Protocol for Destaining Silver Stained Gel Bands for use in Digestion

1. Destain the band using a 1:1 mixture of 30 mM Potassium Ferricynide/100 mM Sodium Thiosulfate solution until the brown color disappears. The solution may need to be changed if it takes >5 minutes. DO NOT let the band sit in this solution too long.
2. Wash for 5-10 minutes at least 5 times with H_2O, you want solution to be clear.
3. Stop here if sample will not be digested right away and store in water or just freeze the gel piece alone.
4. Do the following only at the time of actual digestion (usually done by the Proteomics Core Staff):
    a. Add 40uL or enough 100mM Ambic to cover the gel, incubate for 5 min.
    b. Repeat step 1.a.
    c. Proceed with step 1) f) of the Mann protocol for In-gel Digestion.

60mM Potassium Ferricyanide

a. 0.19752g Potassium Ferricyanide + 10 ml H_2O
b. This solution can be stored in a amber colored glass or plastic bottle for several months
c. Mix 1:1 with the 200 mM Sodium Thiosulfate solution only when ready to destain bands/spots

200 mM Sodium Thiosulfate

a. 0.31622g + 10 ml H_2O
b. This solution can be stored in a clear colored glass or plastic bottle for several months
c. Mix 1:1 with the 60 mM Potassium Ferricyanide solution only when ready to destain bands/spots

100mM Ambic

a. 100uL of 1M Ambic in 900uL ddH_2O