Protein Digestion for Complex Mixtures

Solutions needed:

*Rapi*Gest SF Powder (Waters Corporation, 5 Pack of 1 ml Vials, 186001860)
OR PPS Silent Surfactant (Protein Discovery Catalog #21011)
50mM Ammonium Bicarbonate
500mM DTT (Dithiothreitol)
500mM IAA (Iodoacetamide – light sensitive)
5 M HCl
200 ng/µl Trypsin in 0.01% acetic acid (modified, sequencing grade, Promega, Cat#V5111, 5 x 20ug)

- Make 0.2% *Rapi*Gest diluted in 50 mM Ammonium Bicarbonate pH 7.8 (1 mg *Rapi*Gest per 500 µl 50 mM Ammonium Bicarbonate pH 7.8).
  *Note: You can also use mM Tris pH 8.5 as the buffer, but if you do so make sure to add twice as much HCl before your MS run.
- Using locking lid microcentrifuge tubes, add 100 µl 0.2% *Rapi*Gest per 100 µl protein mixture (1:1). Final concentration of *Rapi*Gest should be 0.1 % (w/v). If protein is in pellet form add 25-50 µl of 0.1% *Rapi*Gest.
- Vortex the sample.
- Boil sample at 99° C for 5 minutes (use heating cap for microcentrifuge tube).
- Allow sample to cool for a couple of minutes.
- Spin the sample for a minute and add DTT to a final concentration of 5mM.
- Incubate sample at 60° C for 30 minutes.
- Cool the sample to room temperature.
- Add IAA to a final concentration of 15mM.
- Place sample **IN THE DARK** at room temperature for 30 minutes.
- Add Trypsin for a final concentration of 1:100 enzyme:protein.
- Incubate for 1 hour with shaking at 37° C.
- Can store samples at -20° C at this point if needed.
- Prior to mass spectrometry run, add 5 M HCl to a final concentration of 200mM.
- Incubate at 37° C for 45 minutes while shaking.
- Spin sample at 14,000 rpm, 4°C for 10 minutes.
- A cloudy pellet should appear. Separate your supernatant form the pellet into a fresh Eppendorf tube.
- Spin again if needed to make sure you have completely removed the cloudy material.
- Also you can add 5% acetonitrile or Buffer A (5% acetonitrile, 95% water, 0.1% formic acid) if you are worried your sample may still clog your column.