

Broccoli (*Brassica oleracea*) histone purification.

Keith Earley, Amy Caudy and Craig Pikaard.

Biology Department, Washington University, St. Louis, MO. 63130

1. Using a razor blade, collect 100 g of proliferating floral meristem tissue from broccoli (cut at the very top of the stalks).
2. Grind tissue in 200 mL of Honda buffer (20 mM HEPES-KOH pH 7.4, 10 mM $MgCl_2$, 0.44 M sucrose, 1.25% Ficoll (type 400), 2.5% Dextran T40, 0.5% Triton X-100, 0.5 mM DTT, 1.0 mM PMSF, and Plant Protease Inhibitors from Sigma) using 6 pulses of 5 seconds each at top speed in a Waring blender.
3. Filter through 2 layers of Miracloth, squeezing out excess liquid from plant material.
4. Spin 15 minutes at 7500 rpm, using the Beckman JA-10 rotor.
5. Pour off supernatant, keeping pellet of chloroplasts, nuclei, and starch.
6. Gently resuspend pellet by pipetting up and down in 100 mL 300mM buffer (20 mM HEPES-KOH pH 7.5, 0.3 M KCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 10% glycerol, 1 mM DTT, 1 mM PMSF, and Plant Protease Inhibitors from Sigma). Continue gently swirling by hand for 10 minutes at 4° C.
7. Pellet nuclei for 15 minutes at 17,500 x g in Beckman JA-20 rotor.
8. Resuspended in 100 mL of HAP buffer (50 mM $NaPO_4$ buffer pH 6.8, 0.6 M NaCl, 1 mM DTT, 1 mM PMSF) and stirred gently for 10 minutes at 4° C.
9. While stirring, add 10 g of dry Bio-Gel HTP powder (Bio-Rad).
10. Stir for 30 minutes (more HAP buffer may be needed)
11. Wash the resin with 3x with HAP buffer, spin between washes (batch method)
12. Pour into Column to collect resin. Allow buffer to wash through the resin.
13. Additional washes can be added if needed.
14. Elute the core histones with a step of ice cold HAP elution buffer (50 mM $NaPO_4$ buffer pH 6.8, 2.5 M NaCl, 1 mM DTT, 1 mM PMSF), collecting 2mL fractions. Place on ice as soon as elutions are collected. Monitor the protein concentration either by absorbance at 280 nM or by Bradford assay. Pool the peak fractions.
15. Combine fractions with histones and place in dialysis tubing.
16. Concentrate the core histones using solid sucrose methodology by placing dialysis tubing (6-8 kd cut-off size) with histones in solid sucrose.
17. Dialyze supernatant overnight in a bucket of salt ice water against 2 liters of LSB (20 mM HEPES-NaOH pH 7.5, 0.1 M NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF).
18. Freeze histones in small aliquots at -80 for future use.