Ed Kelly’s Silver Stain Protocol, updated July 16th, 2010.

Step 1. FIX🡪50% methanol, 10% acetic acid; soak with shaking >20 minutes.

Step 2. Remove SDS🡪10% ethanol, 5% acetic acid; 3 x 10 minute washes.

Step 3. Oxidant🡪0.0034 M potassium dichromate (200 mg/200 mL), 0.0032 N nitric acid (40.6 µL/200 mL); soak with shaking 5 minutes.\*

Step 4. Wash🡪ddH2O until gel is clear.

Step 5. Silver Stain🡪0.012 M silver nitrate (0.41 g/200 mL); soak with shaking 30 minutes.

Step 6. Developer (3x300 mL)🡪0.28 M sodium carbonate (29.68 g/L), 6.65 mM formaldehyde (0.5 mL 37% solution/L). The developing steps must be closely watched for optimal staining. A cloudy precipitate forms rapidly during the first step, but much more slowly in the second step. The final development must be watched to avoid over staining the gel. It may not be necessary, depending on the staining intensity at the end of the second developing step. Higher molecular weight proteins tend to stain before lower ones. At some point in the final developing step, the background intensity will increase at about the same rate as the band intensity. Stop the reaction at this time to avoid dark grey background.

Step 6. STOP🡪5% acetic acid

\*Potassium Dichromate is highly toxic-wear gloves and lab coat when preparing solution and dispose of the solution as hazardous waste.