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Protein Gel Staining Protocols

BioSafe Coomassie appears to stain the broadest spectum of proteins. It has about 2 orders of magnitude and the sensitivity down to 10 ng. SYPRO Ruby gives little background staining and very sensitive (1 ng). The stain is linear over 3 orders of magnitude and allows detection of glycoproteins, lipoproteins, low MW proteins and metalloproteins that are not stained well by other stains. Silver Staining is also very sensitive (1 ng), but the linearity is only 1 order of magnitude. Mass spectrometric compatible silver staining procedures should be used.

BioSafe Coomassie:

1. wash gels three times with MilliQ water, each time 5 min.
2. add 50 ml BioSafe stain (for Criterion gel) and shake for 1 h to overnight.
3. discard the stain, wash gel several times with water. The gels can be stored in water for several days

SYPRO Ruby:

1. gel fixing solution: 10% methanol, 7% acetic acid.
2. wash gels for 30 min in the gel fixing solution.
3. remove the fixing solution and add 50 ml SYPRO Ruby stain (for Criterion gel).
4. stain the gel with gentle agitation for at least 3 h. Gels can be left overnight.
5. rinse the gel with the fixing solution for 30 min to 1 h.
6. wash gel in MilliQ water before imaging.

Silver staining (Sigma ProteoSilver Plus kit):

1. **Solutions**:

- Fixing solution: 50% ethanol, 10% acetic acid in ultrapure water, 100 ml
- 30% ethanol in ultrapure water, 100 ml
- Sensitizer solution: add 1 ml of ProteoSilver Sensitizer to 99 ml of ultrapure water (**use within 2 h**).
- Silver solution: add 1 ml of ProteoSilver Silver to 99 ml of ultrapure water (**use within 2 h**).
- Developer solution: add 5 ml ProteoSilver Developer 1 and 0.1 ml ProteoSilver Developer 2 to 95 ml ultrapure water (**use within 20 min**).

2. **Procedure**: all steps are at room temperature and on a shaker at 65 rpm.

1. Fix the gel in a clean tray with 100 ml Fixing solution (for Criterion gels) for 40 min to overnight.
2. Decant the fixing solution, and wash with 100 ml 30% ethanol for 10 min.
3. Decant the ethanol solution, wash the gel with 200 ml water for 10 min.
4. Decant the water and incubate the gel with 100 ml Sensitizer solution for 10 min.
5. Decant the sensitizer and wash the gel twice with ultrapure water, each time with 200 ml water for 10 min.
6. Decant the water, and equlibrate the gel with 100 ml Silver solution for 10 min.
7. Decant the silver solution and wash the gel for 1 to 1.5 min with 200 ml ultrapure water (Do not exceed 1.5 min).
8. Decant water and develop the gel in 100 ml Developer solution for 3 to 7 min until the desired staining intensity is achieved.

9. Add 5 ml of ProteoSilver Stop solution to the developer solution to stop the developing reaction and shake for 5 min.

10. Decant the Developer/Stop solution and wash the gel with 200 ml ultrapure water for 15 min.

11. Store the gel in fresh ultrapure water.