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CIDC_S16_NMR_Celegans_Extraction_Protocol

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A sample preparation protocol for lyophilized C. elegans samples to be analyzed by NMR spectroscopy

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zirconia beads **BioSpec Products Catalog #11079110zx**

Isopropanol **Fisher Scientific Catalog # A4614**

Methanol **Fisher Scientific Catalog #A4564**

Water **Fisher Scientific Catalog #W64**

Homogenization 7m 30s

1 Remove lyophilized *C. elegans* samples from **-80 °C** freezer

2 Add approximately **200 µL** of **1.0 mm** Zirconia beads (BioSpec Cat. No. 11079110zx) to each sample.

3 1m 30s

FastPrep-96
High-throughput bead beating grinder and lysis system
MP Biomedicals **116010500**

QuickFlex™ Sample Holder
Adapter for 96 x 2 mL tube holder on FastPrep-96
MP Biomedicals **116010570**

Using a FastPrep-96™ instrument (MP Biomedicals) equipped with QuickFlex™ Sample Holder (or similar equipment) homogenize samples at **420 rcf** for **00:01:30**

4 1m 30s
Place samples on dry ice for **00:01:30** to avoid overheating due and sample degradation.

5 1m 30s
Using a FastPrep-96™ instrument (MP Biomedicals) equipped with QuickFlex™ Sample Holder (or similar equipment) homogenize samples at **420 rcf** for **00:01:30**

6 1m 30s
Place samples on dry ice for **00:01:30** to avoid overheating due and sample degradation.

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1m 30s

7 Using a FastPrep-96™ instrument (MP Biomedicals) equipped with QuickFlex™ Sample Holder (or similar equipment) homogenize samples at **420 rcf** for **00:01:30**

8 Store on dry ice and proceed with Extraction (Section 2) or store at **-80 °C** until ready to **00:00:00** process.

Sequential non-polar (i) and polar(ii) extraction 7m 30s

9 Add **500 µL** of 100% IPA chilled to **-20 °C** to each homogenized sample containing: (1) lyophilized *C. elegans* material and (2) zirconia beads

1m 30s

10 Vortex each sample for **00:00:30** to **00:01:00**

35m

11 Let sample sit at **Room temperature** for **00:15:00** to **00:20:00**

12 Add an additional **500 µL** of 100% IPA chilled to **-20 °C** to each homogenized sample containing: (1) lyophilized *C. elegans* material, (2) zirconia beads, and (3) **500 µL** IPA.

1m 30s

13 Vortex each sample for **00:00:30** to **00:01:00**

35m

14 Let sample sit at **Room temperature** for **00:15:00** to **00:20:00**

15 Store samples **Overnight** (~12 hours) at **-20 °C**

30m

16 Centrifuge samples for **00:30:00** at **20800 rcf, 4°C**

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17 Transfer via Pasteur pipette the supernatant of each centrifuged sample to a new 2 mL tube for the analysis of non-polar molecules.

18 Place all non-polar extracts in a CentriVap at **Room temperature** and monitor until completely dry.

CentriVap
Benchtop Centrifugal Vacuum Concentrator
Labconco **7810010**

19 Add **1 mL** of 80:20 Methanol:Water (MeOH:H_2O) chilled to **4 °C** to each tube containing (1) the remaining worm pellet and (2) zirconia beads.

20 Shake samples at **4 °C** for **00:30:00** 30m

21 Centrifuge samples for **00:30:00** at **20800 rcf, 4°C** 30m

22 Transfer via Pasteur pipette the supernatant of each centrifuged sample to a new 2 mL tube for the analysis of polar molecules.

23 Place all polar extracts in a CentriVap at **Room temperature** and monitor until completely dry.

24 Remaining worm pellet and zirconia beads can be stored at **-80 °C** for future protein and/or carbohydrate analysis or discarded appropriately.

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