Silver Staining Protocol Compatible with Mass Spectrometry

I. Convenient Recipes
   a. Sensitization Solution (0.02% sodium thiosulfate) – 0.1 g sodium thiosulfate in 500 mL MilliQ water
   b. Staining Solution (0.1% silver nitrate) - 0.5 g silver nitrate in 500 mL MilliQ water (use glass container only!)
   c. Developing Solution (0.04% formalin in 2% sodium carbonate) - 125 μL formalin (37% formaldehyde solution) and 15 g sodium carbonate in 500 mL MilliQ water

II. Staining Procedure
   a. Immediately following electrophoresis, remove gel from plates and notch the upper right corner. Place gel in the fixing solution (50% methanol: 12% glacial acetic acid) and gently shake overnight.
   b. Discard the fixing solution. Wash the gel with 50% methanol for 20 minutes.
   c. Discard the wash solution. Wash the gel with MilliQ water for 20 minutes.
   d. Repeat wash in step IIC.
   e. Discard wash solution. Place gel in sensitization solution (0.02% sodium thiosulfate) for 1 minute.
   f. Discard sensitization solution. Wash the gel with MilliQ water for 1 minute.
   g. Discard wash solution. Wash the gel a second time in MilliQ water for 1 minute.
   h. Discard the wash solution. Place the gel in the staining solution (0.1% silver nitrate, chilled) for 20 minutes.
   i. Discard the staining solution. Wash the gel with MilliQ water for 1 minute.
   j. Discard the wash solution. Wash the gel a second time with MilliQ water for 1 minute.
k. Discard the wash solution. Place the gel in the **developing solution** (0.04% formalin in 2% sodium carbonate). Time is variable depending on desired staining intensity.

l. Discard developing solution. Place gel in **stopping solution** (5% glacial acetic acid) for 10 minutes.

m. Discard stopping solution. Store gel in 1% glacial acetic acid at 4°C.

**Notes:**

- Wear clean non-latex gloves at all times to avoid keratin contamination.
- Wash Pyrex dish(es) with detergent and wash with copious volumes of deionized water prior to starting silver staining protocol.