

# ***Tannin-Binding Proteins Detected by Electrophoresis on Native Gels***

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## ***Introduction***

This method allows detection of proteins in saliva or other samples which selectively bind to tannins. Either precipitable or nonprecipitable complexes can be detected with this method, as with gel shift assays for other protein ligands. The method described here was specifically developed for assessing saliva, and is modified from Austin et al., *J. Chem. Ecol.* 15, 1335-1347 (1989).

## ***Saliva***

Should be frozen immediately after collection. Add PMSF (phenyl methyl sulfonyl fluoride) at a final concentration of about 40-50 ug/mL saliva to prevent proteolysis during storage. PMSF can be prepared as a stock solution at 10 mg/mL in isopropyl alcohol and stored at room temperature; it is unstable in aqueous solution.

**Caution: PMSF is very toxic.**

## ***Incubation of saliva with tannin***

These amounts are based on ruminant saliva--adjustments to compensate for the concentration of proteins in other samples may be necessary.

1. Prepare a tannin solution containing antioxidant by diluting 10 uL of 100 mM EDTA in 1 mL 50% methanol/50% water. Add 0.009 g ascorbic acid and mix to dissolve the acid. A bit may remain undissolved. Add tannin to make a 20 ug/uL stock solution (condensed tannin) or a 5 ug/uL stock solution (gallotannin). I make about 75 uL of stock solution at a time. (The solution cannot be saved as the tannin oxidizes too readily).
2. Prepare a 1:10 dilution of the stock tannin solution.
3. Mix the saliva with tannin: Dispense 30 uL samples of saliva into a series of microfuge tubes. To each, add a total of 10 uL of the 50% methanol solution containing 0-200 ug condensed tannin or 0-50 ug gallotannin.
4. Mix the solutions and incubate overnight at 4C.
5. Prepare the samples to go on the gel. Mix each sample with 10 uL of bromophenol blue/glycerol/buffer. Centrifuge each sample 3 min at about 3000 rpm.

## ***Prepare the gels***

Make native gels, using the Laemli recipes without SDS. Use 12% acrylamide. I use a Hoefer minigel system 0.75 mm thick gels about 5 x 8 cm, and a sample comb with 10 lanes.

Apply 10 ul sample (supernatant--there may be substantial precipitate, or may be none) to each lane.  
Run the gels. Fix and stain with silver stain.