

## Acrylamide gel protein detection methods

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**Coomassie Brilliant Blue (R-250)** – sensitivity of approximately 100 ng. This is an inexpensive, very reliable quantitative staining method.

1. Place acrylamide gel into staining dish and pour enough CBB stain (0.1% CBB R-250, 20% methanol, 0.5% acetic acid) to cover gel.
2. Incubate at room temperature on orbital agitator for at least one hour. Staining can continue overnight if more convenient.
3. Decant used stain and briefly rinse gel with MilliQ water to remove residual stain.
4. Destain excess CBB in 30% methanol with continuous agitation.

**Colloidal Coomassie Brilliant Blue (G-250)** – slightly better sensitivity than R-250 and is compatible with mass spectrometric analysis.

1. Wash SDS from gel with three consecutive washes (10 min each) in MilliQ water. Longer washes may be necessary for 18 and 24 cm gels. It is critical to remove residual SDS from gel prior to staining.
2. Add enough Colloidal Coomassie stain (20% ethanol, 1.6% phosphoric acid, 8% ammonium sulfate, 0.08% Coomassie Brilliant Blue G-250) to cover gel.
3. Incubate at room temperature on rotary agitator for at least six hours.
4. Decant stain and rinse gel twice with MilliQ water (1 min each).
5. Destain gel with MilliQ water until background is low (4 hours).

**Silver** – sensitivity of 5-10 ng, compatible with mass spectrometry analysis. Not a quantitative staining method for some types of modified polypeptides including glycosylated proteins. Based upon the method of Shevchenko et al. (1996) *Anal. Chem.* 68:850-858. Method is suitable for 7, 11 and 14 cm gels, double volumes for 18 and 24 cm gels. To ensure even staining of gel and proper agitation use an orbital shaker.

1. Fix gel in 100 mL of 20% ethanol, 1% acetic acid for 1 hr.
2. Wash gel in 20% ethanol for 10 min.
3. Pretreat gel with 100 mL 0.02% sodium thiosulfate for 1 min.
4. Decant and rinse gel in MilliQ water for 1 min.
5. Add 100 mL of 0.1% silver nitrate solution and agitate for 15-20 min.
6. Decant and rinse gel in MilliQ water for 1 min.
7. Decant and add 100 mL of developing solution (2% sodium carbonate, 40  $\mu$ L 37% formaldehyde). Develop until protein spots are visible (5-10 min)
8. Stop development with 5% acetic acid (5-10 min) and replace with MilliQ water

**Fluorescent detection methods** – sensitivity of 10-20 ng, compatible with mass spectrometry analysis. Sensitive, broad quantitative range stains that require little destaining. The Sypro series of stains are expensive but easy to use. Alternatively,

ruthenium II can be synthesized inexpensively and gives similar sensitivity to Sypro Ruby (Rabilloud et al. 2001, Proteomics 1:699-704).

### **Ruthenium II**

1. Synthesize ruthenium II stain according to Rabilloud et al., 2001.
2. Fix gel in 30% ethanol, 1% acetic acid for 2 h or overnight
3. Wash gel four times 30 min each in 20% ethanol to remove acetic acid. It is necessary to remove any residual acetic acid to prevent quenching of signal.
4. Stain gel for 3-6 hr in 20% ethanol containing 0.1-0.2  $\mu$ M ruthenium II (5-10  $\mu$ L of stock solution/L).
5. Wash gel twice 10 min each in MilliQ water. Gel can be stored in water.

### **Notes**

- Staining of protein must be performed with labware that has never been in contact with nonfat milk, BSA, or any other protein blocking agent to prevent carryover contamination.
- Always use non-latex gloves when handling gels or IPG strips, keratin and latex proteins are potential sources of contamination.
- Never re-use Coomassie stain, abundant proteins will partially leach out and contaminate subsequent gels.