



### SUMS Solution Digest Protocol

1. Perform a  $-80^{\circ}\text{C}$  acetone protein precipitation by using 4X's the volume.
  - a. Add 4X's the volume of  $-80^{\circ}\text{C}$  acetone to samples and let sit on dry ice for 60 min. Vortex intermittently.
2. Set the centrifuge to  $4^{\circ}\text{C}$  quick cool and spin at 10000G for 10 min.
3. Remove Eppendorf(s) from centrifuge and carefully pipet off top layer of acetone, being careful not to disturb protein pellet.
4. Speed Vac for 5-10 min to ensure all acetone has been boiled away.
5. Reconstitute protein pellet with  $15\mu\text{l}$  8M Urea 100mM Ammonium bicarbonate stock and  $20\mu\text{l}$  Protease max solution.
  - a. For Protease max solution dilute 1 aliquot stock by adding  $60\mu\text{l}$  50mM Ammonium bicarbonate and use for 3 Solution digests ( $20\mu\text{l}$  each). Once Protease is added, vortex on low level
6. Sonicate for 10 minutes to ensure protein is solubilized
7. Add  $1.6\mu\text{l}$  of 500mM DTT stock to each sample
8. Incubate at  $55^{\circ}\text{C}$  for 30 minutes
9. Remove eppendorf(s) and let adjust to room temperature
10. Add  $3.2\mu\text{l}$  of 1M acrylamide to each sample
11. Let incubate at room temperature for 30 minutes
12. Add  $63\mu\text{l}$  of 50mM Ammonium bicarbonate for a final volume of  $98\mu\text{l}$
13. For the addition of trypsin note the protein amount beforehand. Try to add Trypsin/Lys-C at a 1:50 - 1:100 protease to total protein ratio. One trypsin stock vial equals  $5\mu\text{g}$  trypsin, so we will work for  $100\mu\text{g}$  total protein.
  - a. Add the appropriate amount of trypsin to each eppendorf (using new tip each time, mix solution with pipet) and incubate at  $37^{\circ}\text{C}$  overnight.
14. After the overnight digest, quench the tryptic digest and acidify the solution by adding  $10\mu\text{l}$  of 50% formic acid/water stock dilution. Using a new tip each time, mix solution with pipet.
15. Centrifuge at 10000G for 2 minutes.
16. Samples are ready for stage tip purification.

**Supplies:**

- Promega – Trypsin/Lys-C Mix, Mass Spec Grade, 20 µg V5071 (20µg) or V5073 (5x20µg)  
[http://www.promega.com/products/protein-expression-and-mass-spectrometry/peptidases-and-services-for-mass-spectrometry/trypsin\\_lys\\_c-mix\\_-mass-spec-grade/](http://www.promega.com/products/protein-expression-and-mass-spectrometry/peptidases-and-services-for-mass-spectrometry/trypsin_lys_c-mix_-mass-spec-grade/)
- Promega - ProteaseMAX™ Surfactant, Trypsin Enhancer, 1mg, V2071 (1mg) or V2072 (5x1mg)  
[http://www.promega.com/products/protein-expression-and-mass-spectrometry/peptidases-and-services-for-mass-spectrometry/proteasemax-surfactant\\_-trypsin-enhancer/](http://www.promega.com/products/protein-expression-and-mass-spectrometry/peptidases-and-services-for-mass-spectrometry/proteasemax-surfactant_-trypsin-enhancer/)
- Acetone
- 500mM DTT
- 1M Acrylamide
- 50mM Ammonium Bicarbonate
- 100mM Ammonium Bicarbonate