CELL/BACTERIA PREPARATION FOR LCMS

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BACKGROUND

- For preparation of cells/bacteria for endometabolite and exometabolite analysis using Liquid Chromatography-Mass Spectrometry (LCMS)

NOTES

- Perform all steps on ice
- Extraction solvent must be prepared in advance
    - Use equal amounts of HPLC grade methanol and ethanol
- Never use reagent grade methanol or ethanol (must be HPLC grade)
- Recovery standards must be added after the initial 200µL extraction
- Internal standards, added at the end of the extraction or prior to running the LCMS, are typically isotopically labeled amino acids or peptides (e.g. deuterated amino acids), but should be chosen with respect to the compounds of interest (i.e. they should be similar)

EQUIPMENT

- Equipment:
    - Centrifuge
    - Vortex
- Materials:
    - 2:2:1 HPLC grade MeOH:EtOH:H_2O (endometabolite extraction solvent)
    - 1:1 HPLC grade MeOH:EtOH (exometabolite extraction solvent)
    - Cell lifters (cut in half)

PROTOCOL

- Preparatory Work:
    - Label tubes in preparation for extractions
        - For endometabolite tubes, place 2 metal beads in each
        - For exometabolite tubes, add 80µL 1:1 HPLC grade MeOH:EtOH to each

1. From each of the endometabolite wells, collect 20µL of supernatant and add to labeled exometabolite tube (already containing 80µL 1:1 HPLC grade MeOH:EtOH)
    a. Place on ice or in the fridge while other steps are completed
2. Pipette off remaining supernatants and wash adherent cells 2x with PBS
    a. Save supernatants for ELISA and freeze at -80°C if required
3. Add 200µL of cold 2:2:1 MeOH:EtOH:H_2O to each well
    a. Lift cells using cell lifter (cut in half)

b. Pipette off the 200µL solution into the endometabolite tubes (already containing 2 beads)
c. Place all tubes on ice while others are being collected
d. Re-use cell lifter by wiping with ethanol
e. Once all samples are collected, vortex all endometabolite tubes for 2 minutes

4. Store all samples at -80°C or continue with extraction (give samples to Fan Fei from the McCarry Lab)