# *User Bulletin No. 35*

## *Model 431A Peptide Synthesizer*

November 1993 (updated 07/2002)

## SUBJECT: *FastMoc*™ 0.25 and 0.10 on the Model 431A

HBTU [2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate] was first described as a coupling reagent in peptide synthesis in 1984 by Dourtoglou et al[1]. HBTU did not have wide spread use, however, until 1989 when Knorr et al published the use of HBTU and other uronium salts in peptide synthesis[2,3]. In 1990, Applied Biosystems introduced its *FastMoc*™ chemistry, which uses HBTU activation in an Fmoc solid phase peptide synthesis in its automated peptide synthesizers[4,5]. These cycles were described for the Model 431A Peptide Synthesizer in User Bulletin 33 and later included in Model 431A Users Manual, version 2.00.

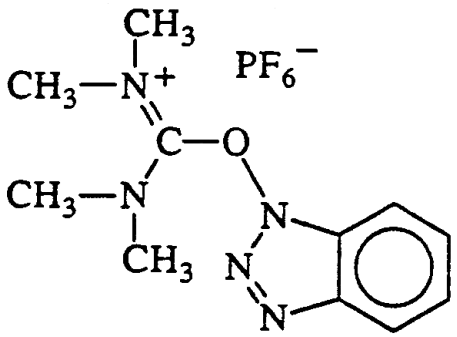


**Figure 1.** HBTU

This User Bulletin describes a new version of the *FastMoc* cycles for the Model 431A. Some of the changes are:

- The two scales, 0.10 and 0.25 mmol, are identical except for the times in some of the steps. This makes it easier to change between scales if the model 431A is not equipped with SynthAssist™ software.
- The new cycles use 2 M DIEA instead of 100% DIEA. We have found that a more reproducible delivery of DIEA is obtained when the entire 0.5 mL measuring loop is used to deliver 2.0 M DIEA. This approach has been used with *FastMoc* cycles on the Model 430A for two years and is currently being used on the Model 433A.
- The delivery of the 0.45 M HBTU is reduced by 1 second. The reason for the change is that a recent report has shown that, following Fmoc removal, the newly exposed $NH_2$ groups can react with HBTU resulting in a guanidinium-like adduct; the latter can terminate synthesis[6]. We are avoiding this side reaction by adding only 0.9 mmol HBTU to the 1 mmol Fmoc amino acid and adding the DIEA to the

cartridge with the HBTU and Fmoc-amino acid to initiate the activation before transferring the activated amino acid to the reaction vessel.

- The loading and capping procedure has been simplified to using modules HF instead of using modules hefffghef.
- The only two places where dichloromethane is used is in the loading procedure and in the final DCM wash.
- The N-methylpyrrolidone used in washing the amino acid cartridge is saved in the activator and used to wash the resin after coupling. This reduces the solvent waste.
- An optional acetic anhydride capping procedure is described which can be used after every coupling cycle.

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To distinguish between the old and new *FastMoc* cycles, the new 0.25 mmol cycles will be called *FastMoc* 0.25 instead of Std Scale *FastMoc* and the new 0.10 mmol cycles will be called *FastMoc* 0.10 instead of Small Scale *FastMoc*. A comparison of the old and new *FastMoc* cycle is shown in Table 1. These new *FastMoc* 0.25 and *FastMoc* 0.10 cycles are identifical to the unmonitored *FastMoc* cycles on the Model 433A Peptide Synthesizer.

**Table 1.** Comparison of FastMoc Cycles

| | **Std Scale *FastMoc* (old)** | ***FastMoc* 0.25 (new)** |
|---|---|---|
| **Operation and Reagents** | Time (min) | Time (min) |
| Piperdine Deprotection | 13 | 15 |
| NMP Washes | 6 | 5 |
| Coupling | 30 | 21 |
| NMP Washes | 6 | 4 |
| Resin sample | (2) | (2) |
| Total Time (no RS) | 55 | 45 |
| **Resin** | 0.25 mmol | 0.25 mmol |
| **Fmoc Amino Acid** | 1.00 mmol | 1.00 mmol |
| **DIEA concentration** | 100% | 2 M |
| **Reaction Vessel** | Standard | Standard |
| **Waste per Cycle (no RS)** | 160 mL | 100 mL |

| | **Small Scale *FastMoc* (old)** | ***FastMoc* 0.10 (new)** |
|---|---|---|
| **Operation and Reagents** | Time (min) | Time (min) |
| Piperdine Deprotection | 7 | 10 |
| NMP Washes | 2 | 3 |
| Coupling | 9 | 9 |
| NMP Washes | 2 | 2 |
| Resin sample | (6) | (2) |
| Total Time (no RS) | 20 | 24 |
| **Resin** | 0.10 mmol | 0.10 mmol |
| **Fmoc Amino Acid** | 1.00 mmol | 1.00 mmol |
| **DIEA concentration** | 100% | 2 M |
| **Reaction Vessel** | Small | Small |
| **Waste per Cycle (no RS)** | 50 mL | 50 mL |

## Reagent and Bottle Positions

As Table 2 illustrates, the reagent changes from previous *FastMoc* descriptions are with bottle #7 and with bottle #4 (if the capping solution is used).

**Table 2.** Bottle Positions

| Bottle # | Reagent | Part Number |
|---|---|---|
| 1 | Piperdine | 400629 |
| 2 | ------- | |
| 4 | 0.50 M acetic anhydride,<br>0.125 M DIEA and<br>0.015 M HOBt in NMP | 400660, 400136<br>400662 & 400580 |
| | or | |
| | 0.1 M DMAP | 400631 |
| 5 | 0.45 M HBTU/HOBt in DMF | 401132 |
| 6 | Methanol | 400470 |
| 7 | 2.0 M DIEA in NMP | 401517 or<br>400136 & 400580 |
| 8 | 1.0 M DCC in NMP | 400663 |
| 9 | Dichloromethane | 400142 |
| 10 | N-Methylpyrrolidine | 400580 |

## Preparation of Special Reagents

***WARNING*** *POTENTIAL CHEMICAL HAZARDS. Some chemicals used on the Model 431A are hazardous and can cause injury. Please familiarize yourself with the information provided in the MSDSs in the Safety Supplement of the Model 431A Manual. When preparing the following chemicals, always wear gloves, protective clothing and eye protection and work in a fume hood.*

### Bottle 7: 2.0 M DIEA

The 2.0 M DIEA can be purchased (part no. 401517) or prepared according to the following directions:

1. Pour 69 mL DIEA (part no. 400136) into a clean, dry, 250 mL graduated cylinder.
2. Add NMP (part no. 400580) to a volume of 200 mL.
3. Pour this solution into a clean, dry, 200 mL bottle for position 7.
4. Place the gasket on the bottle and screw the bottle into the ratchet cap at position 7.
5. Run Flow Test 7 to observe the flow of the solution through the measuring loop.

### Bottle 4: 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP.

To make 400mL of this capping solution, combine the following:

1. Into a clean, dry, 100 mL graduated cylinder, add 19 mL acetic anhydride (part no. 400660), 9 mL of DIEA (part no. 400136) and 6 mL of 1 M HOBt/NMP (part no. 400662). (Alternatively you may add 0.8 g solid HOBt)
2. Add NMP to a volume of 100 mL.
3. Pour this solution into a clean, dry, 500 mL bottle and add another 300 mL of NMP. Mix the solution.
4. Place the gasket on the bottle and screw the bottle into the ratchet cap at position 4.
5. Run Flow Test 4. The value should be approximately 1.7 mL.

The capping solution will turn slightly yellow after a couple of weeks. This is due to the HOBt. While the effectiveness of the capping solution has not been reduced, it is a good practice to make a fresh solution at least once every two weeks.

If you are loading HMP resin, bottle position 4 will have the 0.1 M DMAP/DMF solution attached. In this case, you will need to pause the synthesis after the loading is complete, remove the DMAP solution, wipe the tube with a tissue, add the capping solution to bottle position 4, and flush the #4 line using Fxn 17 (#4 B VB), followed by Fxn 10 (GAS B VB), Fxn 14 (#10 B VB) and Fxn 10 (GAS B VB).

Before the next synthesis is started, you will want to thoroughly clean the #4 bottle position if you are going to use DMAP for loading. Remove the acetic anhydride solution, wipe the line with a tissue and place a bottle containing about 20 mL NMP on bottle position #4. Empty the bottle by activating Fxn 17 (#4 B VB). Remove the bottle, wipe the line again and add the bottle of DMAP. Run a flow test #4 for the DMAP solution. The value for 0.1 M DMAP/DMF is approximately 2.2 mL.

### Bottle 5: 0.45 M HBTU

HBTU is purchased as part of the HBTU activation kit (part no. 401132). The activation kit contains a bottle of solid HBTU (100 mmol), a bottle of 0.5 M HOBt in DMF and two HBTU delivery line filters. Store the reagents in the HBTU activation kit at 0-4° C until you are ready to use them. Mix the reagents together just prior to use.

To prepare 0.45 M HBTU/HOBt in DMF

1. Pour 200 mL of 0.5M 1-hydroxybenzotriazole in N,N-dimethylformamide into the 450 mL bottle containing 100 mmol dry HBTU.
2. Dissolve the HBTU with gentle swirling. The increased volume due to the HBTU reduces the concentration of the two species from 0.50 M to 0.45 M.
3. Locate the Bottle 5 delivery line and thoroughly dry it with a lint-free tissue.
4. Firmly press the polyethylene delivery line filter onto the end of the Bottle 5 delivery line. Screw Bottle 5 into the ratchet cap on the peptide synthesizer.

The 0.45 M HBTU/HOBt/DMF solution is stable at room temperature for at least 6 weeks, as determined by use testing. After a few days, the solution turns yellow. This color change does not have any adverse affect on the efficiency of the reagent.

## Flow Tests

Flow Test 10 is used to set the lower regulator. Flow Test 1, 7 and 11 should also be run. If you are using unloaded HMP resin, you should also run Flow Test 4 and 8.

The delivery of 0.45 M HBTU is determined by using Flow Test 13 if you have software version 2.00, or by writing your own flow test module if you have software version 1.12.

When using Test 13 in **software version 2.00**:

If Flow Test 13 is 2.3 - 2.5 g, then use 7 sec for Fxn 94 in Module A.

If Flow Test 13 is 2.1 - 2.3 g, then use 8 sec for Fxn 94 in Module A.

If Flow Test 13 is 1.9 - 2.1 g, then use 9 sec for Fxn 94 in Module A.

When using **software version 1.12**, write and test the following module and determine the time for Fxn 94 in module A the same way as was done with Flow Test 13:

| Step | Fxn# | Function Name | Time | Add | |
|---|---|---|---|---|---|
| 1 | 5 | NEEDLE DWN | 10 | 0 | |
| 2 | 10 | GAS B VB | 1 | 0 | |
| 3 | 78 | PRS #M | 10 | 0 | |
| 4 | 94 | USER FXN A | 9 | 0 | User Fxn A has valves 12, 15, 27 & 30 activated. |
| 5 | 60 | MIX CART | 5 | 0 | |
| 6 | 61 | VENT CART | 3 | 0 | |
| 7 | 6 | NEEDLE UP | 10 | 0 | |
| 8 | 7 | EJECT CART | 10 | 0 | |
| 9 | 8 | ADVAN CART | 10 | 0 | |
| 10 | 10 | GAS B VB | 10 | 0 | |

## Loading and Benzoic Anhydride Capping

If you are starting with HMP resin, you must first load the C-terminal amino acid on the resin. This is accomplished using DCC activation with 0.1 eq of DMAP as the catalyst. Because this loading is not a 100% complete, the unreacted hydroxyl groups need to be capped. This capping is traditionally done with benzoic anhydride using DMAP as the catalyst. The hydroxy groups need to be capped since HBTU activated Fmoc-amino acids are able to react with the hydroxy groups on the resin (5).

The loading and capping is accomplished by using modules HF. One Fmoc amino acid cartridge is used followed by a cartridge containing approximately 3 mmol of benzoic anhydride (0.60-0.70g).

We strongly suggest you use benzoic anhydride to cap after loading. If you choose not to cap, then you should use the hef modules (for 0.25 mmol) or hefff modules (for 0.10 mmol). These cycles were written without benzoic anhydride capping. The reason capping was not included in these loading cycles is that these cycles were written to

be used with DCC/HOBt activation of the Fmoc amino acids, and HOBt activated amino acids do not react as readily with hydroxy groups as do HBTU activated Fmoc-amino acids.

## Capping After Each Coupling Cycle

Another capping procedure that is often done in SPPS is the capping of unreacted amines after each coupling cycle. This capping is traditionally done using a solution of acetic anhydride. **On peptides where the coupling efficiencies are 99% or better, capping is not necessary.** However, on difficult peptides, capping may be needed if single or double couplings are not successful. An example of a difficult peptide where capping is helpful is HIV Protease (85-99), Ab 95 (ab is aminobutyric acid). Table 3 shows the coupling efficiencies of a synthesis with no capping and a synthesis with capping. The capping solution is 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP. The coupling efficiency is determined by the ninhydrin analysis[7].

**Table 3.** Percent Coupling of HIV Protease (85-99), Ab 95 with and without Capping

| | | Synthesis with no capping | Synthesis with capping | |
|---|---|---|---|---|
| Cycle | Amino acid | Percent Coupling | Percent Coupling | Percent Coupling and Capping |
| Phe resin | | | | |
| 1 | Asn(Trt) | 99.8 | 99.7 | 99.7 |
| 2 | Leu | 99.5 | 99.5 | 99.5 |
| 3 | Thr(tBu) | 99.6 | 99.5 | 99.5 |
| 4 | Ab | 99.6 | 99.5 | 99.5 |
| 5 | Gly | 99.6 | 99.7 | 99.7 |
| 6 | Ile | 99.4 | 99.5 | 99.5 |
| 7 | Gln(Trt) | 99.6 | 99.7 | 99.7 |
| 8 | Thr(tBu) | 99.5 | 99.6 | 99.6 |
| 9 | Leu | 99.7 | 99.7 | 99.7 |
| 10 | Leu | 99.6 | 99.6 | 99.7 |
| 11 | Asn(Trt) | 91.8 | 93.4 | 99.6 |
| 12 | Arg(Pmc) | 87.4 | 91.1 | 99.6 |
| 13 | Gly | 94.9 | 98.8 | 99.6 |
| 14 | Ile | 90.6 | 98.7 | 99.6 |

In the synthesis without capping, it appears that cycles 11 to 14 have very poor couplings, but when the synthesis is done with capping, only cycles 11 and 12 show poor couplings. Evidently, cycle 13 and 14 are not difficult, but only appear difficult if the unreacted peptide from cycle 11 and 12 is not capped.

## Advantages of Version 2.00 Software

The *Fastmoc* cycles are much simpler to use if your Model 431A has the 2.00 version software. Your Model 431A has this software if it has serial number 9109416 or above or if you have purchased the SynthAssist Software Package. If you buy the version 2.00 software separately, you also need to purchase the version 2.00 Users Manual.

One reason to have version 2.00 software is for convenience. We have included Fxn 94 (#5 TO CART) and Fxn 96 (CRT TO RVc) in the 2.00 version software. If you have the 1.12 software you need to write these as User Functions. Another convenience is that the Flow Test for the HBTU delivery to the cartridge is included in version 2.00 as Flow Test 13.

## Module Descriptions for 0.10 mmol cycles

**Module A - Dissolving Amino Acid** **Total time = 7.6 minutes**

At the beginning of module A, the amino acid cartridge name is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1g) and 0.9 mmol of 0.45 M HBTU/HOBt in DMF (2.0g) are added to the cartridge. The amino acid is dissolved by mixing for 6 minutes.

**Module B - Piperdine Deprotection** **Total time = 2.9 minutes**

This module begins with one NMP wash of the resin. A 20% piperdine/NMP solution is introduced and allowed to deprotect for 1 minute. The RV is drained and a second treatment with 20% piperidine is performed. The valve blocks are then rinsed thoroughly. The resin will continue to deprotect for an additional 7.6 minutes during module A.

**Module C - Acetic Anhydride Capping** **Total time = 5.9 minutes**

About 5 mL of the 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP solution is added to the resin and vortexed for 5 min.

**Module D - NMP Washes** **Total time = 2.5 minutes**

The RV is drained and the resin is washed 4 times with NMP. During each NMP wash, the resin sample line is rinsed with NMP. There is an add time of 1 in the loop function, so after cycle 4, the number of washes is 5, and after cycle 14, the number of washes is 6, and so forth.

**Module E - Add 2.0 M DIEA and Transfer to RV** **Total time = 2.1 minutes**

At the beginning of the module, 1 mL of 2 M DIEA in NMP is added to the cartridge, to initiate the activation of the amino acid. The activated amino acid is then transferred from the cartridge to the RV.

**Module F - Clean cartridge, Couple, Drain and NMP Washes** **Total time = 9.3 minutes**

During this module, the amino acid cartridge is washed 2 times with NMP. This NMP is transferred to the Activator Vessel and used later in the module to wash the resin in the RV. Coupling is occurring during this cartridge washing, and the coupling is then

continued for another 4.5 minutes. The RV is drained and the resin is washed with the NMP from the Activator Vessel.

### Module G - Resin Sample — Total time = 1.4 minutes

This module takes a resin sample. Place a blank tube between each resin sample tube. The tarred resin sample test tube should contain MeOH and a few drops of acetic acid.

### Module H - Loading and Benzoic Anydride Capping — Total time = 51 minutes

This module is used to load the first amino acid onto the HMP resin. The 1.00 mmol Fmoc amino acid is dissolved in an NMP/DCM mixture and transferred to the RV. This is followed with 1 mL of 1.0 M DCC/NMP and 0.1 equivalent of DMAP. The resin is mixed for 30 min. The RV is drained and the resin is washed 2 times with 50% MeOH and 50% DCM and 5 times with DCM. The resin is then capped with approximately 3 mmol of benzoic anydride (0.60-0.70g) which has been placed in one cartridge.

The loading and capping is done with modules HF. It requires 1 Fmoc-amino acid cartridge and 1 cartridge containing approximately 3 mmol of benzoic anhydride (0.60-0.70g).

Module H is modified for Arg, Asn, Gln and His the following way:

| | **Fmoc-His(Bum)** | **Fmoc-Arg(Mtr)**<br>**Fmoc-Arg(Pmc)** | **Fmoc-Gln(Trt)**<br>**Fmoc-Asn(Trt)** |
|---|---|---|---|
| Step 14 (#9 CART) | 0 sec | 0 sec | 4 sec |
| Step 15 (#10 CART) | 8 sec | 7 sec | 4 sec |

*IMPORTANT  Do not load with Fmoc-Asn or Fmoc-Gln.*

### Module I - 10 Minute Wait — Total time = 10 minutes

Module I is used in the last cycle to extend the total deprotection time to 12 minutes.

## Module Descriptions for 0.25 mmol cycles

### Module A - Dissolving Amino Acid — Total time = 7.6 minutes

At the beginning of module A, the amino acid cartridge name is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1g) and 0.9 mmol of 0.45 M HBTU/HOBt in DMF (2.0g) are added to the cartridge. The amino acid is dissolved by mixing for 6 minutes.

### Module B - Piperdine Deprotection — Total time = 8.8 minutes

This module begins with one NMP wash of the resin. A 18% piperdine/NMP solution is introduced and allowed to deprotect for 3 minutes. The RV is drained and a second treatment with 20% piperidine is performed. The valve blocks are then rinsed thoroughly. The resin will continue to deprotect for an additional 7.6 minutes during module A.

### Module C - Acetic Anhydride Capping — Total time = 6.4 minutes

About 12 mL of the 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP solution, is added to the resin and vortexed for 5 min.

### Module D - NMP Washes — Total time = 4.6 minutes

The RV is drained and the resin is washed 5 times with NMP. During each NMP wash, the resin sample line is rinsed with NMP. There is an add time of 1 in the loop function, so after cycle 4, the number of washes is 6, and after cycle 14, the number of washes is 7, and so forth.

### Module E - Add 2.0 M DIEA and Transfer to RV — Total time = 2.2 minutes

At the beginning of the module, 1 mL of 2 M DIEA in NMP is added to the cartridge, to initiate the activation of the amino acid. The activated amino acid is then transferred from the cartridge to the RV.

### Module F - Clean cartridge, Couple, Drain and NMP Washes — Total time = 22.2 minutes

During this module, the amino acid cartridge is washed 3 times with NMP. This NMP is transferred to the Activator Vessel and used later in the module to wash the resin in the RV. Coupling is occurring during this cartridge washing, and the coupling is then continued for another 15 minutes. The RV is drained and the resin is washed with the NMP from the Activator Vessel.

### Module G - Resin Sample — Total time = 1.7 minutes

This module takes a resin sample. Place a blank tube between each resin sample tube. The tarred resin sample test tube should contain MeOH and a few drops of acetic acid.

### Module H - Loading and Benzoic Anydride Capping — Total time = 54 minutes

This module is used to load the first amino acid onto the HMP resin. The 1.00 mmol Fmoc amino acid is dissolved in an NMP/DCM mixture and transferred to the RV. This is followed with 1 mL of 1.0 M DCC/NMP and 1.0 equivalent of DMAP. The resin is mixed for 30 min. The RV is drained and the resin is washed 2 times with 50% MeOH

and 50% DCM and 5 times with DCM. The resin is then capped with approximately 3 mmol of benzoic anydride (0.60-0.70g) which has been placed in one cartridge.

The loading and capping is done with modules HF. It requires 1 Fmoc-amino acid cartridge and 1 cartridge containing approximately 3 mmol of benzoic anhydride (0.6.-0.70g).

Module H is modified for Arg, Asn, Gln and His the following way:

| | **Fmoc-His(Bum)** | **Fmoc-Arg(Mtr)**<br>**Fmoc-Arg(Pmc)** | **Fmoc-Gln(Trt)**<br>**Fmoc-Asn(Trt)** |
|---|---|---|---|
| Step 14 (#9 CART) | 0 sec | 0 sec | 4 sec |
| Step 15 (#10 CART) | 8 sec | 7 sec | 4 sec |

*IMPORTANT Do not load with Fmoc-Asn or Fmoc-Gln.*

### Module I - 10 Minute Wait — Total time = 10 minutes

Module I is used in the last cycle to extend the total deprotection time to 17 minutes.

| Description | Modules | Comments |
|---|---|---|
| Single coupling with no resin samples | BADEF | Requires 1 amino acid cartridge |
| Single coupling with resin samples | BADEFG | Requires 1 amino acid cartridge |
| Single coupling with $Ac_2O$ capping and no resin samples | BADEFCD | Requires 1 amino acid cartridge |
| Single coupling with $Ac_2O$ capping with resin samples after coupling | BADEFGCD | Requires 1 amino acid cartridge |
| Double coupling with no resin samples | BADEIADEF | Requires 2 amino acid cartridges |
| Double coupling with resin samples after second coupling | BADEIADEFG | Requires 2 amino acid cartridges |
| Double coupling with resin samples after the first and second couplings | BADEIADGEFG | Requires 2 amino acid cartridges |
| Double coupling with $Ac_2O$ capping and no resin samples | BADEIADEFCD | Requires 2 amino acid cartridges |
| Double coupling with $Ac_2O$ capping with resin samples after both couplings | BADEIADGEFGCD | Requires 2 amino acid cartridges |
| Final Deprotection | BIDc | |
| Loading and Capping | HF | Requires 1 amino acid cartridge and another cartridge containing approximately 3 mmol of benzoic anydride (0.6-0.7g) |

## Examples for Run Editor

### Example 1: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on HMP resin, with removal of the final Fmoc group and resin samples, the Run Editor will be:

Cy: 1 Rpt: 1 M: HF
Cy: 2 Rpt: 9 M: BADEFG
Cy: 11 Rpt: 1 M: BIDc

### Example 2: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on HMP resin, with removal of the final Fmoc group and no resin samples, the Run Editor will be:

Cy: 1 Rpt: 1 M: HF
Cy: 2 Rpt: 9 M: BADEF
Cy: 11 Rpt: 1 M: BIDc

### Example 3: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on Fmoc-Leu resin with removal of the final Fmoc group and no resin samples, the Run Editor will be:

Cy: 1 Rpt: 9 M: BADEF
Cy: 10 Rpt: 1 M: BIDc

### Example 4: Angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

To synthesize Angiotensin on Fmoc-Leu resin, leaving the final Fmoc group on the peptide and taking resin samples, the Run Editor will be:

Cy: 1 Rpt: 9 M: BADEFG
Cy: 10 Rpt: 1 M: Dc

### Example 5: Substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$

To synthesize Substance P on Rink amide resin with removal of the final Fmoc group and resin samples, the Run Editor will be:

Cy: 1 Rpt: 11 M: BADEFG
Cy: 12 Rpt: 1 M: BIDc

# Chemical Usage

Tables 4 and 5 summarize the chemical usage of the 0.10 and 0.25 mmol *FastMoc* cycles for an instrument with average flow rates. The data is for single couples, with no resin samples and no acetic anhydride capping.

**Table 4.** Chemical Usage for 0.10 mmol Synthesis

| Bottle Position | Chemical | Bottle Capacity | mL per Cycle cycle 1 | mL per Cycle cycle 10 | mL per Cycle cycle 20 | mL per Cycle cycle 30 | Cycles per Bottle* |
|---|---|---|---|---|---|---|---|
| 1 | Piperidine | 200 | 2.0 | 2.0 | 2.0 | 2.0 | 100 |
| 5 | HBTU/HOBt | 230 | 2.0 | 2.0 | 2.0 | 2.0 | 110 |
| 7 | 2 M DIEA | 200 | 1 | 1 | 1 | 1 | 200 |
| 10 | NMP | 4000** | 45 | 53 | 61 | 69 | 60 |
| Waste | ___ | 9463 | 50 | 58 | 66 | 74 | 120 |

**Table 5.** Chemical Usage for 0.25 mmol Synthesis

| Bottle Position | Chemical | Bottle Capacity | mL per Cycle cycle 1 | mL per Cycle cycle 10 | mL per Cycle cycle 20 | mL per Cycle cycle 30 | Cycles per Bottle* |
|---|---|---|---|---|---|---|---|
| 1 | Piperidine | 200 | 5.0 | 5.5 | 6.0 | 6.5 | 35 |
| 5 | HBTU/HOBt | 230 | 2.0 | 2.0 | 2.0 | 2.0 | 110 |
| 7 | 2 M DIEA | 200 | 1 | 1 | 1 | 1 | 200 |
| 10 | NMP | 4000** | 92 | 107 | 121 | 136 | 30 |
| Waste | ___ | 9463 | 100 | 115 | 130 | 145 | 65 |

Note DMAP, DCC and MeOH are used only during the loading cycle. No DCM is used in the coupling cycles. DCM is used only in the loading cycle (Module H) and in the final resin wash (Module C).

* The value for the cycles per bottle is the number of cycles that can be done with the first bottle. The cycles per bottle value is based on a slightly higher flow rate than the mL per cycle values, to ensure that the bottles will not go empty. On a longer synthesis, the second bottle of piperdine and NMP will not last as many cycles because of the add times.

** If the two-bottle configuration of NMP is used, the number of unattended cycles is doubled.

## References


1. Dourtoglou, V., B. Gross, V. Lambropoulou, and C. Zioudrou. 1984. *O*-benzotriazolyl-*N,N,N′,N′*-tetramethyluronium hexaflourophosphate as coupling reagent for the synthesis of peptides of biological interest. Synthesis: 572-574.

2. Knorr, R., A. Trzeciak, W. Bannwarth, and D. Gillessen. 1989. New coupling reagents in peptide chemistry, p. 37-39. *In* Jung, G. and Bayer, E. (Eds.), Peptides 1988. Walter de Gruyter & Company, Berlin.

3. Knorr, R., A. Trzeciak, W. Bannwarth and D. Gillessen. 1989. New coupling reagents in peptide chemistry. Tetrahedron Lett. *30*: 1927-1930.

4. Fields, C.G., D.H. Lloyd, R.L. Macdonald, K.M. Otteson, and R.L. Noble. 1991. Application and HBTU activation for automatic Fmoc solid-phase peptide synthesis. p. 65-66. *In* Giralt, E. and Andreu, D. (Eds.), Peptides 1990. ESCOM, Leiden.

5. Fields, C.G., D.H. Lloyd, R.L. Macdonald, K.M. Otteson, and R.L. Noble. 1991. HBTU activation for automatic Fmoc solid-phase peptide synthesis. Peptide Res. 4:95-101.

6. Gausepohl, H., U. Pieles, and R.W. Frank. 1992. Schiff base analog formation during in situ activation by HBTU and TBTU, p. 523-524. *In:* J.A. Smith and J.E. Rivier (Eds.), Proceedings of the Twelvth American Peptide Symposium. ESCOM, Leiden.

7. Sarin, V.K., S.B.H. Kent, J.P. Tam, and R.B. Merrifield. 1981. Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction. Analytical Biochemistry *117*: 145-157.

## *FastMoc* 0.1 and 0.25 Modules

The modules are written so that only the times need to be changed when switching between 0.10 and 0.25 mmol. The times that need to be changed are in bold type. The Model 431A should be set in Standard Scale Fmoc. This is so Module c in the Std Fmoc cycles will be used with the 0.1 mmol and 0.25 mmol cycles.

**Module A** **Dissolving Amino Acid**

| | | | 0.10 mmol | | 0.25 mmol | |
|---|---|---|---|---|---|---|
| Step | Fxn# | Function Name | Time | Add | Time | Add |
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 4 | PRINT CART | 10 | 0 | 10 | 0 |
| 3 | 6 | NEEDLE UP | 10 | 0 | 10 | 0 |
| 4 | 7 | EJECT CART | 10 | 0 | 10 | 0 |
| 5 | 8 | ADVAN CART | 10 | 0 | 10 | 0 |
| 6 | 5 | NEEDLE DWN | 10 | 0 | 10 | 0 |
| 7 | 14 | #10 B VB | 1 | 0 | 1 | 0 |
| 8 | 9 | GAS T VB | 2 | 0 | 2 | 0 |
| 9 | 65 | #10 CART | 5 | 0 | 5 | 0 |
| 10 | 60 | MIX CART | 5 | 0 | 5 | 0 |
| 11 | 78 | PRS #M | 10 | 0 | 10 | 0 |
| 12 | 18 | #5 B VB | 1 | 0 | 1 | 0 |
| 13 | 94* | #5 TO CART | 8** | 0 | 8** | 0 |
| 14 | 98 | BEGIN LOOP | 6 | 0 | 6 | 0 |
| 15 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 16 | 60 | MIX CART | 30 | 0 | 30 | 0 |
| 17 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 18 | 60 | MIX CART | 30 | 0 | 30 | 0 |
| 19 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 20 | 13 | #10 T VB | 1 | 0 | 1 | 0 |
| 21 | 14 | #10 B VB | 3 | 0 | 3 | 0 |
| 22 | 9 | GAS T VB | 5 | 0 | 5 | 0 |
| 23 | 10 | GAS B VB | 5 | 0 | 5 | 0 |

* When using software version 1.12, Fxn 94 is User Function A which has valves 12, 15, 27 and 30 activated.

** When using software version 2.00, the time is based on Flow Test 13. The value is 1 sec less than described in the version 2.00 Users Manual.

When using software version 1.12, the time is based on Module H.

In both cases, the objective is to deliver 1.9 - 2.1 g of the 0.45 M HBTU solution.

**Module B** **Piperdine Deprotection**

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 58 | INTERRUPT | 0 | 0 | 0 | 0 |
| 3 | 56 | #10 B RV | 3 | 1 | 13 | 1 |
| 4 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 5 | 91 | #10 TO RS | 4 | 0 | 4 | 0 |
| 6 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 7 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 8 | 2 | VORTEX ON | 3 | 0 | 3 | 0 |
| 9 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 10 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 11 | 42 | DRAIN RV | 8 | 1 | 15 | 1 |
| 12 | 56 | #10 B RV | 3 | 0 | 11 | 2 |
| 13 | 79 | PRS #1 | 10 | 0 | 10 | 0 |
| 14 | 51 | #1 B RV | 5 | 0 | 8 | 1 |
| 15 | 56 | #10 B RV | 4 | 0 | 4 | 0 |
| 16 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 17 | 98 | BEGIN LOOP | 2 | 0 | 6 | 0 |
| 18 | 2 | VORTEX ON | 15 | 0 | 15 | 0 |
| 19 | 3 | VORTEX OFF | 15 | 0 | 15 | 0 |
| 20 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 21 | 42 | DRAIN RV | 10 | 1 | 18 | 1 |
| 22 | 56 | #10 B RV | 3 | 0 | 12 | 2 |
| 23 | 79 | PRS #1 | 5 | 0 | 5 | 0 |
| 24 | 51 | #1 B RV | 5 | 0 | 10 | 1 |
| 25 | 56 | #10 B RV | 4 | 0 | 4 | 0 |
| 26 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 27 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 28 | 93 | GAS TO RS | 3 | 0 | 3 | 0 |
| 29 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 30 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 31 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 32 | 98 | BEGIN LOOP | 2 | 0 | 2 | 0 |
| 33 | 13 | #10 T VB | 1 | 0 | 1 | 0 |
| 34 | 14 | #10 B VB | 3 | 0 | 3 | 0 |
| 35 | 9 | GAS T VB | 5 | 0 | 5 | 0 |
| 36 | 10 | GAS B VB | 5 | 0 | 5 | 0 |
| 37 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 38 | 1 | WAIT | 1 | 0 | 180 | 0 |

## Module C — Acetic Anhydride Capping

This module is written with the acetic anhydride solution in bottle number 4. If DMAP catalyzed loading is being done, then the DMAP must be removed after the loading steps and the acetic anhydride solution added. Follow the procedure on page 8.

| | | | 0.10 mmol | | 0.25 mmol | |
|---|---|---|---|---|---|---|
| Step | Fxn# | Function Name | Time | Add | Time | Add |
| 1 | 77 | PRS #4 | 10 | 0 | 10 | 0 |
| 2 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 3 | 52 | #4 B RV | 15 | 1 | 35 | 2 |
| 4 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 5 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 6 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 7 | 93 | GAS TO RS | 3 | 0 | 3 | 0 |
| 8 | 89 | RS to FC | 1 | 0 | 1 | 0 |
| 9 | 2 | VORTEX ON | 300 | 0 | 300 | 0 |
| 10 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 11 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 12 | 91 | #10 TO RS | 3 | 0 | 3 | 0 |
| 13 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 14 | 42 | DRAIN RV | 10 | 1 | 20 | 2 |

## Module D — NMP Washes

| | | | 0.10 mmol | | 0.25 mmol | |
|---|---|---|---|---|---|---|
| Step | Fxn# | Function Name | Time | Add | Time | Add |
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 3 | 42 | DRAIN RV | 10 | 1 | 18 | 2 |
| 4 | 98 | BEGIN LOOP | 4 | 1 | 5 | 1 |
| 5 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 6 | 50 | #10 RV-DRN | 2 | 0 | 3 | 0 |
| 7 | 42 | DRAIN RV | 5 | 1 | 5 | 1 |
| 8 | 56 | #10 B RV | 4 | 1 | 13 | 1 |
| 9 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 10 | 91 | #10 TO RS | 2 | 0 | 2 | 0 |
| 11 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 12 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 13 | 2 | VORTEX ON | 3 | 0 | 5 | 0 |
| 14 | 40 | MIX RV | 2 | 0 | 5 | 0 |
| 15 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 16 | 42 | DRAIN RV | 10 | 1 | 15 | 1 |
| 17 | 99 | END LOOP | 1 | 0 | 1 | 0 |

## Module E — Add 2.0 M DIEA and Transfer to RV

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 3 | 5 | NEEDLE DOWN | 10 | 0 | 10 | 0 |
| 4 | 78 | PRS #M | 10 | 0 | 10 | 0 |
| 5 | 70 | PURGE ML | 2 | 0 | 2 | 0 |
| 6 | 98 | BEGIN LOOP | 2 | 0 | 2 | 0 |
| 7 | 68 | MEAS #7 | 2 | 0 | 2 | 0 |
| 8 | 63 | ML TO CART | 4 | 0 | 4 | 0 |
| 9 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 10 | 60 | MIX CART | 5 | 0 | 5 | 0 |
| 11 | 98 | BEGIN LOOP | 4 | 0 | 4 | 0 |
| 12 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 13 | 96* | CRT TO RVc | 6 | 0 | 6 | 0 |
| 14 | 2 | VORTEX ON | 3 | 0 | 3 | 0 |
| 15 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 16 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 17 | 65 | #10 CART | 0 | 0 | 3 | 0 |
| 18 | 60 | MIX CART | 0 | 0 | 3 | 0 |
| 19 | 98 | BEGIN LOOP | 3 | 0 | 3 | 0 |
| 20 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 21 | 96* | CRT TO RVc | 5 | 0 | 5 | 0 |
| 22 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 23 | 40 | MIX RV | 1 | 0 | 2 | 0 |
| 24 | 93 | GAS TO RS | 2 | 0 | 2 | 0 |
| 25 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 26 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 27 | 93 | GAS TO RS | 3 | 0 | 3 | 0 |
| 28 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 29 | 89 | RS TO RC | 1 | 0 | 1 | 0 |
| 30 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 31 | 62 | DRAIN CART | 5 | 0 | 5 | 0 |
| 32 | 40 | MIX RV | 0 | 0 | 0 | 1 |

* When using software version 1.12, Fxn 96 is User Function C which has valves 10, 12, 17, and 22 activated.

## Module F — Clean Cartridge, Drain, and NMP Washes

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 3 | 5 | NEEDLE DWN | 10 | 0 | 10 | 0 |
| 4 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 5 | 98 | BEGIN LOOP | 3 | 0 | 3 | 0 |
| 6 | 67 | #10 SML N | 2 | 0 | 2 | 0 |
| 7 | 62 | DRAIN CART | 5 | 0 | 5 | 0 |
| 8 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 9 | 98 | BEGIN LOOP | 2 | 0 | 3 | 0 |
| 10 | 65 | #10 CART | 18 | 0 | 24 | 0 |
| 11 | 60 | MIX CART | 10 | 0 | 10 | 0 |
| 12 | 24 | CART TO AC | 20 | 0 | 20 | 0 |
| 13 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 14 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 15 | 98 | BEGIN LOOP | 2 | 0 | 2 | 0 |
| 16 | 67 | #10 SML N | 2 | 0 | 2 | 0 |
| 17 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 18 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 19 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 20 | 60 | MIX CART | 5 | 0 | 5 | 0 |
| 21 | 61 | VENT CART | 2 | 0 | 2 | 0 |
| 22 | 98 | BEGIN LOOP | 9 | 1 | 30 | 2 |
| 23 | 2 | VORTEX ON | 15 | 0 | 15 | 0 |
| 24 | 3 | VORTEX OFF | 13 | 0 | 13 | 0 |
| 25 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 26 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 27 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 28 | 91 | #10 TO RS | 5 | 0 | 5 | 0 |
| 29 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 30 | 98 | BEGIN LOOP | 3 | 0 | 4 | 0 |
| 31 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 32 | 28 | GAS T ACT | 4 | 0 | 4 | 0 |
| 33 | 42 | DRAIN RV | 7 | 1 | 15 | 1 |
| 34 | 38 | ACT TO RVo | 6* | 0 | 10* | 0 |
| 35 | 40 | MIX RV | 1 | 0 | 1 | 0 |
| 36 | 2 | VORTEX ON | 3 | 0 | 3 | 0 |
| 37 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 38 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 39 | 22 | DRAIN ACT | 5 | 0 | 5 | 0 |
| 40 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 41 | 42 | DRAIN RV | 7 | 1 | 15 | 1 |

* It might be necessary to adjust this number for your instrument. The objective is to have all the NMP transferred to the RV after the loops are finished.

**Module G** **Resin Sample**

| | | | 0.10 mmol | | 0.25 mmol | |
|---|---|---|---|---|---|---|
| Step | Fxn# | Function Name | Time | Add | Time | Add |
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 14 | #10 B VB | 1 | 0 | 1 | 0 |
| 3 | 56 | #10 B RV | 5 | 1 | 13 | 1 |
| 4 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 5 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 6 | 91 | #10 TO RS | 2 | 0 | 2 | 0 |
| 7 | 93 | GAS TO RS | 2 | 0 | 2 | 0 |
| 8 | 91 | #10 TO RS | 2 | 0 | 2 | 0 |
| 9 | 93 | GAS TO RS | 1 | 0 | 1 | 0 |
| 10 | 91 | #10 TO RS | 4 | 0 | 4 | 0 |
| 11 | 41 | VENT RV | 1 | 0 | 1 | 0 |
| 12 | 98 | BEGIN LOOP | 1 | 0 | 1 | 0 |
| 13 | 39 | RELAY 0 | 1 | 0 | 1 | 0 |
| 14 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 15 | 93 | GAS TO RS | 2 | 0 | 2 | 0 |
| 16 | 91 | #10 TO RS | 4 | 0 | 4 | 0 |
| 17 | 9 | GAS T VB | 3 | 0 | 3 | 0 |
| 18 | 13 | #10 T VB | 1 | 0 | 1 | 0 |
| 19 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 20 | 91 | #10 TO RS | 1 | 0 | 1 | 0 |
| 21 | 48 | GAS T RV | 2 | 0 | 2 | 0 |
| 22 | 87 | TAKE SAMPL | 2 | 0 | 2 | 0 |
| 23 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 24 | 91 | #10 TO RS | 2 | 0 | 2 | 0 |
| 25 | 93 | GAS TO RS | 2 | 0 | 2 | 0 |
| 26 | 91 | #10 TO RS | 2 | 0 | 2 | 0 |
| 27 | 93 | GAS TO RS | 2 | 0 | 2 | 0 |
| 28 | 91 | #10 TO RS | 4 | 0 | 4 | 0 |
| 29 | 93 | GAS TO RS | 4 | 0 | 4 | 0 |
| 30 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 31 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 32 | 91 | #10 TO RS | 3 | 0 | 3 | 0 |
| 33 | 93 | GAS TO RS | 3 | 0 | 3 | 0 |
| 34 | 91 | #10 TO RS | 5 | 0 | 5 | 0 |
| 35 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 36 | 39 | RELAY 0 | 1 | 0 | 1 | 0 |
| 37 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 38 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 39 | 42 | DRAIN RV | 10 | 1 | 18 | 1 |

## Module H — Loading and Benzoic Anhydride Capping

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 1 | 1 | WAIT | 1 | 0 | 1 | 0 |
| 2 | 55 | #9 B RV | 10 | 0 | 20 | 0 |
| 3 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 4 | 90 | #9 TO RS | 6 | 0 | 6 | 0 |
| 5 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 6 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 7 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 8 | 4 | PRINT CART | 10 | 0 | 10 | 0 |
| 9 | 6 | NEEDLE UP | 10 | 0 | 10 | 0 |
| 10 | 7 | EJECT CART | 10 | 0 | 10 | 0 |
| 11 | 8 | ADVAN CART | 10 | 0 | 10 | 0 |
| 12 | 5 | NEEDLE DWN | 10 | 0 | 10 | 0 |
| 13 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 14 | 64 | #9 CART | 4 | 0 | 4 | 0 |
| 15 | 65 | #10 CART | 3 | 0 | 3 | 0 |
| 16 | 60 | MIX CART | 300 | 0 | 300 | 0 |
| 17 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 18 | 42 | DRAIN RV | 10 | 0 | 20 | 0 |
| 19 | 98 | BEGIN LOOP | 5 | 0 | 5 | 0 |
| 20 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 21 | 96* | CRT TO RVc | 8 | 0 | 8 | 0 |
| 22 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 23 | 64 | #9 CART | 5 | 0 | 8 | 0 |
| 24 | 60 | MIX CART | 4 | 0 | 4 | 0 |
| 25 | 78 | PRS #M | 10 | 0 | 10 | 0 |
| 26 | 98 | BEGIN LOOP | 2 | 0 | 2 | 0 |
| 27 | 69 | MEAS #8 | 3 | 0 | 3 | 0 |
| 28 | 43 | ML TO RV | 4 | 0 | 40 | 0 |
| 29 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 30 | 77 | PRS #4 | 10 | 0 | 10 | 0 |
| 31 | 17 | #4 B VB | 1 | 0 | 1 | 0 |
| 32 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 33 | 52 | #4 B RV | 1 | 0 | 1 | 0 |
| 34 | 44 | CART TO RV | 8 | 0 | 10 | 0 |
| 35 | 55 | #9 B RV | 1 | 0 | 2 | 0 |
| 36 | 40 | MIX RV | 1 | 0 | 2 | 0 |
| 37 | 67 | #10 SML N | 5 | 0 | 5 | 0 |
| 38 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 39 | 98 | BEGIN LOOP | 3 | 0 | 3 | 0 |
| 40 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 41 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 42 | 93 | GAS TO RS | 3 | 0 | 3 | 0 |
| 43 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 44 | 2 | VORTEX ON | 600 | 0 | 600 | 0 |
| 45 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 46 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 47 | 6 | NEEDLE UP | 10 | 0 | 10 | 0 |
| 48 | 7 | EJECT CART | 10 | 0 | 10 | 0 |
| 49 | 8 | ADVAN CART | 10 | 0 | 10 | 0 |
| 50 | 5 | NEEDLE DWN | 10 | 0 | 10 | 0 |
| 51 | 64 | #9 CART | 5 | 0 | 5 | 0 |
| 52 | 60 | MIX CART | 180 | 0 | 180 | 0 |

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 53 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 54 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 55 | 90 | #9 TO RS | 6 | 0 | 6 | 0 |
| 56 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 57 | 42 | DRAIN RV | 20 | 0 | 30 | 0 |
| 58 | 98 | BEGIN LOOP | 2 | 0 | 2 | 0 |
| 59 | 55 | #9 B RV | 5 | 0 | 12 | 0 |
| 60 | 54 | #6 B RV | 5 | 0 | 12 | 0 |
| 61 | 40 | MIX RV | 10 | 0 | 10 | 0 |
| 62 | 2 | VORTEX ON | 10 | 0 | 10 | 0 |
| 63 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 64 | 42 | DRAIN RV | 15 | 0 | 25 | 0 |
| 65 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 66 | 98 | BEGIN LOOP | 5 | 0 | 5 | 0 |
| 67 | 55 | #9 B RV | 8 | 0 | 15 | 0 |
| 68 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 69 | 90 | #9 TO RS | 6 | 0 | 6 | 0 |
| 70 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 71 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 72 | 2 | VORTEX ON | 10 | 0 | 10 | 0 |
| 73 | 3 | VORTEX OFF | 1 | 0 | 1 | 0 |
| 74 | 42 | DRAIN RV | 10 | 0 | 20 | 0 |
| 75 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 76 | 98 | BEGIN LOOP | 4 | 0 | 4 | 0 |
| 77 | 41 | VENT RV | 2 | 0 | 2 | 0 |
| 78 | 96* | CRT TO RVc | 6 | 0 | 6 | 0 |
| 79 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 80 | 64 | #9 CART | 3 | 0 | 6 | 0 |
| 81 | 60 | MIX CART | 5 | 0 | 5 | 0 |
| 82 | 77 | PRS #4 | 10 | 0 | 10 | 0 |
| 83 | 17 | #4 B VB | 1 | 0 | 1 | 0 |
| 84 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 85 | 52 | #4 B RV | 2 | 0 | 2 | 0 |
| 86 | 44 | CART TO RV | 8 | 0 | 8 | 0 |
| 87 | 55 | #9 B RV | 1 | 0 | 2 | 0 |
| 88 | 40 | MIX RV | 2 | 0 | 2 | 0 |
| 89 | 88 | RS TO RV | 1 | 0 | 1 | 0 |
| 90 | 93 | GAS TO RS | 4 | 0 | 4 | 0 |
| 91 | 89 | RS TO FC | 1 | 0 | 1 | 0 |
| 92 | 2 | VORTEX ON | 1 | 0 | 1 | 0 |
| 93 | 98 | BEGIN LOOP | 3 | 0 | 3 | 0 |
| 94 | 67 | #10 SML N | 3 | 0 | 3 | 0 |
| 95 | 62 | DRAIN CART | 10 | 0 | 10 | 0 |
| 96 | 99 | END LOOP | 1 | 0 | 1 | 0 |
| 97 | 65 | #10 CART | 5 | 0 | 5 | 0 |
| 98 | 10 | GAS B VB | 2 | 0 | 2 | 0 |
| 99 | 1 | WAIT | 60 | 0 | 60 | 0 |

* When using software version 1.12, Fxn 96 is User Function C which has valves 10, 12, 17, and 22 activated.

## Module I — 10 Min Wait

| Step | Fxn# | Function Name | 0.10 mmol Time | 0.10 mmol Add | 0.25 mmol Time | 0.25 mmol Add |
|---|---|---|---|---|---|---|
| 1 | 1 | WAIT | 600 | 0 | 600 | 0 |

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

Stock No. 343816-002