02/02/21

UNIVERSITY OF SOUTH ALABAMA
MASS SPECTROMETRY
CORE FACILITY

METABOLOMIC EXTRACTION OF TISSUES FOR MS 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 tissue weight for data normalization if necessary **

Biological replicates are preferred but not required. 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.

Tissues should be harvested and immediately snap-frozen in liquid nitrogen and stored at -80°C. If data normalization is necessary, tissue should be weighed prior to submitting to MS facility.

1. Transfer frozen tissues to prefilled 2mL bead beating tubes containing 2.8mm ceramic beads (Fisher Scientific, P/N 15-340-154). ~50-100mg of tissue is preferred.
2. Add 400µl 80% MeOH (Degassed, stored at -80°C, containing heavy labeled internal standards if necessary) and bead beat for 30s.
3. Centrifuge 100 x g at 4°C for 5 min. Transfer 300µl supernatant to 2mL Eppendorf tube and keep cold.
4. Repeat step 2. Centrifuge 100 x g at 4°C for 5 min. Transfer and combine 300µl supernatant with previous lysate and keep cold.
5. Repeat step 2 once more to complete the 3x bead homogenization. Centrifuge 100 x g at 4°C for 5 min. Transfer 400µl of the supernatant and combine with previous lysate and keep cold. The combined lysate should now contain a total of 1mLs.
6. Add additional 200µl cold degassed H_2O, vortex well.
7. Add 800µl cold degassed $CHCl_3$, vortex well. The ratio of MeOH : $CHCl_3$: H_2O should now be 2:2:1.
8. Centrifuge 16,100 x g at 4°C for 15min.
9. Transfer 800µl of the top aqueous phase to clean 1.5mL Eppendorf tube and speed vac to dryness (without heat), being very careful to avoid touching the organic phase with the pipette tip.
10. Dissolve dry samples into 40µl degassed H_2O and vortex well. Centrifuge 16,100 x g at 4°C for 15min. Carefully transfer 30µl of the supernatant, avoiding the bottom of the tube, to a mass spec appropriate vial for analysis.

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.

University of South Alabama Mass Spectrometry Core Facility
1660 Springhill Ave, Mobile, AL 36604, Room 2095
251-445-9860
Usa.Mass@southalabama.edu

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