

10/19/2020

Mitochondria isolation of *C.elegans*

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Prior to Start:

- Label tubes for samples and chill
- Turn on centrifuge so it is ready at 4 degrees.
- Aliquot Mitochondrial Lysis Buffer into conical tubes and keep on ice. For one strain/treatment of worm sample, need ~ 5ml MSME/1 mM PMSF and ~30ml MSME. Add protease inhibitor to Mitochondrial Lysis Buffer (1:1000).

Washing worms:

1. Harvest 15,000-20,000 adult worms into 15 ml conical tube. ABOUT 500ul.
2. Wash 3X in M9 buffer (spin 30 sec at 1000g after each wash).

Prepare mitochondria

- Clean the homogenizer with EtOH and ddH₂O
- Wash worms off and wash three times with M9, spin down 30s @1000g
- Wash once with 10 ml MSME
- Wash once with 2 ml MSME/1 mM PMSF
- worm pellets were suspended in 1 ml MSME/1 mM PMSF.
- Optional : Add 100 ul of a collagenase stock solution, incubated for 20 min at room temperature on roller.
- Transfer to a 5 ml Potter homogenizer and treated for 4 min (120 times) on ice.
- Transfer to a clean Eppendorf tube
 - (mix #1:20ul)
- Spin 10min @200 g to clear off the debris.
 - (pellet #2:20ul/1ml, sup #3: 20ul)
- Transfer supernatant to a new tube
- Spin for 10min @800g
 - (pellet #4:20ul/200ul, sup #5: 20ul)
- Transfer supernatant to a clean tube
- Spin 10 min @12,000 g to get the Mito pellet
 - (pellet #6: 20ul/1ml sup #7:20ul)
- Wash with MSME/1 mM PMSF, spin 10 min @12,000 g
 - (pellet #8: 20ul/200ul sup #9: 20ul)
- Wash with MSME 2 times, spin 10 min @12,000 g
- Pellet was suspended in 60ul MSME with 10 µg/ml proteinase K (Sigma) containing digitonin (Sigma, 0.5% - 1%) or 1% Triton X-100, and incubated on ice for 30 min.
 - (sup #10: 5ul/200ul)

MSME:

- 5 mM Tris-HCl pH 7.4
- 220 mM mannitol (182.17)
- 70 mM sucrose (342.30)
- 2 mM EDTA (292.24)

For 500mL

- 5ml Tris-HCl (500mM)
- 20.03 g mannitol
- 11.98 g sucrose
- 5ml EDTA (200 mM)

500 mM Tris-HCl(pH=7.4):

60.57 g Tris, 20ml HCL, to 1 L

200 mM EDTA

58.45 g EDTA, to 1 L

PMSF, 200mM

For 1ml:

- Dissolve 34.84mg PMSF in 1ml EtOH, keep in -20

Collagenase stock solution

- 33 mg/ml in 5 mM CaCl₂/PBS; Sigma–Aldrich

Digitonin stock solution 10%

- 250mg digitonin + 2.5 ml MilliQ

MSME+D (for 200ul)

- 180ulMSME+ 2ul proteinaseK(1mg/ml) + 20ul Digitonin(10%)