

## Protocol for Protease Max Digestion (~50 ug scale)

### Preparation of Solutions

1. 1% Protease Max: Dissolve 1 mg of protease max (Promega, V207A) in 100 uL of 100 mM TEAB or 50 mM ammonium bicarbonate (ABC). Keep on ice. Aliquot unused solution in 10 uL aliquots and freeze at -20 C.
2. 0.5 M DTT: Dissolve 19 mg of DTT in 250 uL water. Prepare immediately before experiment.
3. 0.55 M IAA: Dissolve 41 mg of IAA in 400 uL of water. Prepare immediately before experiment and keep in the dark.
4. Trypsin (various vendors, sequencing grade): Prepare trypsin at ~0.2 ug/uL. Ensure a 25:1 to 50:1 protein:enzyme ratio for digestion.

**If starting with a pellet**, add 20 uL of 0.2% protease max:50 mM ABC to solubilize. Gently shake for 10-60 min to solubilize. ([PM] = 0.2%)

**If starting with a solution**, add 2 uL of 1% protease max. Gently shake for 10-60 min to solubilize. (Try to get [PM] to ~0.1% for solubilization)

Step	Solution	Volume	Conditions
Dilution	50 mM ABC	Make up to a final vol of 86.3 uL	
Reduction	0.5 M DTT	1 uL	56C for 20 min. Cool before adding IAA.
Alkylation	0.55 M IAA	2.7 uL	RT, dark, 15 min
Solubilization*	1% protease max	1 uL	Gently mix
Digestion	0.2 ug/uL	9	37C for 3 hr, agitate
3 hr later...			
Quench**	10% TFA	5.3	Mix at RT for 5 min

\*Try to get [PM] to be 0.03% for cytoplasmic proteins or 0.05% for membrane proteins before the digestion step.

\*\*Add 10% TFA so the final [TFA] is 0.5%