# **CEM** Application Note

# Peptide Cleavage and Protected Cleavage Procedures

## Introduction

In Fmoc synthesis, the removal of the peptide from the solid support is typically accomplished with trifluoroacetic acid (TFA). Because the side chain protecting groups used in Fmoc synthesis are acid labile, a single step both cleaves the peptide from the resin and removes the protecting groups. Various scavenger molecules are added to the TFA to prevent the cleaved protecting groups from reattaching to the peptide. The particular scavengers used depend on the specific peptide sequence. Common scavengers include water (scavenges t-butyl cations), triisopropyl silane (TIS, scavenges trityl and Pbf cations), ethane dithiol (EDT, scavenges t-butyl cations, reduces oxidation of Cys/Met side chains), dioxa-1,8-octane-dithiol (DODT, scavenges t-butyl cations, suppresses oxidation of Cys/Met side chains), phenol (protects Tyr and Trp side chains from oxidation),



CEM Razor™

and thioanisole (aids in removal of Pbf protecting groups from Arg(Pbf), suppresses oxidation of Cys/Met side chains).

Cleavage can be performed either at room temperature or heated to 38 °C. For heated cleavage, CEM recommends the Razor<sup>™</sup> parallel peptide cleavage system for both single peptides and large batch cleavages. The table below lists CEM's recommended cleavage solution and solvents for both heated and room temperature cleavage. This recommended cleavage solution is a good general-use cleavage solution, having scavengers for most common side chain protecting groups.

Cleavage Solution	Precipitation Solvent
TFA/TIS/H <sub>2</sub> 0/D0DT <sup>1</sup> (92.5/2.5/2.5/2.5)	Diethyl ether or MTBE <sup>2</sup>

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

<sup>2</sup>Anhydrous and ice cold.

### Room Temperature and Heated Cleavage

#### WARNING

The Liberty Blue<sup>®</sup> is not equipped to perform peptide cleavage for better system reliability. All cleavage procedures must be carried out at room temperature outside of the Liberty Blue system or in the Razor<sup>™</sup> parallel peptide cleavage system.

- 1. Remove resin from the reaction vessel.
  - a. Rinse the resin from the vessel into a syringe vessel with DCM. DMF can be used, however DCM allows for easier removal of resin. If DMF is used, the resin needs to be washed thoroughly with DCM prior to cleavage.
- 2. Add approximately 4 mL (or double the volume of the resin bed) of TFA solution to the syringe vessel.
- 3. **For room temperature cleavage**, allow the peptide to cleave for 3 hr at room temperature. If the peptide has multiple Arg residues, increase the cleavage time by 15 min for every additional Arg after the third Arg residue. Do not exceed 5 hr for the total cleavage time.
- 4. **For heated cleavage**, allow the peptide to cleave for 30 min at 38 °C. If the peptide has multiple Arg residues, increase the cleavage time by 5 min for every additional Arg after the third Arg residue. Do not exceed 45 min for the total cleavage time.

- 5. Following cleavage, drain the liquid into a 50 mL centrifuge tube.
- 6. To precipitate the cleaved peptide, add ice cold ether up to 50 mL (or 8 times the amount of drained liquid). If the volume exceeds 50 mL, use a second centrifuge tube. The ether should be ice cold to ensure the maximum amount of peptide precipitates out.
- 7. Centrifuge the peptide solution for 5 min at 3500 rpm or until a white or clear peptide pellet forms on the bottom of the tube. Repeat the centrifuge process if necessary to remove any floating particles.
- 8. Decant the ether, leaving the precipitated peptide in the tube.
  - a. The peptide can be resuspended in ice cold ether and centrifuged again to ensure all protecting groups are gone.
- 9. The peptide can be analyzed immediately on an HPLC or UPLC for purity, or can be lyophilized overnight then analyzed.
  - a. To lyophilize, suspend the peptide gel in 1% acetic acid in deionized water then freeze with liquid nitrogen before placing on the lyophilizer.

#### Protected Cleavage

Resins with hyper-acid sensitive linkers such as CI-TCP(CI) ProTide can be cleaved with 1% TFA to produce protected peptides.

- 1. Remove resin from the reaction vessel.
  - a. Rinse the resin from the vessel into a syringe vessel with DCM. DMF can be used, however DCM allows for easier removal of resin. If DMF is used, the resin needs to be washed thoroughly with DCM prior to cleavage.
- 2. Add approximately 3 mL (or double the volume of the resin bed) of 1% TFA in DCM to the syringe vessel. Allow the peptide 2 min to cleave at room temperature.
- 3. Filter the liquid into a centrifuge tube containing 3 mL of 10% pyridine in MeOH.
- 4. Repeat cleavage and filtration steps four more times for a total of five cleavages.
- 5. Combine all the filtrates in a 125 mL round bottom flask, rinsing each collection tube with additional MeOH.
- 6. Using a rotary evaporator, evaporate the solution to a volume of less than 3 mL.
- 7. Transfer the remaining solution to a clean 50 mL centrifuge tube using a small amount of MeOH (less than 2 mL).
- 8. Add enough ice cold deionized water to bring the volume to 35 mL and precipitate the protected peptide.
- 9. Centrifuge the peptide solution for 5 min at 3500 rpm or until a white or clear peptide pellet forms on the bottom of the tube. Repeat the centrifuge process if necessary to remove any floating particles.
- 10. Decant the water, leaving the precipitated peptide in the tube.
- 11. The peptide can be analyzed immediately on an HPLC or UPLC for purity, or can be lyophilized overnight then analyzed.
  - a. To lyophilize, suspend the peptide gel in 1% acetic acid in deionized water then freeze with liquid nitrogen before placing on the lyophilizer.