

# *Coomassie Stain for PRPs on SDS Gels*

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## *Introduction*

Proline-rich proteins have unusual solubility and staining characteristics. This method of staining takes advantage of those characteristics to stain proline-rich proteins pink or violet; other proteins stain blue. The method is not as sensitive as silver stain, and is not a method for evaluating whether proteins bind tannin, because it is used in SDS gels. We have found that some tannin-binding proteins (deer) do not stain distinctively with this method. We have used the method most successfully with rat saliva. The method given here was adapted from Beeley et al. *Electrophoresis* 12, 1032-1041 (1991).

Separate the proteins on 12% SDS gels, 0.5 or 1.5 mm thick, in Hoeffer minigel apparatus as usual.

## *Stain*

1. Dissolve 0.5 g Coomassie blue R-250 in 200 mL absolute ethanol (or 210 mL 95% ethanol). Add this to a solution of 50 mL glacial acetic acid up to 500 mL with water. You can store this solution and reuse it repeatedly as long as it remains clear and free of particulates.
2. Stain the gels for 2 h in this stain, and destain in 10% acetic acid (10 mL glacial acetic acid up to 100 mL with water).
3. Normal proteins stain blue or violet, while (some) salivary proline-rich proteins eventually destain to form pink bands.
4. It may take 4 days for the pink color to show up.
5. A useful contrast is to run an identical gel and stain with same solution, but destain with 10% acetic acid/10% ethanol (10 mL glacial acetic acid plus 10 mL ethanol up to 100 mL with water). The proline-rich proteins are destained to almost colorless bands, so their absence contrasts nicely with their pink color in the acid destain.