



**Microwave-Enhanced
Science**

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Discover SPS Quick Reference Guide

Suggested Parameters for Fmoc Solid Phase Synthesis

NOTE: All values for Power are suggested, and should be adjusted depending on sample size. The goal is to reach the maximum temperature in 75 seconds for Deprotection and Coupling, and in 120 seconds for Cleavage. The Cleavage temperature should NEVER exceed 43 °C.

Fmoc Deprotection:

Power = 20 W
Maximum Temperature = 75 °C
Time = 3:00
Delta Temperature = 5 °C

Coupling:

Power = 20 W
Maximum Temperature = 75 °C
Time = 5:00
Delta Temperature = 5 °C

TFA Cleavage:

Power = 20 W
Maximum Temperature = 38 °C
Time = 30:00
Delta Temperature = 5 °C

Method Programming

1. Press the Open Folder key.
2. Select "New Method".
3. Press the right arrow key until "Mode = SPS". Press ENTER.
4. Set Power, then press ENTER.
5. Set Maximum Temperature, then press ENTER.
6. Set Method Run Time, then press ENTER.
7. Set Delta Temperature to 5 °C, then press ENTER.
8. Set Stirring to "OFF", then press ENTER. (NOTE: Magnetic stirring is not recommended with solid phase synthesis resins.)
9. Set Cooling to "OFF", then press ENTER.
10. Set "Next Stage = (N)", then press ENTER.
11. Set "Save Method = (Y)", then press ENTER.
12. Create a method name using the arrow keys, highlight "Exit", and press ENTER. The method is now saved in the software.

Reagents for Fmoc Solid Phase Synthesis

Activator Base Solution			
Number of Couplings	0.1 mmol Scale		
	mL DMF	mL DIEF	final Vol.
5	19	1	20
10	38	2	40
Per Coupling:	4 mL		

Note: Do not add Activator Base Solution to Amino Acid/Activator until immediately before coupling.

Deprotection Solution				
Number of Couplings	0.1 mmol Scale			
	mL DMF	mL Piperidine	g HOBt	final Vol.
5	28	7	0.49	35
10	56	14	0.98	70
Per Decoupling:	7 mL			

Note: HOBt is optional, but is strongly recommended for sequences susceptible to aspartimide formation.

Cleavage Solution
General cleavage: 95% TFA / 2.5% TIS / 2.5% H ₂ O
For peptides containing Cys, Met, Trp, and/or Arg: 82.5% TFA / 5% thioanisole / 5% H ₂ O / 5% phenol / 2.5% EDT

Amino Acid and Activator			
Amino Acid	MW	0.1 mmol Scale	
		g AA	g HBTU
Fmoc-Ala-OH	311.3	0.156	0.190
Fmoc-Arg(Pbf)-OH	648.8	0.324	0.190
Fmoc-Asn(Trt)-OH	354.4	0.298	0.190
Fmoc-Asp(OtBu)	411.5	0.206	0.190
Fmoc-Cys(Trt)-OH	585.7	0.293	0.190
Fmoc-Gln(Trt)-OH	610.7	0.305	0.190
Fmoc-Glu(OtBu)-OH	425.5	0.213	0.190
Fmoc-Gly-OH	297.3	0.149	0.190
Fmoc-His(Trt)-OH	619.7	0.310	0.190
Fmoc-Ile-OH	353.4	0.177	0.190
Fmoc-Leu-OH	353.4	0.177	0.190
Fmoc-Lys(Boc)-OH	468.5	0.234	0.190
Fmoc-Met-OH	371.5	0.186	0.190
Fmoc-Phe-OH	387.4	0.194	0.190
Fmoc-Pro-OH	337.4	0.169	0.190
Fmoc-Ser(tBu)-OH	383.4	0.192	0.190
Fmoc-Thr(tBu)-OH	397.5	0.199	0.190
Fmoc-Trp(Boc)-OH	526.6	0.263	0.190
Fmoc-Tyr(tBu)-OH	459.6	0.230	0.190
Fmoc-Val-OH	339.4	0.170	0.190

Protocol for Fmoc Solid Phase Synthesis (0.1 mmol scale)

Setup the system and swell the resin.

1. Ensure the system is in Open Vessel mode.
2. Lock the attenuator in place.
3. Ensure that a 50 mL centrifuge tube has been inserted in the 250 mL bottle and that the lid is secure.
4. Insert the thermowell into the clip and attach the clip to the reaction vessel, ensuring that the tip of the thermowell is completely immersed in the solvent.
5. Place the appropriate mass of resin in the reaction vessel, and add 7 mL DMF to the resin. Allow the resin to swell for 15 minutes.
6. Drain the DMF. Rinse down any resin stuck to the side of the reaction vessel with additional DMF.

Deprotect the amino acid.

1. Add 7 mL Deprotection Solution to the resin.
2. Insert the fiber-optic probe into the thermowell. Place the reaction vessel into the holder and insert into the microwave cavity.
3. Load the Deprotection method, and press the Play button to start the run.
4. When the method has finished, remove the reaction vessel from the holder and drain the reagents to waste.
5. Rinse the resin five times with 7 mL DMF.

Is there another
amino acid in
the sequence?

YES

NO

Couple the next amino acid.

1. Dissolve the appropriate amino acid and activator in 4 mL Activator Base Solution. Transfer the solution to the reaction vessel.
2. Insert the fiberoptic probe into the thermowell. Place the reaction vessel into the holder and insert into the microwave cavity.
3. Load the Coupling method, then press the Play button to start the run.
4. When the method has finished running, remove the reaction vessel from the holder and drain the reagents to waste.
5. Rinse the resin five times with 7 mL DMF.

Cleave the completed peptide from the resin.

1. Rinse the resin five times with 7 mL DCM.
2. Add the appropriate Cleavage Cocktail to the resin.
3. Insert fiber-optic probe into the thermowell. Place the reaction vessel into the holder and insert into the microwave cavity.
4. Load the Cleavage method, and press the Play button to start the run.
5. When the method has finished running, drain the solution into the centrifuge tube. Wash the resin once with DCM.