Protocol for Cell Signaling Digestion

Preparation of Solutions

1. 20 mM Hepes, pH 8.0
2. Urea Lysis Buffer: 20 mM Hepes pH 8.0, 8 M urea, 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate
   Adjust the inhibitors accordingly for your experiment
3. 1.25 M DTT: Dissolve 48 mg of DTT in 250 uL water. Prepare immediately before experiment.
4. 1 M IAA: Dissolve 47.5 mg of IAA in 250 uL of water. Prepare immediately before experiment and keep in the dark.
5. Trypsin (sequencing grade for low scale, Worthington for large scale): Prepare trypsin at ~1 ug/uL. Ensure a 25:1 to 50:1 protein:enzyme ratio for digestion.

Step	Solution	1-2 mg scale	10-20 mg scale	Condition
		Volume	Volume	
Lysis	Urea Lysis Buffer	1 mL	10 mL	Resuspend pellet
Reduction	1.25 M DTT	4 uL	36 uL	55C for 30 min
Alkylation	1 M IAA	10 uL	100 uL	RT, dark, 15 min
Dilution*	20 mM Hepes pH 8.0	3 mL	30 mL	NA
Digestion	1 ug/uL	40-80 uL	400-800 uL	37C, overnight, agitate
The next day…				
Quench**	10% TFA	455	4.55 mL	Vortex 10 min, then high speed spin to remove particulate

*Adjust accordingly so all samples have the same volume at the end of the digestion, depending how much trypsin is added to each sample. The final [urea] needs to be no higher than 2M before digestion.

**Add 10% TFA so the final [TFA] is 1%