

PROTOCOL FOR SILVER STAINING OF SDS-PAGE GELS (BLUM & OAKLEY METHOD)

- **Make ALL solutions fresh!!** Most of these solutions must be used within hours of being made!

- In all solutions containing formaldehyde (Fm), add the Fm **just before adding the solution to the gel.** Do not mix the Fm hours in advance!

- Avoid **at all times** manipulating the gel with your hands (even gloved hands) as much as possible! Use tweezers and be extremely careful during the changes of solutions.

(the following recipe is good for one 7x10cm gel)

- 1) Remove the gel from the glass plates and put it in a **CLEAN** plastic tray (pre-clean the tray with a small amount of detergent and LOTS of ddH₂O).
- 2) Wash the gel 5 min with ddH₂O.
- 3) Fix the gel 1h at room temperature, in 50ml of 50% methanol + 12% acetic acid, containing 1/2000 of 37% formaldehyde (25 µl for 50ml solution).
- 4) Wash the gel 3x20 min in 50% ethanol (prepare at least 100 ml in advance).
- 5) Wash the gel 2 min in 0.8mM sodium thiosulfate (~10mg sodium thiosulfate in 50ml water). Prepare 50 ml of sodium thiosulfate but reserve ~2 ml for step 9.
- 6) Wash the gel in ddH₂O 3x30 seconds.
- 7) Incubate the gel in (silver nitrate + formaldehyde) x 15 minutes. Prepare the silver nitrate in advance (100mg silver nitrate in 50ml water) and **keep it in the dark** until the moment of use. Add 38 µl formaldehyde/50ml solution just before adding the solution to the gel.
- 8) Wash the gel in ddH₂O 3x30 seconds
- 9) Develop the gel in (sodium carbonate+tiosulfate+formaldehyde) until the bands are visible. Prepare in advance ~50ml of 6% w/v sodium carbonate in water. Just before using, add 1/50 vol. of sodium thiosulfate from step 5 and 1/1000 of formaldehyde. Ex., for one gel prepare 3g Na₂CO₃ in 50ml water and then add 1 ml of sodium thiosulfate and 25 µl formaldehyde.
- 10) Wash very quickly in ddH₂O 2x60 seconds
- 11) To stop the reaction, place the gel in (50% methanol +12 % acetic acid) x 15 minutes
- 12) Wash the gel in 50% methanol x 20 minutes
- 13) For long-term storage leave the gel in (40% methanol + 5% glycerol) x at least 2 hours before imaging or drying between cellophane sheets.