**Silver Nitrate Stain**

Solutions/Buffers to be made

A] Fixative: Acetic acid 10%, ethanol 30%

* Measure 100 ml glacial acetic acid
* Measure 300 ml ethanol
* Fill to 1000 ml with MilliQ water

B] Rinse: 20% ethanol

C] Sensitizer: 0.02% sodium thiosulfate

* Weigh 0.2 gm sodium thiosulfate
* Dissolve in 1000 ml MilliQ water

D] Silver Nitrate: 0.2% silver nitrate

* Measure 2.0 gm silver nitrate
* Dissolve in 1000 ml MilliQ water

E] Developer: must be prepared fresh [make at step 6 of the SOP]; 3% sodium carbonate, 0.025% formaldehyde, sodium thiosulfate [10 mgm/L]

* Measure 15 gm sodium carbonate
* Measure 25 ml of stock sodium thiosulfate [see step C]
* Measure 125 µl 37% formaldehyde
* Fill to 500 ml with MilliQ water

F] Stop: Tris, acetic acid

* Measure 50 gm Tris base
* Measure 25 ml glacial acetic acid
* Fill to 1000 ml with MilliQ water

Standard Operating Procedure

1. Soak the gel in fixative [see step A] for at least one hour. Then, change the solution for a minimum of another hour; however, overnight is ok.
2. Rinse the gel in rinse solution [see step B] for 20 minutes
3. Rinse the gel in MilliQ water for 10 minutes [minigel] or 20 minutes [13 x 16 cm gel]
4. Soak the gel in sensitizer solution [see step C] for 1 minute
5. Rinse the gel in MilliQ water 3 times for 20 seconds each rinse
6. Soak the gel in silver nitrate solution [see step D] for 45 minutes.
   1. Make the developer at this time!
7. Rinse the gel with MilliQ water for 5-10 seconds
   1. This is a critical step…SECONDS AND NOT MINUTES
8. Soak the gel in the developing solution [see step E] until bands are adequate
   1. 3-4 minutes
      1. DO NOT over-expose or under-expose
9. Soak the gel in stop solution [see step F] for a minimum of 15 minutes, then store in fresh MilliQ water

After the SOP is complete, the gel staining process is complete. It does not require destaining like a coomassie blue stained gel. The bands can then be image and compared.