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CIDC_S16_LC_MS_Celegans_Extraction_Protocol

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A sample preparation protocol for lyophlized C. elegans samples to be analyzed via LC-MSMS

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C. elegans, C elegans caenorhabditis elegans, LC-MSMS, LC-MS/MS, LC-MS, liquid chromatography, mass spectrometry, metabolomics, lipidomics

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MATERIALS

Glass beads, acid-washed, 425-600 µm (30-40 U.S. sieve) **Sigma Aldrich Catalog #G8772-100G**

Acetonitrile **Sigma Aldrich Catalog #34998**

Methanol HPLC **Fisher Scientific Catalog #9093-03**

Eppendorf tubes 1.5 mL uncolored **Eppendorf Centrifuge Catalog #022363204**

2-Propanol **Fisher Scientific Catalog #A417-4**

Water Optima™ LC/MS Grade Fisher Scientific **Contributed by users Catalog #10728098**

2.0mm zirconium oxide beads (Next Advance SKU ZROB20) **Contributed by users Catalog #ZROB20**

Homogenization

1 Samples are removed from **-80 °C**

2 (3) 2.0mm zirconium oxide beads and ~ **75 µL** volume of 0.5mm glass beads are added to each sample tube.

3 Samples are placed in Tissuelyser II using adapter trays chilled at -80C and homogenized at 1800rpm for **00:03:00** .

4 Samples are now homogenized.

Extraction

5 **750 µL** of 100% isopropanol is added to the 1.5mL Eppendorf tube containing the homogenized sample. The sample tube is lightly vortexed to create a suspension of homogenized sample and the resulting slurry is transferred to a new 2.0mL Eppendorf tube, leaving the beads behind in the original tube. This step is repeated so that a total of **1.5 mL** of solvent is transferred to the new 2.0mL Eppendorf tube.

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6 Samples are vortexed for **00:01:00** and then extracted over night at -20C

7 Samples are placed in the centrifuge and spun at max speed (22100G) for **00:05:00**

8 Supernatant of each sample is transferred to a new 2.0 mL Eppendorf labeled for RP chromatography and dried down using steps 14 through 18

9 The second round of the sequential extraction is **1.5 mL** of 80/20 methanol/water per added directly to the pellet remaining after centrifugation.

10 Samples are shaken using the Fisher Scientific Isotemp High Speed Shaker at 1500rpm for **00:30:00**

11 2.0mL Eppendorfs are placed in the centrifuge and spun at max speed (22100G) for **00:05:00**

12 Supernatant of each sample is transferred to a new 2.0 mL Eppendorf labeled for HILIC chromatography and dried down using steps 14 through 18

13 Pellets are dried for 1 hour and stored at **-80 °C**

Sample drying/storage

14 Samples are placed in a Labconco CentriVap concentrator and monitored until they have completely dried (roughly 4-5 hours)

15 Once dry, samples are stored at **-80 °C** until they are to be run on the LC-MS instrument.

16 When preparing samples to run on LC-MS, reconstitute samples with **75 µL** of 100% isopropanol for reverse phase and 80/20 methanol/water for HILIC.

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17 Vortex for 1 minute, then centrifuge at max speed (22100G) for **00:05:00**

18 Transfer to LC-MS vial

19 After LC-MS analysis, samples can be stored at -80C until ready for IMS analysis

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