Worm Lysis Protocol

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Materials Needed:

2X worm lysis buffer (see recipe below)
liquid nitrogen or dry-ice/EtOH bath
Millipore ultra-free-MC centrifugal filter unit (0.6μm)
pestle (disposable plastic ones for microcentrifuge tubes)
M9 (see recipe below)
sand, white quartz
gel loading tip

Procedure:

1. Add worms to a microcentrifuge tube using M9.
2. Remove maximum amount of liquid and flash freeze the animals in liquid nitrogen (or dry-ice and EtOH bath).
   * Samples can be stored at -80oC.
3. Estimate the pellet volume and quickly add 1:1 volume of worm lysis buffer.
4. Use a Pipetman to measure the total volume and correct the volume to your 1:1 estimation in the previous step. This step is useful to help in concentration consistency if preparing several samples.
5. Let sample sit on ice for 15 minutes. Up to 30 minutes on ice is ok.
6. Monitor the efficiency of lysing by sampling a small aliquot of extract (1μL) under a microscope. If there are still intact worm bodies, add sand (up to 50% of the volume) and vortex.
7. Transfer extract to a new microcentrifuge tube by using a gel-loading tip (long-thin tip).
8. Spin at 3000rpm (1000xg) to remove debris for 1 minute.
9. Transfer extract to a Milipore spin column (0.6μm) and spin for 15 minutes at 5000xg.
10. Repeat step 9 until all the sample is passed through the filter, adding more lysis buffer as necessary.
11. Measure protein concentration.

Recipes:

<u>2x Worm Lysis Buffer</u>
* Common stock in -20°C stock freezer (Cook 3103)
50mM Tris (pH 7.4)
5mM $MgCl_2$
2% Triton X-100 (use 0.5% for native samples)
0.2mM PMSF (Sigma P-7626)
1μg/mL Leupeptin (Sigma L-0649)
5x Protease Inhibitor Cocktail (use at 5x concentration)

<u>M9</u> (1L)
* Common stock in worm room.
5.8g $Na_2HPO_4 \cdot 7H_2O$
3.0g KH_2PO_4
5.0g NaCl
0.25g $MgSO_4 \cdot 7H_2O$
ddH_2O to 1L
• Filter (0.22μm) and bottle.