

Acid Extraction of *Drosophila melanogaster* Nuclei

1. Flies are flash frozen on liquid nitrogen and dismembered by several pulse vortexes.
2. Fly heads are isolated using pre-chilled (-80 C) size exclusion sieves (fly head recovery averages ~7% whole-fly starting weight).
3. Fly heads are pulverized in liquid nitrogen using a ceramic mortar and pestle.
4. After the liquid nitrogen has evaporated, the homogenate is mixed (gently vortexed) with 5ml of buffer A + NP in a 15ml falcon tube.
5. The homogenate is then transferred to a 15ml dounce homogenizer (Wheaton) and disrupted with 20 strokes of the loose pestle (type B). The homogenate is filtered through one layer of Miracloth (Habco Product, NJ). Squeeze the Miracloth to remove excess liquid trapped in the cloth.
6. 3ml of buffer A + NP is used to rinse homogenizer and the suspension is filtered through the same Miracloth.
7. The filtrate is loaded over 2ml of buffer AS and centrifuged in Sorvall HB-4 swinging bucket rotor at 4 C, 3000 rpm (1,500g) for 5 min.
8. The recovered pellet is resuspended in 5ml of buffer A + NP, transferred to a 7ml dounce homogenizer, and dispersed with 10 strokes of the tight pestle (type A). The mixture is loaded over 2ml of buffer AS and centrifuged as before.
9. The recovered pellet is resuspended in 5ml of buffer A, transferred to a 7ml dounce homogenizer and dispersed with 10 strokes of the loose pestle (type B).
10. The nuclei are collected by centrifugation in the same rotor at 3000rpm for 5 min. The pellet is washed with another 5ml of buffer A and centrifuged at 4000 rpm (2600g) for 5 min.
11. The pellet is resuspended in 5-10 volumes of lysis buffer: 10mM HEPES, pH 7.9, 1.5mM MgCl₂, 10mM KCl, 0.5mM dithiothreitol, 1.5mM PMSF –Add PMSF and DTT just prior to use of the buffer
12. Add hydrochloric acid to a final concentration of 0.2M (0.2N). (Use polypropylene tubes)
13. Incubate on ice for 30 min.
14. Centrifuge at 11,000 x g for 10 min. at 4 C.
15. Keep the supernatant fraction, which contains the acid soluble proteins, and discard the acid - insoluble pellet.
16. Dialyze the supernatant against 50-fold excess 0.1M (0.1N) acetic acid, twice for 1-2 hours each at 4 C.
17. Dialyze against water three time (1 hour, 3 hours and overnight) at 4 C. The recovered acid extracted nuclear protein can be stored at -80.

BUFFERS

	<u>A + NP</u>	<u>AS</u>	<u>A</u>
KCl	60mM	60mM	60mM
NaCl	15mM	15mM	15mM
EDTA	13mM	1mM	1mM
EGTA	0.1mM	0.1mM	0.1mM
Tris Cl pH 7.4	15mM	15mM	15mM
Spermine	0.15mM	0.15mM	0.15mM
Spermidine	0.5mM	0.5mM	0.5mM
DTT	0.5mM	0.5mM	0.5mM
NP-40	0.5%	---	----
Sucrose	--	0.3M	----