SUMS In-Gel digest protocol

1. Cut gel pieces in 1mm x 1mm squares.
2. Ensure gel pieces are at neutral pH by adding 50-100 µl 100mM Ammonium bicarbonate, let sit for 10 minutes and discard)
3. Add 10 µl 50mM dithiothereitol (DTT) and 100 µl 50mM Ammonium bicarbonate
4. Incubate at 55 ˚C for 30 minutes.
5. Discard solution phase, let temperature of eppendorf's adjust to room temperature
6. Add 10 µl 100mM acrylamide and 100 µl 50mM Ammonium bicarbonate (pH 8-8.2)
7. Incubate at room temperature for 30 minutes.
8. Discard solution phase
9. Wash gel pieces with 100-150 µl 50mM Ammonium bicarbonate/ 50% acetonitrile for 10 minutes, vortex every 5 minutes. (Disregard this step for Silver stained samples)
    a. Depending on intensity of stain, repeat step 9 until the gel pieces are clear.
    b. If after doing this twice and the stain has not dissipated and 100 µl of 50mM Ammonium bicarbonate for 1 min, then discard. Repeat step 6.
10. Discard solution phase and dry samples in speed vac for 5-10 minutes.
11. Add 5pmol promega sequencing grade trypsin in 50mM Ammonium bicarbonate, 0.02% Protease Max, to each sample and spin down.
    a. Add 200 µl of 50 mM Ammonium bicarbonate to 1 Protease max stock vial.
    b. Add 75 µl of the newly diluted Protease Max stock to the Trypsin stock.
        1. **Non-Silver Stained**
           10 µl of diluted Trypsin stock will be 5pmol tryspin.
           Add 10 µl of diluted Trypsin stock to each eppendorf for in-gel digestion.
        2. **Silver Stained**
           Add 1.6 µl of diluted Trypsin to each eppendorf for in-gel digestion
    c. Add a minimal amount of 0.02% Protease Max diluted in 50mM Ammonium bicarbonate (typically 15-40 µl) just to cover gel pieces.
12. Incubate overnight in 37˚C oven (or Incubate 1-2 hour at 50˚ C)
13. Spin Down and pull off all liquid, and transfer to a newly labeled mini-eppendorf (0.7mL).
14. Add 40-50 µl 1% formic acid, 66% acetonitrile 33% 100mM Ammonium bicarbonate
15. Incubate for 10 minutes at 37˚ C.
16. Spin at 10,000G for 2 minutes
17. Extract all solution
18. Speed vac total combined extract to dryness. Save gel pieces & store at -4˚ C until analysis is confirmed.

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/proteases-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/proteases-and-services-for-mass-spectrometry/proteasemax-surfactant_-trypsin-enhancer/

•50mM DTT
•100mM Acrylamide
•50mM Ammonium Bicarbonate
•100mM Ammonium Bicarbonate