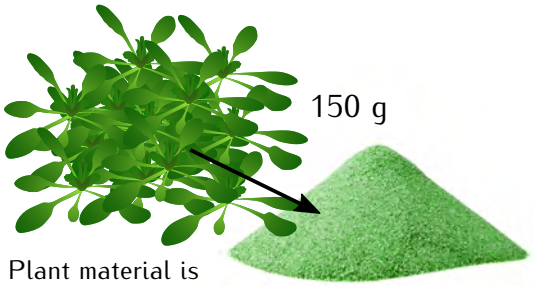
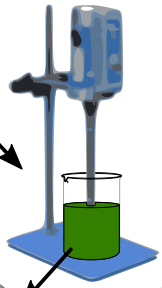


150 g

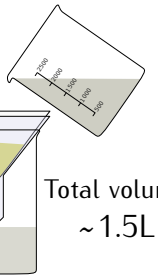
Plant material is frozen and homogenized in liquid nitrogen

Suspenssion in 1.5L of 4.5% PCA



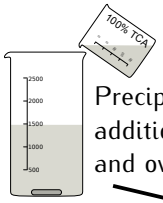
Homogenization in rotor-stator homogenizer for 30 min. at max. speed

×2



Removal of cellular derbies by centrifugation (15 min., 15 000×g) and filtration of supernatant through Miracloth

Total volume ~1.5L

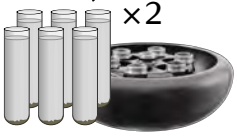


Precipitation of protein by addition of TCA to 25%, v/w and overnight incubation

Total volume ~2L

Centrifugation (25 000×g, 30 min., 30 ml glass tubes, 13 centrifugations were required to collect precipitate from the whole sample)

Protein precipitates rinsed twice by adding 30 ml of -20°C acetone and centrifugartion (15 min., 25 000×g)



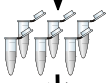
×2

Six glass tubes containing precipitates vacuum dried and stored in -20°C

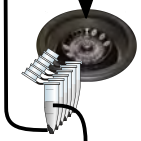
Pellet in each tube suspended in 500 µl of 4.5%G by vortexing and 15 min. sonication in ice-colded BioRuptor UCD-200 at maximal power operated in 30 s cycles (ultrasound source operating 15 s in each cycle).

Suspenssion transfered to microcentrifuge tubes, then glass tubes rinsed twice with 500µl of 4.5%G

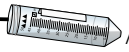
suspenssion of pellets



Suspenssion sonicated in BioRuptor (as before) and incubated for 30 min. in termomixer (1400 rpm.)



Centrifugation for 15 min. at 25 000×g supernatants pooled in new tube



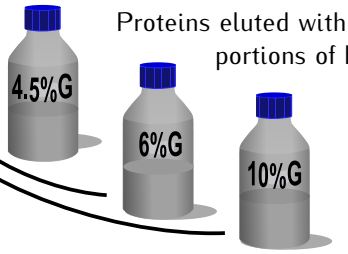
Addition of 2 ml of 50% Bio-Rex 70 in 4.5%G

Incubation for 4h with mixing by inversion

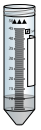
Total volume at this stage ~12.5 mL

Suspension transfered to column, resin allowed to seetle (aproximatly 40 min.)

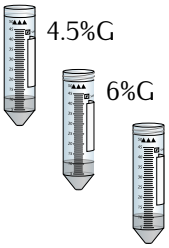
10 ml



Proteins eluted with 10 ml portions of buffers



Unbound proteins



4.5%G

6%G

10%G

Fractions collected, frozen in liquid nitrogen, vacuum dried and stored in -20°C

Histone H1

HPLC