Silver Staining protocol:

This protocol is valid for both SDS-PAGE and native PAGE gels. If not better, these home-made solutions work as good as commercially available ones.

NOTE: all solutions must be made fresh just prior to use and all steps are performed at room temperature on shaker unless otherwise stated.

After running the gel in electrophoresis apparatus:

1- Fix the gel (fixative solution, 50 % Methanol, 10% Acidic Acid, 50 μL Formalaldehyde /100 mL solution) for one hour to overnight. *There is NO need to wash gel prior to this step. But, if you decided to do so, rinse it with water for few seconds.*

2- Wash gel with 50 % ethanol three times (20 minutes each), or twice for minigels. *Washing with longer time won't enhance the final product; however, washing with less time will interfere with the later steps resulting in bad image at the end.*

3- Treat gel for precisely **ONE** minute with hypo solution (Sodium Thiosulfate solution 20 mg/100 mL). *Shake by hand. SAVE 2 ml FOR LATER STEP.*
4- *Over treating with the hypo solution will result in darker gel at the end.*

5- Wash with water three times (20 seconds each). *Shake by hand.*

6- Treat gel with Silver Nitrite solution (200 mg/100 mL) for 30 minutes. *Over treating will result in unwanted artifacts in final stage.*

7- Wash with water three times (20 seconds each). *Shake by hand*

8- Develop gel in 100 mL developing solution (6 g Sodium Carbonate, 2 mL of the hypo solution, 50 μL Formalaldehyde). This step can take from one minute to 30 minutes, depending on protein concentrations. *If you overdevelop, gel will turn dark brown which is not useful for figure making or presentation at the end.*

9- Once developed, stop with 5% Acidic Acid. *Untimely stopping will result in darkening the gel. Once you feel you see what you look for, stop developing the gel immediately.*

10- Store gel in fixative solution. *Do not store for long time, try to dry the gel as soon as you can.*