



UNIVERSITY OF SOUTH ALABAMA
MASS SPECTROMETRY
CORE FACILITY

PREPARATION OF CELL PELLETS FOR METABOLOMICS ANALYSIS

****Proper sample preparation is crucial for successful MS results. Please consult the MS facility regarding sample preparation prior to bringing your samples for analysis. Researcher will be responsible for providing total protein/cell counts for data normalization if necessary. ****

Cells should be grown in media free of phenol red. Please advise the MS facility what treatments have been applied to the cells to confirm this will not interfere with MS analysis. Biological replicates are preferred but not required. Approximately 5 million cells will be needed for analysis. It is ideal that any solvents used for sample preparation be freshly degassed to prevent oxidation of reduced compounds if these are to be measured.

Collection of Cell Pellets

1. Add 3-5mLs H₂O to cell plate to wash the cells, remove all H₂O from the plate.
2. Add 3mLs H₂O to plate, cells should be scraped and transferred to a clean centrifuge tube.
3. Gently centrifuge at 1,000 rpm to pellet the cells and discard the entire H₂O supernatant, being careful not to disturb the cell pellet.

Liquid-liquid Extraction

1. Add 400µl -80°C MeOH directly to cell pellets.
2. Sonicate at 4°C 3x for 10 seconds each.
3. Add 400µl CHCl₃ and vortex well.
4. Add 200µl H₂O and vortex well.
5. Centrifuge samples at 13,000rpm for 15 minutes at 4°C.
6. Carefully transfer 400µl of the top aqueous phase containing the metabolites to a clean 1.5mL Eppendorf, being very careful to avoid touching the organic phase with the pipette tip.
7. The samples should be frozen at -80°C and subjected to lyophilization using a freeze dryer. When drying, a small hole should be punctured into the lid of the closed Eppendorf tube to allow the solvent vapors to escape.
8. Store dry samples at -80°C until analysis by MS.

Samples are placed in a queue upon receipt. A completed requisition form must be provided along with the samples. Results will be provided by email and/or PDF attachment using the email address provided.

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Please visit our website at www.southalabama.edu/colleges/com/research/mass-spectrometry.html