CEM
Microwave-Enhanced Science

Liberty/Liberty1 User Guide

Recommended Parameters

Deprotection

Temperature: 75 °C
Time: 30 sec (Initial)
3 min (Deprotection)

Coupling

Temperature: 75 °C
Time: 5 min

Microwave Power

Specific power settings will vary depending on scale and the individual microwave. The power should be set so that the temperature reaches **75 °C in 70 sec,** and the maximum temperature should not exceed 85 °C. The Run History file records the temperature during each microwave step, and should be reviewed periodically. Microwave methods can be adjusted in the Microwave Editor (Setup>Microwave Editor).

Special Coupling Cycles

Cysteine and Histidine

Cysteine and histidine are susceptible to racemization at elevated temperature. **By default, Cys and His are coupled at 50 °C** to minimize racemization. If a non-default cycle is selected for Cys or His, ensure a 50 °C microwave method is used for the coupling.

When using non-natural amino acids where racemization is a concern, the coupling temperature should be lowered to 50 °C. (See also CEM Application Note BIO-0003.)

Arginine

Arginine is susceptible to γ-lactam formation, greatly reducing the coupling efficiency. **By default, Arg is coupled using a modified double coupling** cycle. If a non-default cycle is selected for Arg, ensure that it is a double coupling method. (See also CEM Application Note BIO-0006.)

Standard Concentrations

The Liberty/Liberty1 uses stock solutions of all reagents. The default cycles are designed to deliver the proper volumes to give 5 eq of amino acid and activator and 10 eq of activator base for each coupling.

Reagent	Standard	0.05 mmol Scale*
Activator	0.5 M	0.25 M
Activator Base	2 M	1 M
Amino Acid	0.2 M	0.2 M
Deprotection (Piperidine)	20%	20%

***NOTE: Due to the size of the sample loops used for reagent delivery, when working on 0.05 mmol scale activator and activator base should be made at half the standard concentration. Other reagents do not need to be diluted.**

Potential Side Reactions

Aspartimide Formation

Aspartimide formation is a common side reaction that occurs in peptides containing Asp followed by Asn(Trt), Gly, Thr, or Ser. The use of **5% piperazine with 0.1 M HOBt** for the deprotection solution can reduce the amount of aspartimide formation. When using the UV monitoring option, HOBt shows significant UV absorbance. The use of **Fmoc-Asp(OMpe)-OH** in place of the more common Asp(OtBu) can reduce aspartimide formation without the addition of HOBt.

Tetramethyl Guanidinium Capping

For extended coupling times (10 minutes or longer), one potential issue is the tetramethylguanidinium capping of the free amine by HBTU (or other uronium-type activators). This side reaction is detected as a truncation of target +101 on mass spec. Capping by activator can be minimized by using a slight excess of other reagents (for example, 5 eq amino acid/4.5 eq HBTU/10 eq DIEA), or by using alternate activator strategies (such as DIC/HOBt).

Resin Selection

Most resins that are used for conventional peptide synthesis are compatible with microwave peptide synthesis.

Mesh Size

Only 100-200 mesh resin should be used with the Liberty/Liberty1. The use of 200-400 mesh resins will result in clogging of the reaction vessel frit and damaged to the system.

Acid-Sensitive Resins

Acid-sensitive resins, such as 2-chlorotrityl and HMPB, require special conditions during microwave synthesis. All couplings with these resins should be done at 50 °C, and no HOBt should be added to the deprotection solution.

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Reaction Vessel Selection

To select the appropriate reaction vessel, first look at the scale to ensure the volumes are reasonable, then look at the mass of resin required. If using **more than 3 g of resin** on the Liberty, use the **Skip Resin Loading** option and manually load the resin into the reaction vessel, then manually add solvent to swell.

The 10 mL reaction vessel should be used for 0.05 mmol syntheses (up to 0.5 g of resin).

The 35 mL (standard) reaction vessel should be used for 0.1 - 0.25 mmol syntheses (up to 1 g of resin).

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

Reagents and Stability

Amino Acids

Most amino acids are stable in solution for up to **two weeks**. After that, the amino acid will begin to crystallize, which can cause damage to the instrument. Notably, His is only stable for one week. Val, Ile, and Leu will begin to crash out sooner than other amino acids.

Activator/Base

Activators are stable for up to **one week**. Uronium-type activators (HBTU, HCTU, HATU) are light-sensitive, and should be **stored in amber bottles**. DIEA in NMP is stable for up to **two weeks**.

Deprotection

Deprotection solution (both 20% piperidine and 5% piperazine) are stable for up to **one month**.

Cleavage Cocktail

Cleavage cocktails are only stable for 24 hours. They should be discarded after **one day**.

Alternative Reagents

Piperidine/Piperazine

5% Piperazine can be used as an alternative to 20% piperidine. Under conventional synthesis conditions, piperazine requires extended deprotection times, but in the microwave full deprotection by piperazine is accomplished in the same time as piperidine.

HOBt/HOBt•H_2O/OxymaPure

Anhydrous HOBt is considered explosive. The HOBt monohydrate can be used as a direct substitute for anhydrous HOBt and is stable to ship. OxymaPure can also be used as a direct substitute for HOBt. When using OxymaPure for coupling with DIC, some discoloration of the vent line on the reaction vessel may occur; this will not affect the operation of the Liberty/Liberty1.

EDT/DODT

DODT (dioxa-1,8-octane-dithiol) is a less malodorous alternative to EDT (ethane dithiol), and an be directly substituted for EDT without any difference in cleavage quality.

Using DIC/HOBt

In cases where synthesis with HBTU/DIEA yields poor quality peptides, DIC/HOBt is a good alternative approach. When coupling amino acids using DIC/HOBt, CEM recommends putting **0.5 M DIC in DMF on the Activator position**, and **1 M HOBt in DMF on the Activator Base position**. This will give 5 eq DIC and 5 eq HOBt in the final reaction mixture.

Reagent Quality

The quality of reagents used for synthesis, including the age of reagents, will have a significant impact on the quality of peptides produced. Solvents should be higher than ACS grade. DMF should not be used if it is more than six months old. TFA that has discolored (is no longer clear) should not be used; this can result in incomplete side chain deprotection. Amino acids should be stored at room temperature for no longer than six months, or in a freezer for no longer than a year.

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Peptide Cleavage and Analysis

Cleaving Peptides

Typically, cleavage is accomplished with trifluoroacetic acid (TFA). Scavenger molecules are added to the TFA to prevent the cleaved side chain protecting groups from reacting with the peptide. The particular scavengers used depend on the specific peptide sequence.

For general use, CEM recommends 92.5% TFA / 2.5 % TIS / 2.5% DODT / 2.5% Water.
Other Suitable Cocktails:

- 82.5% TFA / 5% TIS / 5% Water / 5% Phenol / 5% EDT
- 82.5% TFA / 5% Thioanisole / 5% Water / 5% Phenol / 5% EDT
- 88% TFA / 5% TIS / 5% water / 2% Phenol
- 90% TFA / 5% Thioanisole / 3% EDT / 2% Anisole

At room temperature, peptides should be cleaved for 2 to 4 hours. Peptides containing multiple Arg residues will require longer cleavage times (3 hours or longer), because of the difficulty in removing the Pbf protecting group. **In the microwave, peptides should be cleaved at 38 °C for 30 minutes** to ensure complete deprotection.

Common Mass Differences

Side Chain Protecting Groups

Incomplete cleavage can result in mass additions due to incomplete removal of side chain protecting groups.

Mass Difference	Reason
+100	Boc
+56	tBu
+242	Trt
+252	Pbf

Side Reactions

Mass Difference	Reason
-18	Aspartimide
+67	Aspartimide
+101	Tetramethylguanidium

N-terminal Modifications

Mass Difference	Reason
+223	Fmoc
+42	Acetyl

Amino Acid Deletions

Mass Difference	Reason	Mass Difference	Reason
-71	-Ala	-113	-Leu
-156	-Arg	-128	-Lys
-114	-Asn	-131	-Met
-115	-Asp	-147	-Phe
-103	-Cys	-97	-Pro
-128	-Gln	-87	-Ser
-129	-Glu	-101	-Thr
-57	-Gly	-186	-Trp
-137	-His	-147	-Tyr
-113	-Ile	-99	-Val

Salts

Because peptides are charged molecules, when analyzing peptides by ESI-MS, the target often is detected as a salt.

Mass Difference	Reason
+23	Na^+ salt
+39	K^+ salt
+114	TFA salt
+46	Formic salt

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Spare Parts and Further Information

Reaction Vessels

Part Number	Name	Description	Picture
167260	30 mL Reaction Vessel Body	Standard 30 mL Teflon reaction vessel body that connects to the standard reaction vessel attenuator	
542415	125 mL Reaction Vessel Body	Large 125 mL Teflon reaction vessel body that connects to the standard reaction vessel attenuator	
167765	10 mL Reaction Vessel Body	Small 10 mL Teflon reaction vessel that connects to the 10 mL vessel attenuator	

Filters

Part Number	Name	Description	Picture
167485-M	Dip Tube Filters	Replacement filters for all reagent dip tubes (bag of 50 filters)	
172150-M	Inline Filters	Replacement filters for F1, F2, and F3 positions (box of 25)	

For More Information

- PepDriver/PepDriver1 contains comprehensive Help Text with details about setup and operation of the system, as well as general information about microwave chemistry and peptide chemistry. **The Help Text can be accessed by clicking the Help button on the PepDriver/ PepDriver1 main screen.**
- The Liberty Operation Manual (PN 600178) and the Liberty1 Operation Manual (PN 600192) have detailed information about setup and operation of the system, as well as a full list of spare parts.
- For information about routine maintenance procedures, including monthly checklists, see the Liberty Maintenance Manual (PN 600119) or the Liberty1 Maintenance Manual (PN 600765).
- For the latest information about CEM's products, including references and application notes, visit the CEM website at **http://www.cem.com**. The CEM website also hosts a peptide synthesis discussion forum, where CEM users can interface with other users from around the world.
- CEM has a dedicated group of peptide chemists with access to a full laboratory. For applications support, contact CEM at **(800) 726-3331 (US/Canada) or (704) 821-7015 and ask for "peptide support"**, or by email at **peptides@cem.com**. For service, contact CEM Service at **(800) 726-5551 (US/Canada) or (704) 821-7015 and ask for "Liberty/Liberty1 Service"**.