

C. ELEGANS WHOLE CELL EXTRACT PREPARATION

Reagents:

Buffer C
Protease Inhibitors (Leupeptin, PMSF, Pepstatin A)
50 ml conical tube
centrifuge that can spin 50ml conical tubes 3000rpm
nutator or shaker
1xPBS
methanol/dry ice bath
sonicator with microtip
Bio-Rad Protein Assay Dye Reagent Concentrate (cat#500-0006)

Procedure:

Starting with a 10cm plate of worms:

- 1) Wash plate with ~5ml 1xPBS and pour off into 50ml conical tube.
- 2) Let worms pellet by gravity or spin at 3000 rpm for 2min.
- 3) Nutate/mix 1 hour to wash bacteria off worms.
- 4) Spin 3000 rpm 2 min (repeat steps 3+4 optional to ensure clean extract).
- 5) Resuspend worm pellet in ~20ml 1xPBS.
- 6) Spin 3000 rpm 10min.
- 7) Transfer pellet to an eppendorf tube.
- 8) Spin 5 sec to pellet worms; remove supernatant.
- 9) Add 1:1 volume of Buffer C with protease inhibitors to worm pellet.
- 10) Freeze/thaw 3x in methanol/dry ice.
- 11) Sonicate ~10 bursts with the microtip.
Settings: microtip: 5-6; duty cycle; 40-50; timer: hold.
- 12) Spin ~5sec. Transfer supernatant to new tube.
- 13) Determine protein concentration by BioRad assay.

Buffer C:

20mM HEPES pH 7.9
25% (v/v) glycerol
0.42 M NaCl
1.5 mM MgCl₂
0.2 mM EDTA
--add 0.5mM DTT before use

Protease Inhibitors

-add to a final concentration of 1µg/ml or 0.1mM
Leupeptin
PMSF
Pepstatin A

Phosphatase Inhibitors:

1mM sodium fluoride (100mM stock)
1mM sodium molybdate (100mM stock)

