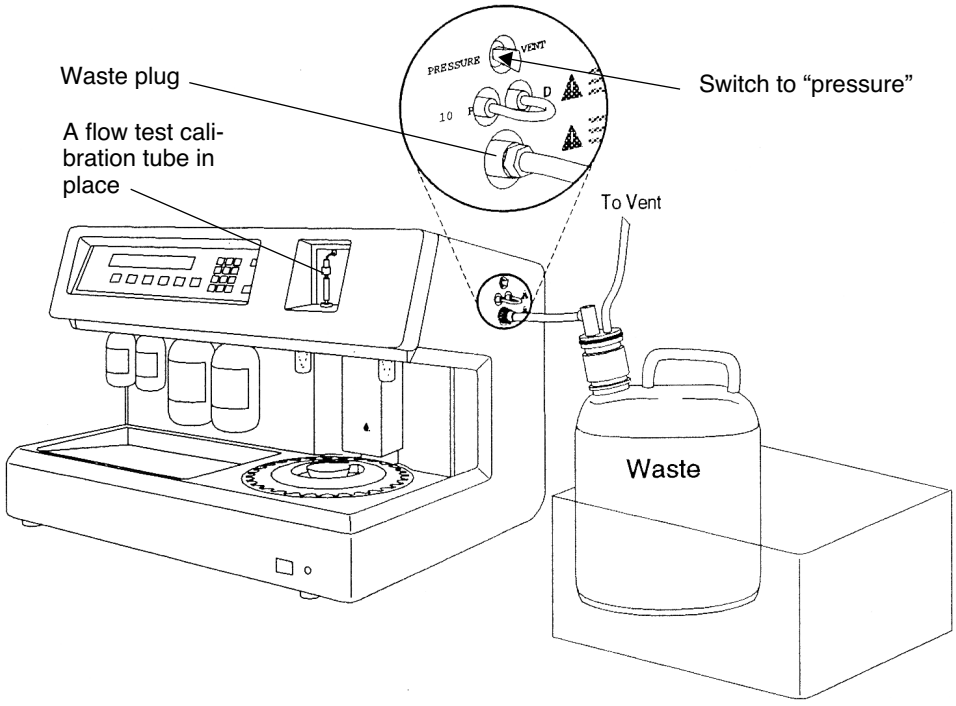



**Figure 11. The Pressure/Vent Switch on the right side panel of Synergy**

2. Press the ***OK*** soft key.
3. Check that a flow test calibration tube is in place on the front of Synergy (Figure 11). Press the ***OK*** soft key.

| ON NEXT MENU, SEE USER'S MANUAL IF PRESSURE | |
|---|---|
| NOT BETWEEN 3.5–5.5 psi | OK |

4. Read the internal pressure reading that appears after the words "***Current pressure***". The internal pressure should be 3.5 ± 5.5 psi.

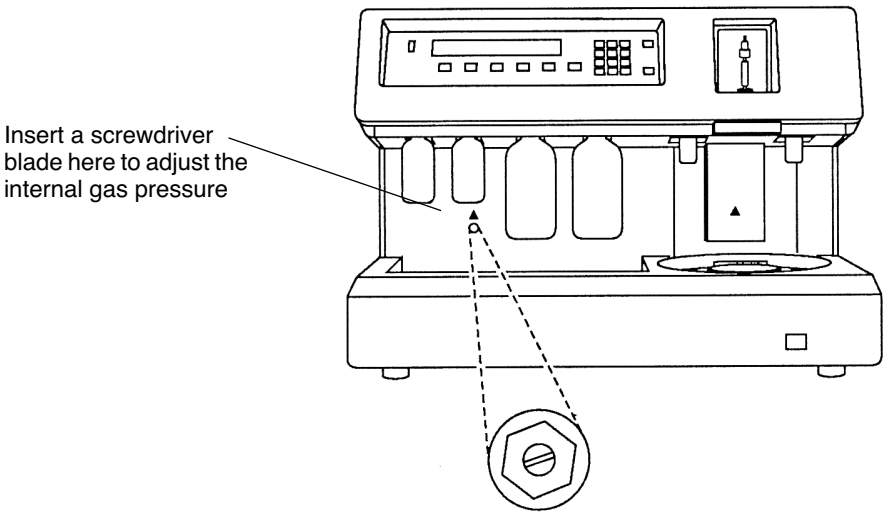



**Figure 12. Adjusting the internal gas pressure**

**If the pressure is below 3.8 psi**, use a flat-edged screwdriver to adjust the internal pressure to 5.0 ± 0.5 psi. Insert the screwdriver blade into the small hole located behind the reagent bottles (Figure 12). A quarter turn clockwise raises the pressure reading by about 0.2 psi. Turn the screw clockwise, **in one direction only**, and wait 30 seconds for the pressure reading to stabilize.

*IMPORTANT* *Turn the internal gas adjustment screw in one direction only and then wait 30 seconds for the pressure to stabilize.*

**If the pressure is above 5.4 psi**:

a. Note the current internal gas pressure reading that appears after the words ***Current pressure*** on the LCD. The internal gas pressure should initially be set at 5.0 ± 0.5 psi. If a quarter turn counterclockwise lowers the pressure reading by about 0.2 psi, estimate how far you will need to turn the internal gas adjustment screw. **Do not make this adjustment until you have completed steps b and c of this procedure.**

b. Use a 5/8 in. wrench to remove the plug in the Waste fitting (Figure 11).

c. Set the Pressure/Vent switch to "Vent" to vent the excess pressure.

d. Turn the pressure adjustment screw counterclockwise to lower the pressure.

e. Use a 5/8 in. wrench to replace the plug in the Waste fitting.

f. Set the Pressure/Vent switch to "Pressure" and wait 30 seconds. Observe the value displayed after the words "***Current pressure***" on the LCD. If the pressure is still too high, repeat steps a-f.

5. Press the ***OK*** soft key when the internal pressure is set.

## Amino Acid Column Wheel Test

This test confirms that the autosampler jaws and the wheel that holds amino acid columns are working properly. During this test, the wheel rotates until a wheel test fixture in position one moves into the jaw assembly, and the jaws close down on the test fixture.

***WARNING*** **PERSONAL INJURY HAZARD.** ***The autosampler jaw is operated by a pneumatic valve under high pressure. Keep your fingers away from the jaws during this wheel test.***

| | |
|---|---|
| **The next menu will test the wheel** | |
| **and jaw assembly.** | **OK** |

1. Press the ***OK*** soft key.

| | |
|---|---|
| **Put a wheel test fixture in** | |
| **position one on the wheel.** | **START** |

2. Place a wheel test fixture (Figure 13) in position 1 (one) on the amino acid column wheel.

*IMPORTANT* *Place only one test fixture on the wheel for this wheel test.*

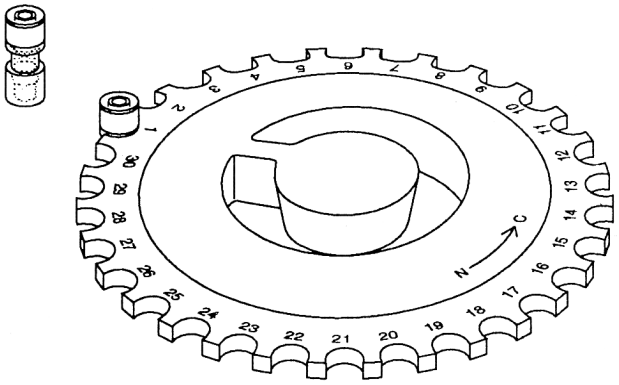



**Figure 13. Placing a wheel test fixture in position 1 (one) on the wheel**

3. Place the wheel on top of the hub on the right side of Synergy. Slowly rotate the wheel until the protruding pin on the hub slips into the corresponding groove in the bottom of the wheel.

4. Press the ***START*** soft key.

   The wheel should now rotate so that position 1 moves into the jaw assembly. Next, a series of tests check the motor and the sensors that activate the jaws.

   If a mechanical failure is detected during the Wheel Test, an error message appears on the screen. You may read about error messages on page 7-20 of the Troubleshooting section of the User's Manual.

   **Test passed**

   **OK**

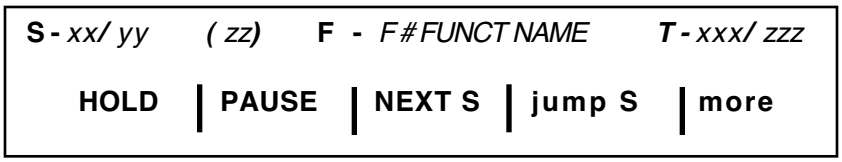
5. This display appears if the autosampler jaw assembly and wheel are working. Press the ***OK*** soft key to continue the installation.

   If the LCD displays the message "***Test Failed***," press the ***OK*** soft key and repeat the wheel test. If the test continues to fail, call Applied Biosystems Technical Support.

## Internal Gas Delivery Leak Test

This test confirms that all connections in the internal gas delivery system are tight and free of leaks.


1. Leave the wheel, with the calibration test fixture-wheel in position one, on Synergy. Press the ***START*** key.




   This LCD display is called the Run Monitor Menu. When it appears, a test for leaks in the internal gas system automatically follows. This particular pressure leak test takes 6 minutes if no pressure test failures occur.

   To read about pressure test failures, see page 62 of this Installation Guide.


2. When this display appears, press the ***OK*** soft key to continue installation.

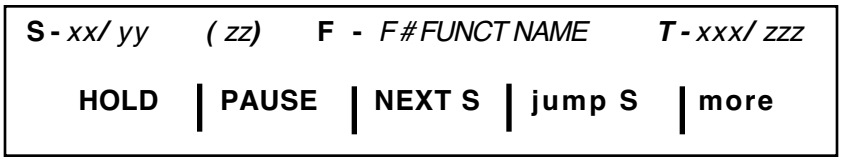
## Vent Test and Waste Bottle Installation

This particular vent test is only performed during the installation procedure, while the waste port is plugged. It is never performed during routine operation of Synergy.

The vent test checks the internal ventilation system for leaks and the external waste system for blockages. It conducts a series of five pressure tests on the valve blocks, both transfer vessels, and the waste lines.


1. **Verify that the plug is still inserted in the Waste port before beginning this test** and then press the ***START*** soft key.


2. Switch the Pressure/Vent switch above the Waste port to "Vent."
3. Press the ***OK*** soft key.




4. The Run Monitor Menu appears and a pressure test automatically begins. After a minute, the following display appears:

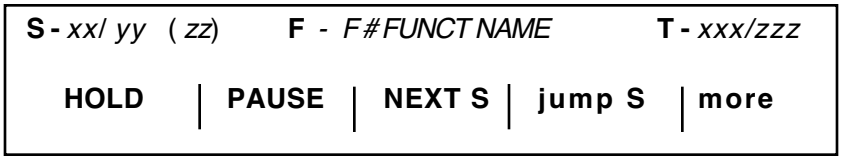

5. Switch the Pressure/Vent switch to "Pressure" and press the ***OK*** soft key. The pressure test continues for two more minutes. When the pressure test is completed, the display gives more instructions. If the LCD has not displayed "Pressure test failed," go on to step 6.

   **If the LCD displays the message: "Pressure test failed" before step 6** of this procedure, there is a leak that you must locate before installing the

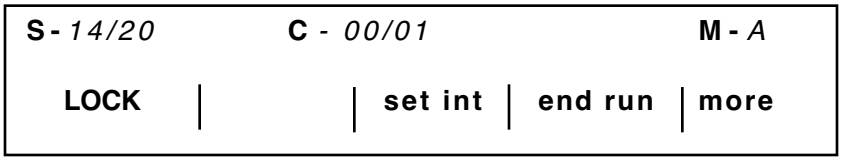
waste bottle. Instead of performing steps 6–10 of this written procedure, follow these troubleshooting steps:

a. Press the ***OK*** soft key after the "Pressure test failed" message appears.

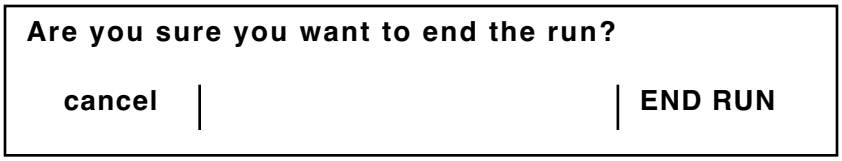
b. Verify that the waste port is tightly plugged. **The waste line should not be attached.**




c. Press the ***more*** soft key.

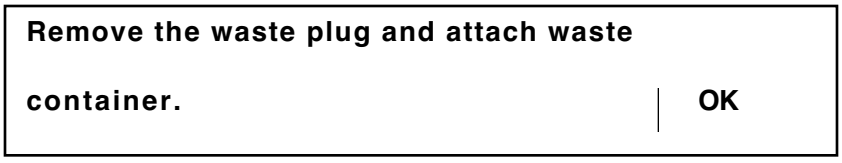



d. Press the ***end run*** soft key.




e. Press the ***END RUN*** soft key.


f. Press the ***OK*** soft key. The Vent Test LCD, shown at the top of page 28 returns. Restart the Vent test. If the pressure test fails again, call Applied Biosystems Technical Support.



6. Use a 5/8 in. wrench to remove the plug from the Waste port. **Save the waste plug for future Synergy Shutdowns**, described in Chapter 2 of the Synergy User's Manual.

7. Place the plastic compression nut on the shorter tube from the waste bottle cap assembly. Slip the tube into the Waste port and screw the plastic compression nut over the Waste port fitting (Figure 14). Using a wrench, ***gently*** tighten an additional 1/4 turn. Be sure to recheck this fitting when running reagents (p. 32).

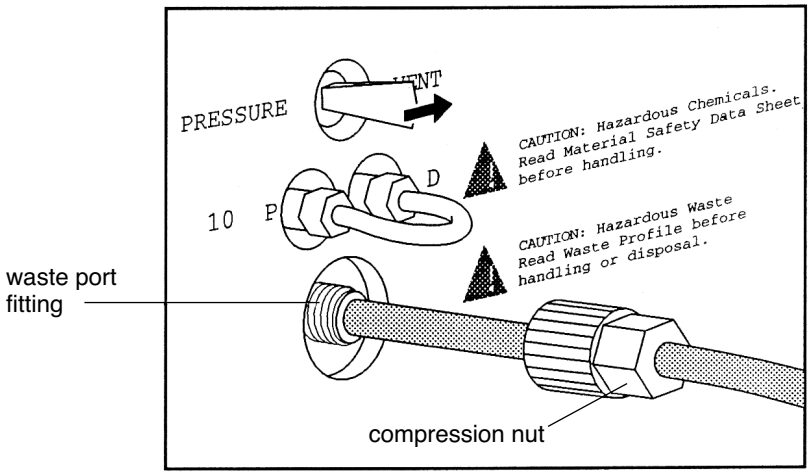



**Figure 14. Attaching the waste tubing at the waste port**

8. Attach the longer tube from the cap assembly to the external ventilation system described in the Pre-Installation Manual and in the ***Laboratory Ventilation Requirements*** on page 1-8 of the Synergy Safety Supplement. Check that the waste line runs up to the external ventilation, without any downward dips where waste can accumulate. If necessary, cut off excess line to allow proper flow.

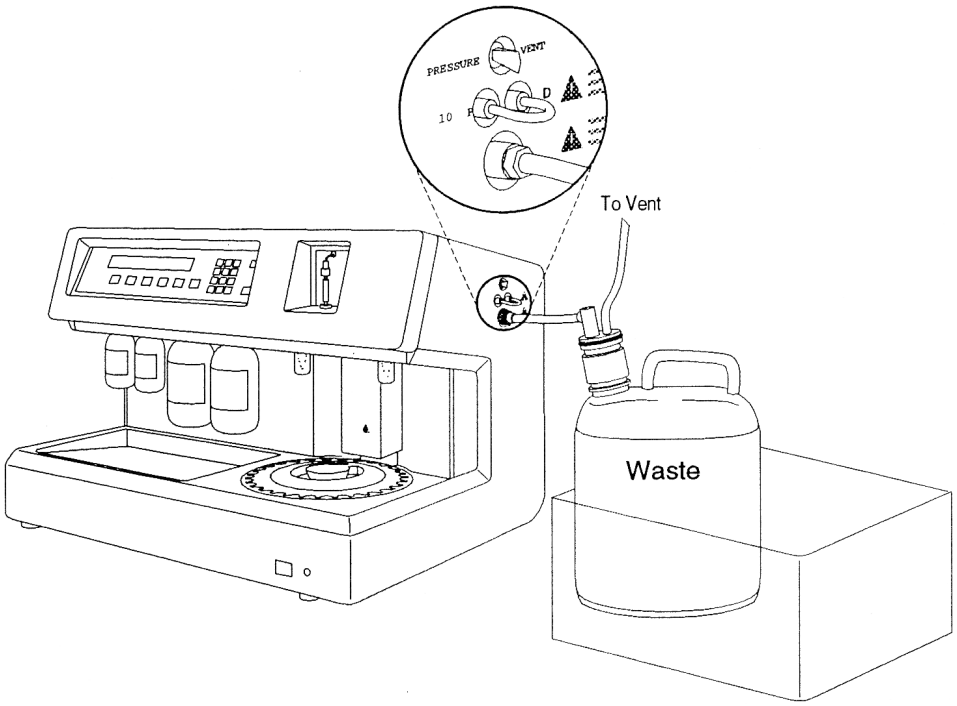



**Figure 15. External waste bottle assembly on Synergy**

9. Place the waste bottle in a safety carrier (Figure 15). Screw the cap assembly on to the waste bottle.

10. Press the ***OK*** soft key. A one-minute pressure test checks for possible blockage in the waste delivery system.

| **Test passed.** | |
|---|---|
| | **OK** |

If no blockages in the waste delivery system are found, this display appears. Press the ***OK*** soft key to continue installation.

| **Waste line is clogged.** | |
|---|---|
| | **OK** |

**If this message appears**, an obstruction in the waste delivery system has been detected. Press the ***OK*** soft key. The LCD then displays the message: ***Test failed***.

Verify that the waste plug (a hexagonal nut) has been removed from the waste port. Visually check the lines attached to the waste cap for blockage.

If you can see a crimp or blockage in either of the two waste lines

a. Detach the waste line from Synergy and clear the obstacle from the line. When you remove the waste line, you can hear a soft hiss of escaping pressurized air.

b. Replace the hexagonal nut that plugged the Waste port.

c. Set the Pressure/Vent switch to Pressure.

d. Press the ***OK*** soft key and repeat the Vent test.

**If you cannot see an obstacle in either of the two waste lines,** or if the vent test fails repeatedly, call Applied BiosystemsTechnical Support for assistance.

## Connect External DMF Reagent Bottle

***WARNING*** **POTENTIAL CHEMICAL HAZARD.** ***Regard all chemicals on the synthesizer, including liquid in the lines, as hazardous. Wear protective eyeglasses, gloves and a laboratory coat when working with these chemicals. If a spill occurs, clean it up in accordance with instructions in the MSDSs or Waste Profile found in the Synergy User's Manual.***


1. Switch the Pressure/Vent switch to "Vent."
2. Use a 1/4-in. wrench to remove the loop in the side port labeled "D." Save this loop for use in the Synergy Shutdown Procedure, described in Chapter 2 of the Synergy User's Manual.
3. Locate the cap assembly for the DMF (N, N-dimethyl formamide) bottle. Two lines of tubing run out of the cap assembly for the DMF bottle.

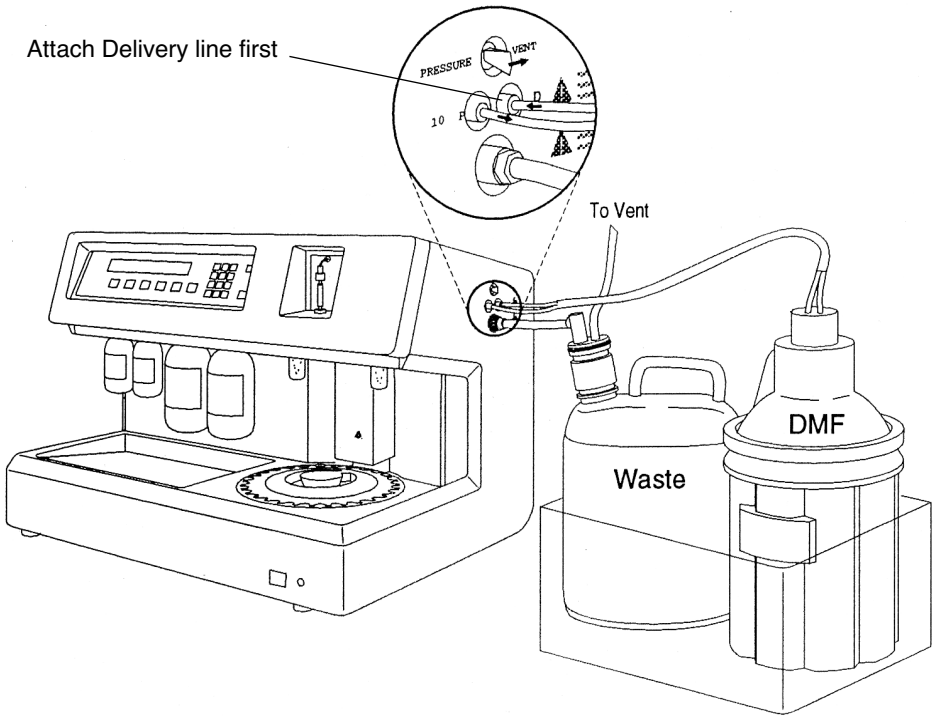



**Figure 16. Switch to Vent and attach the DMF bottle**

4. First, attach the tubing line labeled “D” to the D port. Turn the fitting until it is finger tight, then use a 1/4-in. wrench to turn the fitting an additional quarter turn.

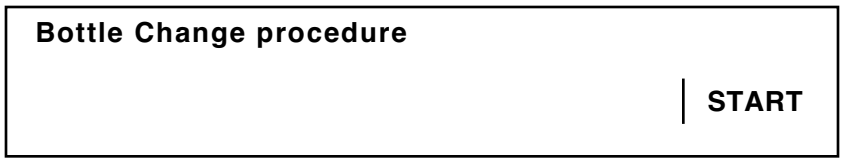
5. Next, remove the loop in the port labeled “P”. Attach the tubing line labeled “P” to the P port. Turn the fitting until it is finger tight, then use a 1/4-in. wrench to turn the fitting an additional quarter turn.

***WARNING*** **CHEMICAL HAZARD.** ***DMF is flammable and an irritant to skin and eyes. Avoid all skin contact with and inhalation of DMF. The MSDS can be found in the Synergy User’s Manual.***

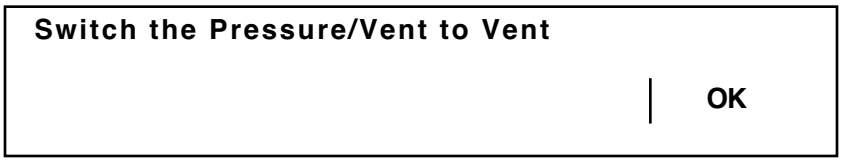
6. Feed the cap through the top piece of the safety carrier. The top piece can be fitted onto the bottom piece after the cap is in place.

7. Place the DMF bottle in a safety carrier in front of the waste bottle (Figure 16). Screw the cap assembly tightly on the DMF reagent bottle, and press the ***OK*** soft key.

## Bottle Change Procedure




1. Press the ***START*** soft key.




2. Verify that the Pressure/Vent switch is set to "Vent." Press the ***OK*** soft key. After 30 seconds, the next display appears.


3. Remove the 4 empty reagent bottles and the bottle seal for each bottle on the front of the Synergy Peptide Synthesizer. You may have to use tweezers to dislodge the bottle seal from inside the ratchet cap. Discard the bottle seals.

### *0.2 M HBTU Reagent Preparation*

You must combine the contents of two reagent bottles to prepare the HBTU [2-(1- H-benzotriazol-1-yl)-1,1,3,3 -tetramethyluronium hexafluorophosphate] reagent. Instructions for preparing the HBTU reagent follow.

***WARNING*** **CHEMICAL HAZARD.** ***HBTU can cause allergic reactions and skin irritations in sensitive persons. Wear protective clothing and prepare the HBTU solution in a properly vented fume hood. See the MSDS in the Synergy User's Manual.***

a. Pour the contents (40 mL) of the bottle labelled "HOBt/DMSO/NMP (0.2 M 1-hydroxybenzotriazole/Dimethyl sulfoxide/N-Methylpyrrolidone)" into the bottle labelled "Peptide Synthesis Reagent A, HBTU, 8 mmoles".

b. Tightly cap the bottle and invert it several times to dissolve the HBTU.

c. Write the date the HBTU reagent was prepared on the bottle label. The prepared reagent can be used for up to six weeks.

d. Locate the delivery line for the HBTU. **Press a reagent line filter onto the end of the delivery line.**

| | |
|---|---|
| *Note* | *One reagent line filter can be used for five consecutive bottles, or approximately 200 mL, of HBTU reagent* |

4. Place reagent bottles in position in the following manner:

   a. Open the reagent bottle and place a bottle seal on the rim.

   b. With the reagent delivery line inside, hold the bottle straight up and turn it carefully into the threads of the ratchet cap on Synergy (Figure 17). The bottle should fit firmly in place.

| | |
|---|---|
| ***Caution*** | ***Do not overtighten the reagent bottles! If you turn the bottle past the first point of resistance, you may crack the bottle seal or damage the ratchet cap assembly*** |

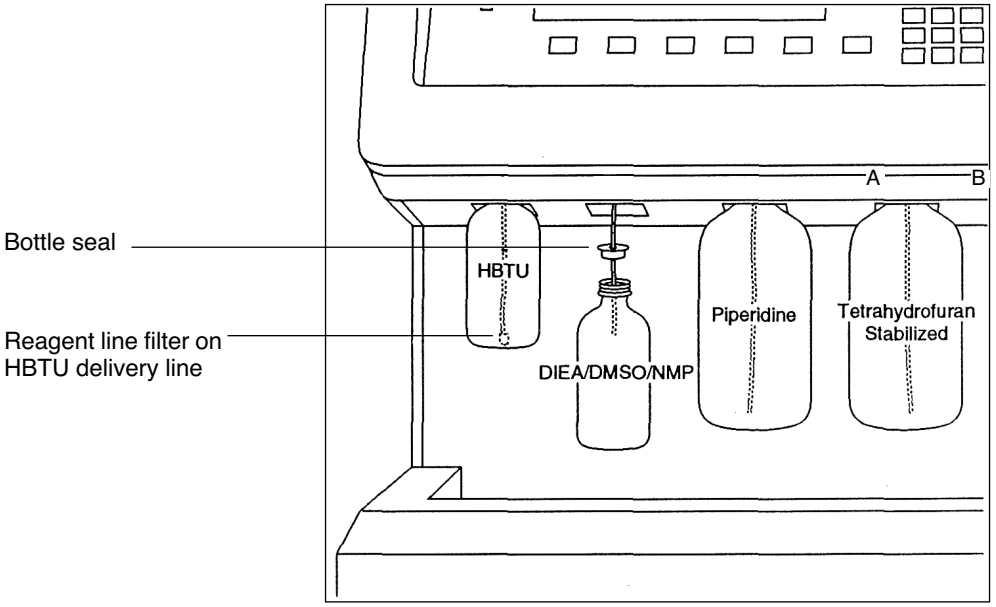
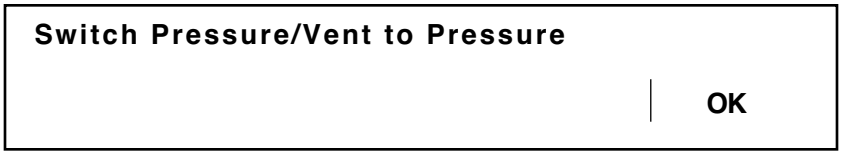



**Figure 17. Reagent bottle positions on the front of the Synergy Peptide Synthesizer.**

When all the reagent bottles are in place, from left to right, facing the front of the synthesizer, the labeled reagent bottles should be HBTU in position A, DIEA/DMSO/NMP (0.4M N,N-diisopropylethylamine/

dimethyl sulfoxide/N-methylpyrrolidone) in position B, Piperidine in position 1, and THF (Tetrahydrofuran) in position 2 (Figure 17).

5. When all the reagent bottles are in place, press the ***OK*** soft key.




6. Switch the Pressure/Vent switch to "Pressure" and press the ***OK*** soft key. A three-minute pressure test follows.


7. When this display appears, all the bottle connections are tight and you can start the bottle priming procedures. Press the ***OK*** soft key.

   If this test fails, one or more of the reagent bottles have not been properly attached to Synergy.

***WARNING*** ***CHEMICAL HAZARD Reagent bottles are pressurized. With the exception of piperidine, always switch the Pressure/Vent switch to "Vent" before unscrewing a reagent bottle on the Synergy Peptide Synthesizer.***

Piperidine is the only reagent that is on its own pressure system, separate from the other bottles. You may open the piperidine bottle without depressurizing the pressure system for the other reagents. When you open the piperidine bottle, you hear a small hiss as pressure is released.

If a pressure leak is detected during the bottle change procedure, switch the Pressure/Vent switch to Vent and check the bottle seal and cap on each reagent bottle, including the DMF bottle.

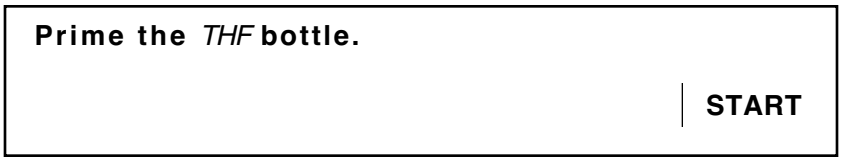
If the pressure test continues to fail, call Applied Biosystems Technical Support.

## Reagent Bottle Priming Procedure

At this stage of the installation, all the reagent bottles are attached to the Synergy Peptide Synthesizer. Now each bottle is individually primed before reagent deliveries are tested and calibrated. One at a time, the reagent bottles are primed in the following order:

- THF
- DMF
- HBTU
- DIEA
- PIP (piperidine)

The LCD tells you which bottle is being primed. After each priming routine, a pressure test checks for leaks in the system.




1. At the beginning of each bottle prime, press the ***START*** soft key. A one-minute pressure check follows.

***Caution*** ***During the prime, you should observe reagent flowing into the external waste bottle. If you detect leaks around the waste port fitting, tighten the compression nut at the fitting.***


2. Press the ***OK*** soft key.
3. Repeat Steps 1 and 2 for each reagent bottle. After the PIP bottle prime is finished, installation continues with flow tests. If the pressure test fails during a priming procedure, check the bottle seal and cap for that bottle.

| | |
|---|---|
| ***WARNING*** | ***CHEMICAL HAZARD Reagent bottles are pressurized. With the exception of piperidine, always switch the Pressure/Vent switch to "Vent" before unscrewing a reagent bottle on the Synergy Peptide Synthesizer.*** |

**If a leak is detected during the piperidine prime routine:** Check that the piperidine bottle and bottle seal is free of cracks and is seated properly on the cap. Replace a faulty bottle seal and check that the bottle cap is tight.

**If a leak is detected during any other reagent prime routine:** Switch the Pressure/Vent switch to Vent and check the bottle's seal and cap. If you cannot locate the source of the leak, call Applied Biosystems Technical Support.

## Flow Tests

At this stage of the installation procedure, you perform nine flow tests to test and calibrate chemical flow through the instrument. For each test, you must perform specific tasks which are described on the LCD. The top line of LCD text tells which chemical flow is being tested. The bottom line of LCD text shows soft key responses.

To perform these flow tests, you must have access to a scale that is accurate to ±1 mg. All flow tests must be successfully performed to complete the installation procedure. When a flow test fails, Synergy automatically repeats the test. If any of these flow tests repeatedly fail, call Applied Biosystems Technical Support for assistance.

***WARNING*** **POTENTIAL CHEMCIAL HAZARD.** ***Regard all chemicals on the synthesizer, including liquid in the lines, as potentially hazardous. Wear protective eye wear, gloves and a laboratory coat when working with the chemicals used on the instrument. If a spill occurs, clean it up in accordance with instructions in the MSDSs or Waste Profile found in the Pre-Installation Manual and in the Synergy User's Manual.***

### Flow Test 1: DMF Delivery to Vessels and Peptide Synthesis Column

This flow test checks three deliveries of DMF: first, through the top valve block; second, through the bottom valve block; and third, through the synthesis column. During this test, you are prompted to tare the left transfer vessel, and then weigh the contents of the left vessel after each of three reagent deliveries and enter the values.

If the reagent delivery weights do not fall within the acceptable ranges, Flow Test 1 fails and you must repeat it before you can continue installation. Since the success of this flow test depends on accurate weights, take these special precautions to insure accuracy and precision:

- Use a scale that is accurate to ± 1mg.
- Always tare the left transfer vessel at the beginning of the flow test.
- Weigh the contents of the vessel **immediately** after reagent delivery to avoid reagent weight loss due to evaporation or spillage.

If you have taken all these precautions and Flow Test 1 fails, repeat it once more. If Flow Test 1 fails the second time, you must adjust the internal gas pressure to calibrate the DMF flow. For this adjustment the gas pressure range is expanded to 4.5 ± 1.0 psi. After you adjust the gas pressure, repeat Flow Test 1 one final time to assure the delivery weights are within range.

*IMPORTANT* ***Always*** *tare the left transfer vessel at the beginning of Flow Test 1,* ***especially if you must repeat the test****.*

```
Run Flow Test:  DMF —>L/R/PSC

  PRINT                              START
```

1. Verify that a flow test calibration tube is in place in the synthesis position (Figure 18). Press the ***START*** soft key.

```
Remove the left vessel and put on scale.

Zero scale then replace the vessel.        OK
```

2. When this display appears, remove the left transfer vessel on the front of Synergy (Figure 18). Tare the scale by placing the vessel on a scale that is accurate to ± 1 mg and setting the scale to zero.
3. Replace the left transfer vessel on Synergy. Press the ***OK*** soft key.

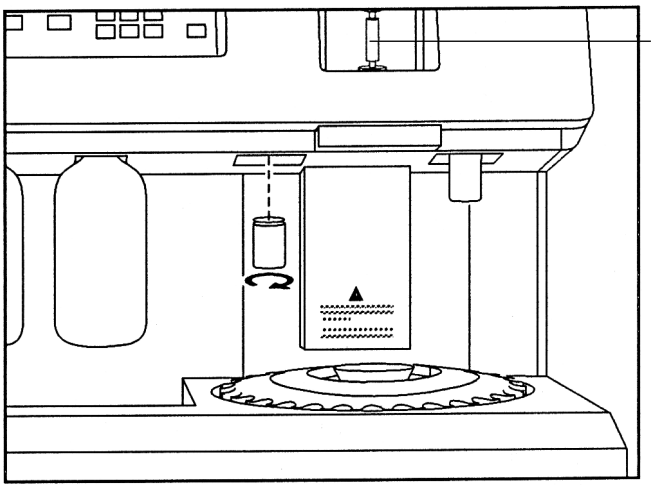



**Figure 18. Remove the left transfer vessel**

```
Remove the left vessel and weigh.    _ mg

                                        OK
```

| Note | *This message appears 3 times during Flow Test 1. When it appears, the left transfer vessel contains DMF.* |
|---|---|

4. Carefully remove the left transfer vessel on the front of Synergy without spilling the contents (Figure 18). **Immediately** weigh the left vessel and use the alphanumeric keys to enter the weight on the LCD. Do not discard the DMF.

   Press the ***OK*** soft key.

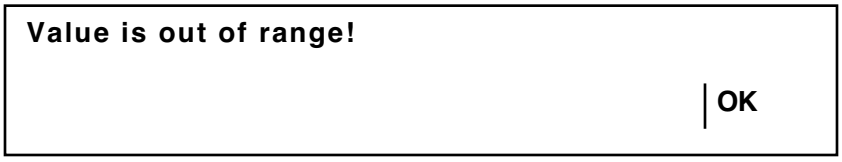
5. Replace the left transfer vessel on Synergy. Press the ***OK*** soft key.
6. Repeat steps 4 and 5 two more times.

   The first two weights both measure DMF deliveries from the valve blocks; they both should be 600 ± 140 mg. The third value results from DMF delivery to the peptide synthesis column (PSC)and should be 550 ± 30 mg.

7. The LCD displays this message at the end of each successful flow test. Press the ***OK*** soft key to continue to the next test. Directions for Flow Test 2 begin on page 45.

**If any one of the DMF delivery weights in Flow Test 1 is out of range,** the LCD displays the message:




When this display appears, press the ***OK*** soft key. The flow test continues to ask for weights until all three deliveries have been weighed. At the end of the test, the LCD displays the message ***Test failed.***

If Flow Test 1 fails, repeat the test one more time from the beginning. During the repeat, check the printer for the value of the **third** DMF delivery

weight. If the repeat of Flow Test 1 fails, you must adjust the internal gas pressure to calibrate the **third** DMF delivery (see page 43).

*IMPORTANT* *Always re-tare the left transfer vessel at the beginning of Flow Test 1.*

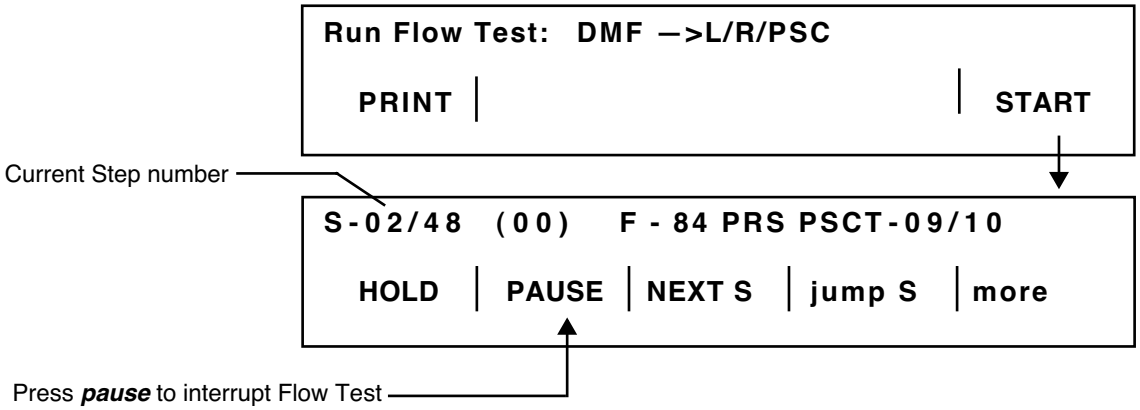
***Adjusting the internal gas pressure to calibrate DMF delivery***

If the repeat of Flow Test 1 fails, adjust the internal pressure to calibrate the **third** DMF delivery. Compare the value of the third DMF delivery weight to the acceptable range (550 ± 30 mg). Optimize this delivery to 550 ± 10 mg, if possible.

**If the third DMF delivery weight was less than 500 mg**, increase the internal gas pressure.

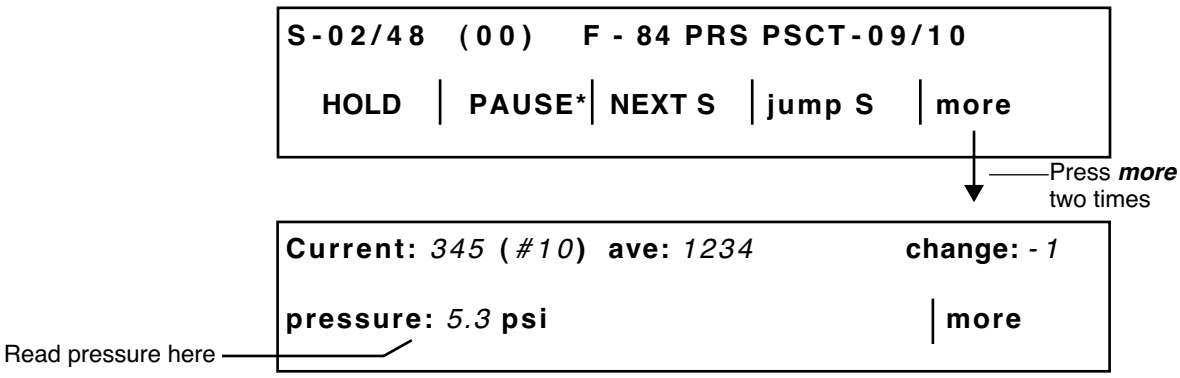
**If the third DMF delivery weight was greater than 600 mg,** decrease the internal pressure.

You can monitor the internal gas pressure when a flow test or synthesis is running by selecting the appropriate Run Monitor Menu on the LCD. The acceptable internal gas pressure range is 3.5 ± 5.5 psi.




1. Press the ***START*** soft key to begin Flow Test 1. **Immediately** press the ***PAUSE*** soft key to temporarily interrupt the flow test.

   When you press the ***PAUSE*** soft key, the current step number should be either ***02*** or ***03***. An asterisk appears (***PAUSE****) to indicate the flow test has been temporarily interrupted.




2. Press the ***more*** soft key **twice**, until the Run Monitor displays the current pressure reading on the bottom line of the LCD.

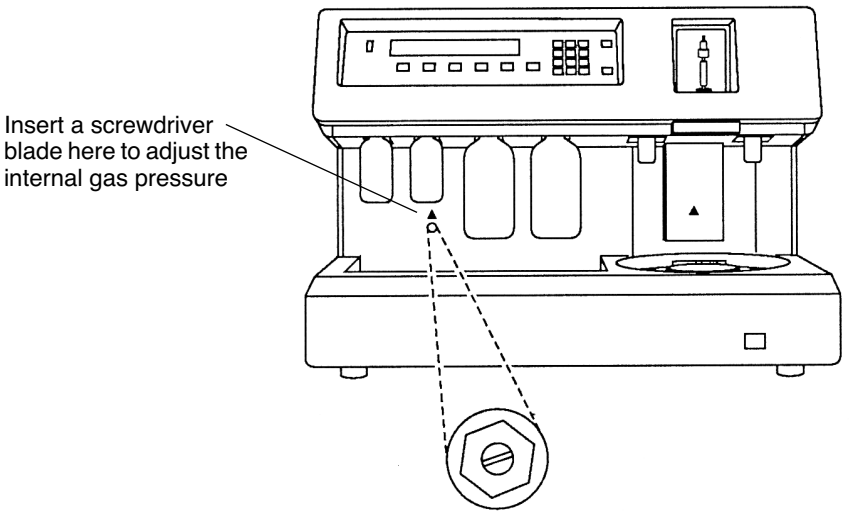



**Figure 19. Adjusting the internal gas pressure**

**If the pressure is too low**, use a flat-edged screwdriver to adjust the internal pressure. Insert the screwdriver blade into the small hole located behind the reagent bottles (Figure 12). Turn the screw a quarter turn clockwise, **in one direction only**, to raise the pressure 0.2 psi. Wait 30 seconds for the pressure reading to stabilize.

*IMPORTANT* *Turn the internal gas adjustment screw in one direction only and then wait 30 seconds for the pressure to stabilize.*

**If the pressure is too high**:

a. Note the current internal gas pressure reading that appears after the words ***pressure*** on the LCD.

b. Set the Pressure/Vent switch to "Vent" to vent the excess pressure.

c. Turn the pressure adjustment screw counterclockwise a quarter turn to lower the pressure by 0.2 psi.

d. Set the Pressure/Vent switch to "Pressure" and wait 30 seconds.

3. After you have adjusted the internal gas pressure, press the ***more*** soft key to return to the Run Monitor that displays the ***PAUSE**** soft key.

4. Press the ***PAUSE**** soft key to continue Flow Test 1.

   As Flow Test 1 continues, repeat the complete procedure to verify that all deliveries are within range. If all delivery weights are within range, continue with Flow Test 2. It is best to have a final delivery of 550 mg ± 10 mg, although it is not required.

   If any delivery weights are not in range, repeat the internal gas pressure adjustment procedure.

**Flow Test 2: Calibrate Flow Test Calibration Tube**

During this test, two deliveries of DMF go into the flow test calibration tube placed in the peptide synthesis column position. You need an indelible ink marker to mark the level of DMF in the column after each flow.

```
Run Flow Test:   CALIBRATE TUBE

                                  | START
```

1. Press the ***START*** soft key. In the two minutes that follow, Synergy performs a brief pressure test and a DMF delivery.

```
Mark the calibration tube.

                                  | OK
```

2. Make a thin, horizontal mark on the flow test calibration tube to indicate the level of the DMF in the column (Figure 20). The mark should be level with the bottom of the meniscus. Press the ***OK*** soft key.

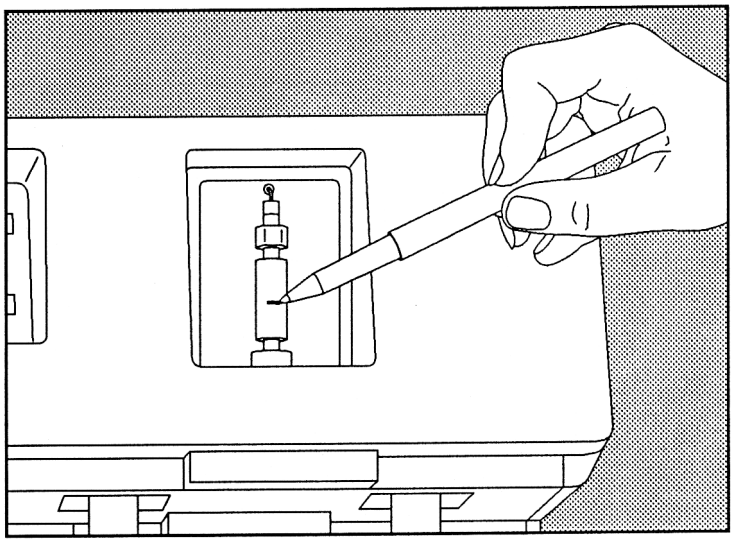


**Figure 20. Marking the flow test calibration tube**

| **Mark the calibration tube.** |
| --- |
| **OK** |

3. After a second DMF delivery, the LCD directs you to make a second mark on the flow test calibration tube. The second mark should be higher than the first mark and level with the bottom of the meniscus. Press the ***OK*** soft key.

*IMPORTANT* *Do not remove this marked flow test calibration tube; you will use it again in the flow tests that follow.*

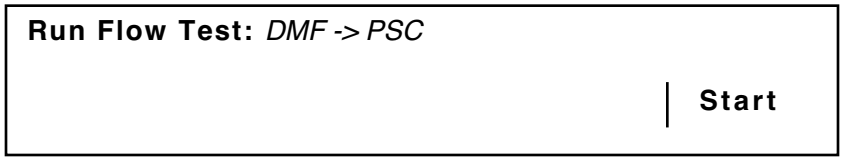
| **Test passed.** |
| --- |
| **OK** |

4. When this display appears, press the ***OK*** soft key to complete the flow test.

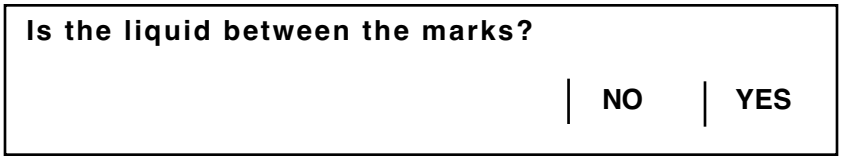
**Flow Tests 3, 4, and 5**

- **DMF → PSC**
- **THF → PSC**
- **PIP → PSC**

The next three flow tests deliver reagent to the flow test calibration tube that you marked in Flow Test 2. During each flow test, you observe the level of reagent delivered to the calibration tube. This level should fall somewhere between the two marks or right on the mark on the tube.




1. Press the ***START*** soft key to begin each flow test. After two minutes, reagent is delivered to the flow test calibration tube in the peptide synthesis column (PSC) position.




2. If the level of reagent in the flow test calibration tube is between the two calibration marks or right on the mark, press the ***YES*** soft key.

   If the level of reagent in any flow test falls outside these two marks, press the ***NO*** soft key.

   After 2 more minutes, the LCD reports if the test was successful.


3. Press the ***OK*** soft key to continue to the next flow test.

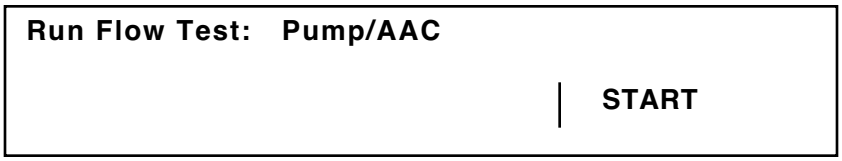
If a flow test fails, check that the ends of the reagent lines in all the bottles are submerged in fluid. Press the ***OK*** soft key and repeat the flow test.

If one of these flow tests fails repeatedly, call Applied Biosystems Technical Support.

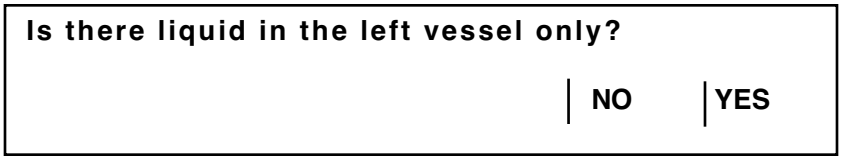
**Flow Test 6: Pump/AAC**

This test first checks the flow of reagent from the left transfer vessel, through the amino acid column (AAC) on the wheel, to the right transfer vessel. Then it checks the flow from the right transfer vessel back to the left transfer vessel. The "pump" in the name of this flow test refers to the configuration of transfer vessels that assures continuous reagent delivery during synthesis.

The calibration test fixture-wheel should be in position 1 (one) on the amino acid column wheel during this test.



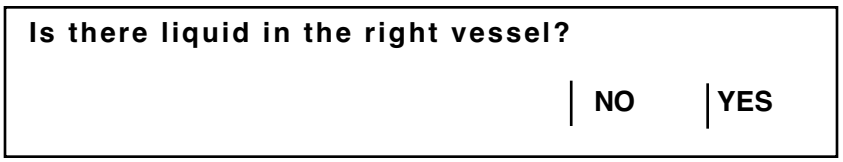

1. Press the ***START*** soft key to begin the flow test. A one-minute pressure test precedes the first reagent delivery.




2. Look at both transfer vessels. You should see liquid in only the left transfer vessel. If you see liquid in the left vessel, press the ***Yes*** soft key.

   If there is no liquid in the left transfer vessel, or if there is liquid in the right transfer vessel, press the ***No*** soft key.

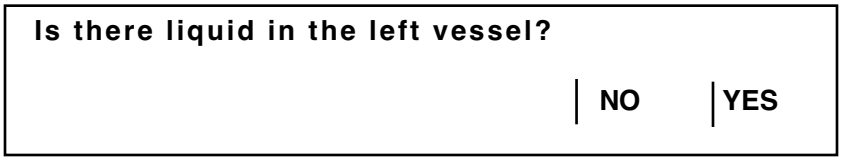
   Watch the liquid as it drains out of the left vessel. At the end of the liquid drain, a few drops may flow back into the vessel.




3. Look at both transfer vessels. You should see liquid in the right transfer vessel, and no more than a drop of liquid in the left transfer vessel. If you see liquid in the right vessel, press the ***Yes*** soft key.

If there is no liquid in the right transfer vessel, press the ***No*** soft key.

Again, watch the liquid as it drains out of the right vessel. At the end of the liquid drain, a few drops may flow back into the vessel.




4. Again, check that there is liquid only in the left vessel, and no more than a drop of liquid in the right vessel. If you see liquid in the left vessel, press the ***Yes*** soft key.

   If there is no liquid in the left transfer vessel, press the ***No*** soft key.


5. When this display appears, press the ***OK*** soft key to continue to the next flow test.

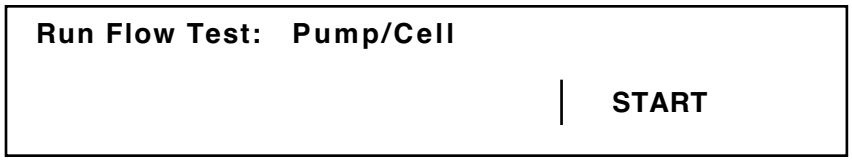
   If you have pressed the ***No*** soft key at any time during this flow test, the LCD displays the message: ***Test failed***. Press the ***OK*** soft key and repeat the flow test.

   If this flow test continues to fail, you may refer to page 4-26 in the Synergy User's Manual or call Applied Biosystems Technical Support.

**Flow Test 7: Pump/Cell**

The "pump" in the name of this flow test refers to the configuration of transfer vessels that assures continuous liquid circulation during synthesis.

During this flow test, two conductivity readings are taken. The first one measures conductivity of DMF delivered to the calibration text fixture-PSC in the peptide synthesis column position. Next, a small amount of HBTU is delivered into the DMF solution and a second reading is taken. The first reading falls within the range of low conductivity readings, the second reading falls within the range of high readings.



1. Press the ***START*** key. A two-minute pressure test precedes the first conductivity reading.


2. Two conductivity measurements are taken during this test, with a two-minute time lapse between each reading. When the conductivity value is within range, the LCD displays the conductivity. Press the ***OK*** key to continue the installation after each display.


3. When this display appears, press the ***OK*** soft key to complete the flow test.


   This message appears when a conductivity value is not within specifications. Press the ***OK*** key to see the value. Press the ***OK*** key again to continue Flow Test 7.

   If the message ***Test failed*** appears, repeat Flow Test 7. If Flow Test 7 continues to fail, call Applied Biosystems Technical Support.

**Flow Test 8: Calibrate HBTU**

| | |
|---|---|
| ***WARNING*** | ***CHEMICAL HAZARD. HBTU can cause allergic reactions and skin irritations in sensitive persons. Wear protective clothing and handle HBTU solution in a properly vented fume hood. See the MSDS in the Synergy User's Manual.*** |

This flow test measures delivery of HBTU (Peptide Synthesis Reagent A) to the left transfer vessel. To perform this flow test, you again tare the left transfer vessel and weigh the contents of the left vessel after each of two reagent deliveries. Then you re-tare the left vessel and weigh one more delivery.

*IMPORTANT* ***Always*** *tare the left vessel when prompted.*

| **Run Flow Test: CALIBRATE HBTU** | |
|---|---|
| | **START** |

1. Press the ***START*** soft key.

| **Remove left vessel and put on scale.** | |
|---|---|
| **Zero scale then replace vessel** | **OK** |

2. Remove the left transfer vessel on the front of Synergy (Figure 18). Place the vessel on a scale that is accurate to ± 1 mg and set the scale to zero.

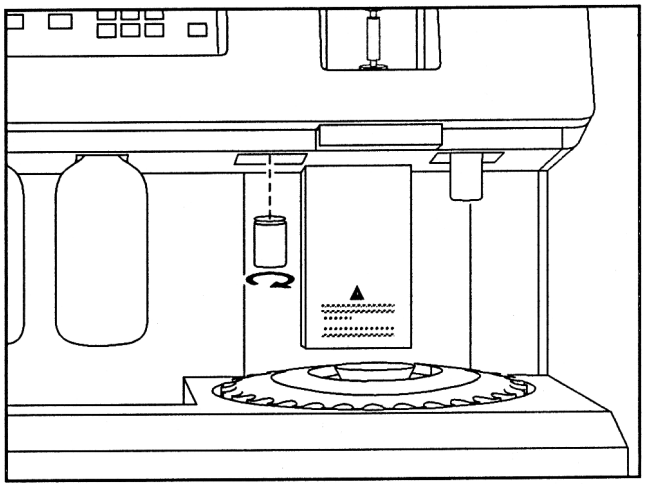


**Figure 21. Remove the left transfer vessel**

3. Replace the transfer vessel and press the ***OK*** soft key.

| **Remove the left vessel and weigh.** | **_ mg** |
|---|---|
| | **OK** |

4. When this display appears, the left transfer vessel contains HBTU. Without spilling the contents, carefully remove the left vessel and weigh

the HBTU delivered. Use the alphanumeric keys to enter this value on the LCD. Press the ***OK*** soft key.


5. Replace the vessel on Synergy. Press the ***OK*** soft key.
6. When prompted, repeat Steps 4 and 5. The second HBTU weight should be greater than the first.


7. Again, remove the left transfer vessel (Figure 18), place it on a scale that is accurate to ±1 mg, and set the scale to zero.
8. When prompted, repeat Steps 4 and 5 a third time. The third weight should be between 340 and 420 mg. Synergy records the time it took to deliver this third amount of HBTU and uses it as the default time for HBTU delivery during synthesis.


9. When this display appears, the flow test has been successfully completed. Press the ***OK*** soft key to continue to the next flow test.

   If you see the message ***Test failed***, press the ***OK*** soft key and repeat the flow test. If this flow test repeatedly fails, call Applied Biosystems Technical Support.

**Flow Test 9: Delivery and Calibration of DIEA**

This flow test measures delivery of DIEA (Peptide Synthesis Reagent B) to the left vessel. As with the previous flow test, you tare the left transfer vessel and then weigh the contents of the left vessel after each reagent delivery.

***WARNING*** **CHEMICAL HAZARD.** ***DIEA (N, N-Diisopropylethylamine) can irritate skin, eyes and mucous membranes. Wear protective clothing and gloves when handling DIEA. See the MSDS in the Synergy User's Manual.***

```
Run Flow Test:    CALIBRATE DIEA

                                  | START
```

1. Press the ***START*** soft key to begin the flow test.

```
Remove the left vessel and put on scale.

Zero scale then replace the vessel.  | OK
```

2. When this display appears, remove the left pump vessel on the front of Synergy (Figure 18). Place the vessel on a scale that is accurate to ±1 mg and set the scale to zero.
3. Replace the left transfer vessel on Synergy. Press the ***OK*** soft key.

```
Remove the left vessel and weigh.    _ mg

                                   | OK
```

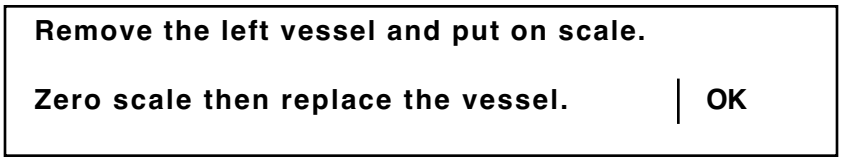
4. When this display appears, the left transfer vessel contains DIEA. Without spilling the contents, carefully remove and weigh the left vessel of DIEA. Use the alphanumeric keys to enter this value on the LCD. Press the ***OK*** soft key.

```
Replace the vessel.

                                  | OK
```

5. Replace the left transfer vessel. Press the ***OK*** soft key.

6. When prompted, repeat steps 4 and 5. The second DIEA weight should be greater than the first.




7. Again, remove the left pump vessel, place it on a scale that is accurate to ±1 mg, and set the scale to zero.
8. When prompted, repeat steps 4 and 5 a third time. The third weight should be between 380 and 460 mg. Synergy records the time it took to deliver this third amount of DIEA and uses it as the default time for DIEA delivery during synthesis.


9. When this display appears, the flow test has been successfully completed. Press the ***OK*** soft key to continue to the Cycle Test.

   If the LCD displays the message ***Test failed***, press the ***OK*** soft key and repeat the flow test. If this flow test repeatedly fails, call Applied Biosystems Technical Support.

## Cycle Test

During the last stage of the Installation Procedure, you load a peptide synthesis column and 3 amino acid columns on the Synergy Peptide Synthesizer. The instrument then performs a pre-determined set of modules and synthesizes LAGV, a 4-mer, test peptide.

| | |
|---|---|
| *IMPORTANT* | *Peptide sequences are written with the N-terminal amino acid on the left and the C-terminal amino acid on the right. On Synergy, however, peptides are synthesized from the C-terminal end towards the N-terminal.*<br>*This means that with LAGV, for example, the C-terminal amino acid, V, is the first amino acid in the sequence and goes in the PSC position. As synthesis progresses, G, A, and L are attached to V, in that order (Figure 23).* |

The printer generates a trace that represents the deprotection and coupling reactions of the synthesis. Compare the trace data from your synthesis with the trace shown in this manual.

```
The next menu will

run the cycle test.          | OK
```

1. Press the ***OK*** soft key to begin the cycle test.

```
Load the PSC-V

      AACs:    1-G    2-A   3-L    START
```

2. Remove the calibration column from the luer fittings at the peptide synthesis column (PSC) position. **Do not unscrew the black plastic bushing attached to the top luer fitting.** Replace the calibration column with the peptide synthesis column labelled "V" (valine). See Figure 22.

| | |
|---|---|
| ***Caution*** | ***Leaks in the peptide synthesis column position can damage the conductivity cell. To assure a leak-proof connection between the peptide synthesis column and the luer fittings, push the luer fittings onto both column ends with a slight twisting motion.*** |

3. Push the top luer fitting into the PSC.

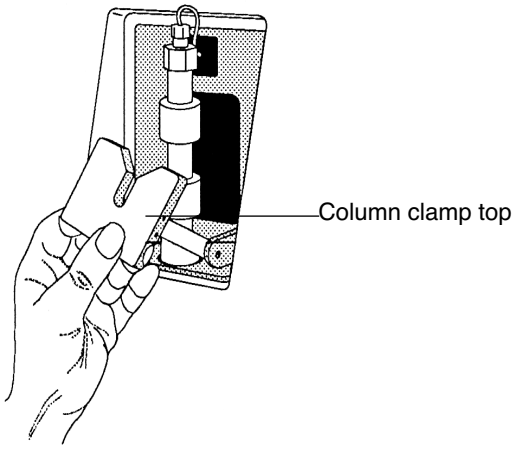



**Figure 22. Pull out the PSC column clamp.**

4. Pull the top of the column clamp out toward you, lift it up until it clears the top luer fitting, and then release it.

| | |
|---|---|
| ***Caution*** | ***Do not attempt to push the column clamp onto the luer fitting without pulling out the clamp top, as this may damage the bottom luer fitting. Do not use the column clamp with the flow test calibration tube.*** |

5. Remove the amino acid column wheel from Synergy and remove the calibration column from position one. Place the amino acid column (ACC) labelled "G" in position 1, place the AAC labelled "A" in position 2, and place the AAC labelled "L" in position 3 (Figure 23).

| | |
|---|---|
| *IMPORTANT* | *AACs and PSCs **cannot** be used interchangeably. PSC labels are white. AAC labels are light blue.* |

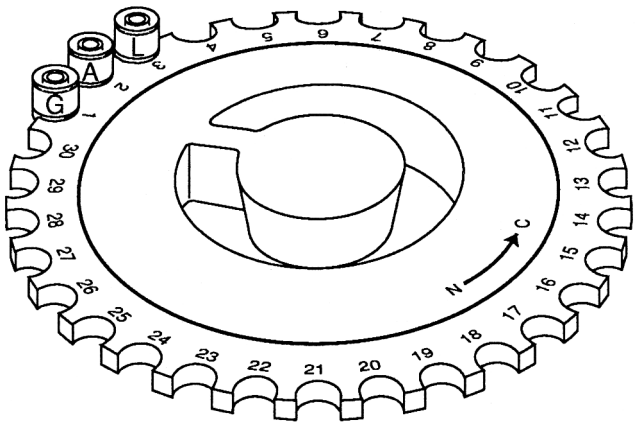



**Figure 23. Load the amino acid columns for the test peptide, LAGV.**

6. Replace the amino acid column wheel on Synergy. Press the ***START*** key to begin the cycle test.

As synthesis progresses, check around the PSC luer fittings and the plastic compression nut at the waste port fitting for leaks. Occasionally, a drop of liquid may begin to form around the middle of the PSC, not at the luer fittings. Wick this moisture away with a tissue, and check frequently during synthesis for any further moisture.

It takes approximately 3 hours to complete this cycle test. During this phase of the installation, pre-programmed modules automatically direct the flow of reagents, monitor pressure, and generate a conductivity trace of the synthesis. You may walk away and leave the instrument unattended during this synthesis.

Run Monitor

| **S -** *xx/ yy ( zz)* | **F -** *F# FUNCT NAME* | **T -** *xxx/zzz* |
|---|---|---|
| **HOLD** \| **PAUSE** | **NEXT S** \| **jump S** | **more** |

Throughout the cycle test, the LCD displays the Run Monitor. The Run Monitor displays the step and function that is currently running.

| **Run ended:** *13:58* | **Run began:** *10:54* |
|---|---|
| **Cycles run: 3** | **OK** |

7. This display appears when the synthesis is completed. Press the ***OK*** soft key.

| | |
|---|---|
| *Note* | *After the synthesis, the AACs still contain white, inert material, but very little amino acid remains. AACs contain only enough amino acid for one synthesis cycle. They cannot be re-used.* |

| | |
|---|---|
| ***WARNING*** | **SOLID HAZARDOUS WASTE** ***Used AACs and PSCs have been exposed to DMF (N, N-dimethyl formamide), THF (tetrahydrofuran) and other hazardous chemicals. Dispose of used columns in accordance with local, state, and federal regulations regarding solid hazardous waste. Read the Synergy Waste Profile in the Synergy User's Manual.*** |

## Examine the Synthesis Trace

| Does the trace look like the example in the manual? | NO | YES |
|---|---|---|

Figure 24 on page 60 shows a typical conductivity trace generated during a synthesis of LAGV. Compare the trace your instrument has generated to Figure 24. The text on page 61 describes the Trace Features of a conductivity trace.

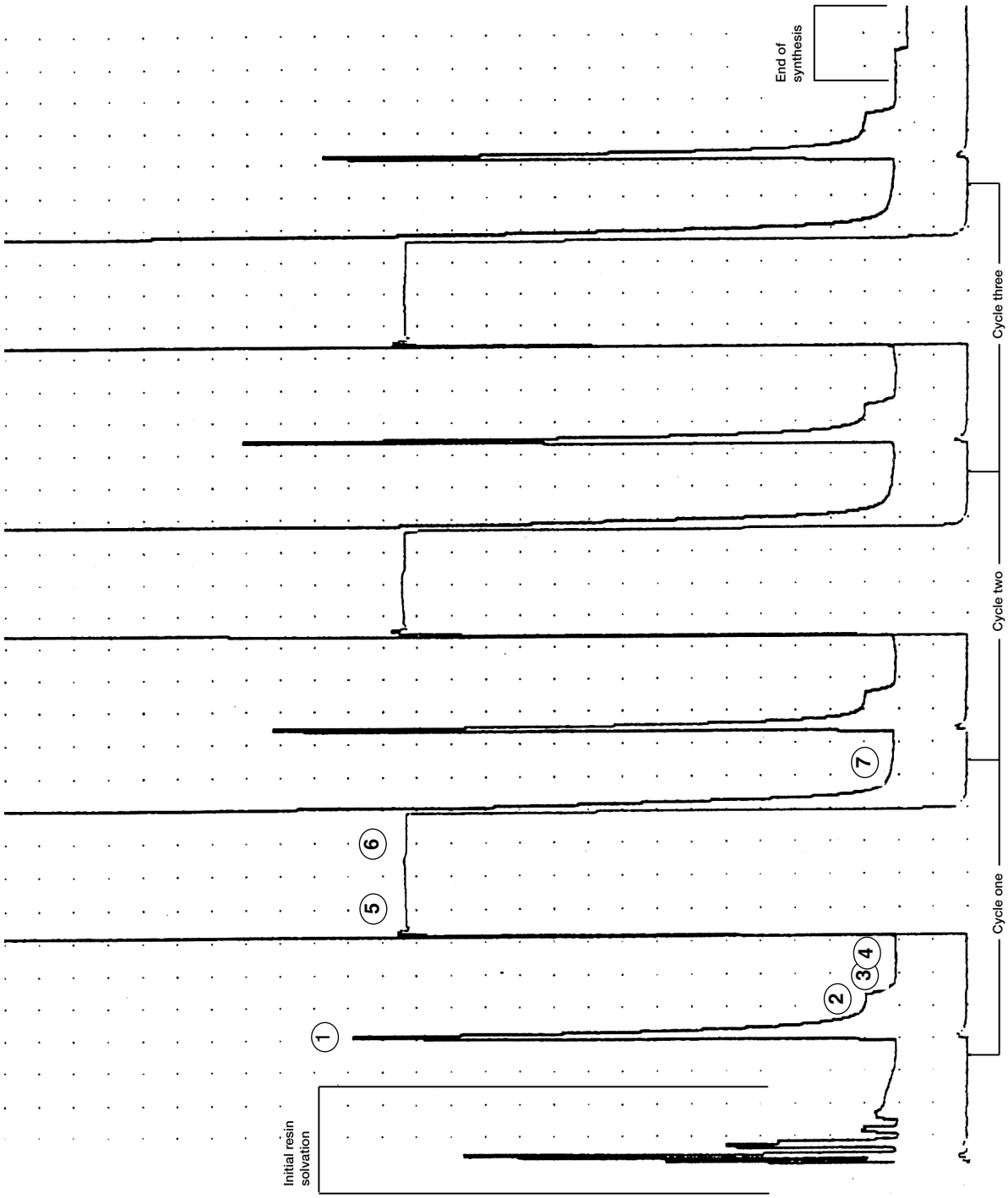



**Figure 24. Seven features of a sample conductivity trace of the synthesis of LAGV on Synergy** (scaled to fit page)

**Conductivity Trace Features**

Note that there are actually two overlapping conductivity traces that reflect two conductivity ranges. Trace Features 1-4 and Trace Feature 7 are in the low conductivity range; Trace Features 5 and 6 are in the high conductivity range.

Typically, at the beginning of every trace, you should observe a series of upward spikes as the resin in the PSC solvates before the DMF baseline is established. Following resin solvation, the trace for the test peptide LAGV should have three deprotection peaks (Trace Feature 1) each followed by a coupling plateau (Trace Features 5 and 6). At the end of the synthesis, the trace should show one more deprotection followed by a drop below the DMF baseline as THF washes through the instrument.

Seven Trace Features on the trace your instrument has generated should closely match the trace in Figure 24 on page 60. Each complete cycle should contain all seven features.

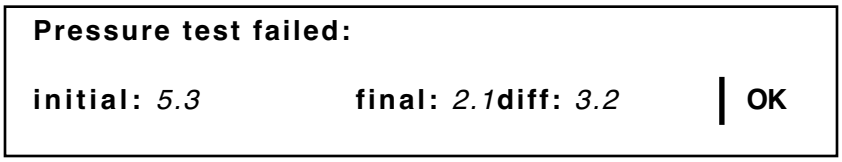
1. **Beginning deprotection:** a single, sharp peak
2. **Deprotection complete:** a level horizontal line slightly higher than the DMF baseline
3. **Piperidine washout:** a return to DMF baseline
4. **Amino acid activation**: a level baseline consistent with point 3
5. **Coupling begins**: a rapid rise followed by a steady, high plateau
6. **Pump/AAC wash:** a level consistent with the plateau at point 5
7. **Coupling solution washout:** a drop that returns to DMF baseline

If the conductivity trace matches the trace shown in Figure 24, press the ***Yes*** soft key to complete the Installation Procedure. When you have completed this Installation Procedure, the printer prints the words “Install finished:” followed by the time and date. Fill out the Warranty card and mail it to Applied Biosystems. Save your installation trace in the front pocket of the Synergy User’s Manual as a record of your successful installation.

If your conductivity trace does not have all seven features for each cycle, call Applied Biosystems Technical Support. Retain your trace and the installation printout for reference, should you have a problem in the future.

## Pressure Test Failures

The Synergy Peptide Synthesizer cannot operate properly with a leak in the pressure system. The pressure system is tested several times during the installation procedure and before every cycle in a synthesis. When a pressure leak is detected, the following sequence of events occurs.




1. When a leak is first detected, the LCD displays a message similar to the one shown here. Press the ***OK*** soft key.


2. This next display features an asterisk (*) after the word ***PAUSE***, which means Synergy has automatically suspended operation.

   The information in the top line identifies the **Step and Function** where the pressure failure occurred. When a pressure failure occurs, this line usually displays Function 89 (-PRS TEST).

   You cannot continue the installation until the leak is eliminated. You may call Applied Biosystems Technical Support for assistance.

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**Headquarters**
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free: +1 800.345.5224
Fax: +1 650.638.5884

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