Trypsin Digestion

Stock solutions:

1. **0.25 µg/ µl Trypsin:** 20 µg Trypsin (Mass Spec grade) + 80 µl 50 mM Acetic acid, store at -20°C
2. **5x digestion buffer***: 250 mM NH_4HCO_3 (MW 79) in 25:75 CH_3CN: H_2O
   For example: 19.8 mg NH_4HCO_3 + 0.25 ml CH_3CN +0.75 ml H_2O
   *Use 25mM Tris buffer (pH 7.8 or pH 8.0) if there is Ca^{2+} in sample.
3. **200 mM DTT** (MW 154): 15.4 mg DTT + 500 µl H_2O
4. **500 mM Iodoacetamide** (IAM, MW 185): 18.5 mg IAM + 200 µl H_2O
5. **20% TFA**: 0.2 ml TFA+ 0.8 ml H_2O

Procedure:

1. Mix sample solution and 5x digestion buffer in 4:1 ratio (**see table).**
2. Add 200 mM DTT (to final 5 mM) (**see table)**, incubate at 70-80°C for 20 min.
3. After cooling to room temperature, add 500 mM Iodoacetamide (to final 12.5 mM) (**see table)**, incubate at room temperature for 15 min.
4. Add a 1:20 weight ratio of trypsin (enzyme to protein, w to w ratio of trypsin; **see table**).
5. Stop reaction by adding 20% TFA (check pH 2~3) (add ~ 3 ul 20% TFA/100ul solution).
6. **Sample can be directly analyzed by LC-MS (more complex samples require de-salting by C18 solid phase extraction). Alternately, the sample can be stored at -20ºC for future use.

Sample name	Protein mg/ml	Sample µl	Protein (µg)	5*-td Buffer	DTT 200mM	IAM 500mM	Trypsin 0.25µg/µl	20% TFA	Total µl
Protein 1	0.05	200	10	50 ul	6.3ul	6.3 ul	2.0 ul	~7.5ul	

* If Rapigest was used, be sure to delete this detergent prior to MS analysis according to the manufactore's manual. Typical deletion involves decomposition and precipitation of the detergent by acidification. The sample can then be centrifuged and the supernatant extracted for LC-MS analysis.